IMPLEMENTATION AND EVALUATION OF STRATEGIES FOR CONTROL OF SCHISTOSOMIASIS AND SOIL TRANSMITTED HELMINTHIASIS IN PEMBA ISLAND, ZANZIBAR.

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Declaration

‘I, Shaali Makame Ame confirm that the work presented in this thesis is my own. Where information has been derived from other sources, I confirm that this has been indicated in the thesis.’

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Abstract

The context of this work in Pemba was (i) the initial reestablishment of School-Based Treatment (SBT) with praziquantel (PZQ) and albendazole (ALB) for control of urogenital schistosomiasis and soil-transmitted helminths (STH), respectively, and (ii) the subsequent implementation of mass drug administration (MDA) with or without additional snail control or behavioural modification which was aimed at evaluation of the possibility of elimination of schistosomiasis. The prime focus of the work described was to use regular parasitological monitoring to evaluate and compare control strategies with regard to the effects of intervention on transmission or morbidity.

Chapters 1 and 2: General Introduction and Material and Methods respectively

Chapter 3: Urogenital schistosomiasis. (i) Use of single annual SBT with PZQ: By assessing urine egg output in new-entry standard (Std)-1 children, neither the intensity nor baseline prevalence of 9.5% was lowered over a two year period of intervention. However, prevalences in St-3 children who had received SBT showed lower prevalences than Std-1 children presumably reflecting PZQ-mediated worm reductions. This supports the WHO recommended use of SBT treatment once/year for areas with prevalence <20% for morbidity control but demonstrates its lack of effect in transmission control. (ii) Use of MDA (twice/year) alone or combined with snail control or behavioural modification:. Testing of Std-3 and Std-4 children in each of 15 shehias allocated to the three different intervention arms revealed an overall reduction in the prevalence of schistosomiasis from 8.3% in 2012 to 5.4% in 2014 (impact:- behavioural control > snail control>MDA). There was a downward trend in the overall mean intensity across all interventions (from 2.71 $\rightarrow$1.83 $\rightarrow$1.71) but no consistent reduction in the proportion of heavy infections among the infected children. ***Recommendations:*** Integrated interventions are likely to be necessary for interrupting transmission of schistosomiasis and eventually leading to elimination. In poor resource areas, such as Zanzibar, it would be difficult to implement fully integrated control measures and so focusing on PC in areas with high transmission coupled with behavioural changes and strengthening of diagnostic capability of health facilities could be essential.

Chapter 4: STH. Faecal examination of the Std-1 children, as above, for the eggs of *Ascaris lumbricoides, Trichuris trichiura* and hookworm, showed a high frequency of polyparasitism. Neither SBT nor intense MDA significantly reduced prevalence of any STH worm in the communities. The worm specific prevalences were slightly reduced overall, most consistently for *Trichuris.* However, there were no significant changes in intensity of infection, which remained light in the majority of infected children. ***Recommendations:*** No impact of either SBT or MDA on transmission to Std-1 children was observed. Integrated approaches e.g. WASH (water, sanitation and hygiene), education and PC are likely to be essential for reduction in transmission.

Chapter 5: The efficiency of drug distribution and associated compliance were monitored during the MDA by individual questionnaire and reports from the community drug distributors (CDDs). Overall coverage rate was high (~80%) but variable between the districts (69.3-88.2%). Based on questionnaire, a significant proportion of individuals were non-compliant with the MDA especially regarding PZQ (10.2%) although this did not associate with knowledge about disease transmission, signs/symptoms or risk factors for schistosomiasis. Only mild adverse effects were reported. ***Recommendations***: For any future MDA careful thought needs to be given to understanding the variation in compliance observed across the different areas of Pemba. There is a need of intensifying sensitization meetings in the communities with focus of discussion on potential side effects which may develop. Engage religious and other influential leaders during sensitization meetings.

Chapter 6: Since the intensive enhanced MDA for schistosomiasis was aimed at evaluating the possibility of elimination of transmission, the role in transmission of the preschool (>3<5yr) children, who were not previously included in PC in Pemba, was assessed. A substantial proportion of these children was infected (7.04%), the prevalence steadily increasing with age. By questionnaire to mothers/care-givers, contact with stream/pond water through washing and visiting with the children were significant risk factors for pre-school infection. ***Recommendations***: In view of the pre-school prevalence demonstrated, the community MDA was extended to <3yr olds. The government should be ready to register and order paediatric formulation of PZQ once it becomes available. Diagnostic capability of the respective health facilities has to be strengthened in terms of training of laboratory personnel and purchase of essential laboratory equipment.

Chapter 7: Implementation of snail control in Pemba prompted study of transmission of schistosomiasis by the snail intermediate host, *Bulinus* spp**.** Only ~1% of field-collected snails shed cercariae but 56% of tested snails were found infected by PCR. DNA sequencing confirmed the presence of *B. nasutus* and *B. globosus*, both of which transmitted schistosomes as judged by cercarial shedding and molecular techniques. ***Recommendations***: Further studies are needed to confirm the potential role of the *B. nasutus* in the transmission of urogenital schistosomiasis in Pemba. For monitoring of schistosomiasis transmission, it would be essential to assess the presence of infection in snail host using molecular techniques especially when elimination is achieved or targeted.

Chapter 8: Increasing intensity of PZQ administration raises concerns about possible selection of drug resistance in *S. haematobium* and prompted studies on the efficacy of PZQ and its effects on parasite genetic diversity in Pemba. It was found that PZQ efficacy was comparable to previous trials. Miracidial samples collected from Std-1 children in 2011 showed diverse, and some novel, haplotypes of the Cox-1 gene of *S. haematobium*. Clustering of the different haplotypes from different areas indicated the role of internal/external migration in the spread of infection. ***Recommendations:*** Praziquantel remains effective and should still be used for the treatment of schistosomiasis at the individual, community or school level in Pemba. More studies are needed to assess susceptibility to PZQ of different *S. haematobium* haplotypes and of the groups (G1 and G2) with which they associate.

Chapter 9: General Discussion: Overall it was concluded that *S. haematobium* and STH infections remain a public health problem in Pemba and that, although PC using SBT helps control parasite burdens, integrated control measures were more effective and would be required to reduce transmission to approach elimination. Valuable experience in implementation and monitoring of such measures (MDA±snail control/behavioural modification) and application of modern genetic analysis tools was gained during the work and further studies on the snail hosts and population genetics of *S. haematobium* proposed.

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Special thanks go to my family for the tolerance shown during the difficult times they faced especially when I was away from home.

Attribution

The study design and planning of the ZHCP and SCORE programmes through which the data herein were derived was undertaken by a group of which I was a member and central advisor. Other members of team were: Dr David Rollinson from the Natural History Museum, London; Dr. Daniel Colley and Dr Carl Campbell from the SCORE program, USA, Dr. Steffi Knopp from University of Basel, Switzerland and Dr Khalfan Abdallah from ZNCP, Zanzibar.

The parasitology field work was carried out with the help of a small group of technicians from the PHL. I was solely responsible for the planning, organisation, day to day supervision and Quality control of this process and was an active member of this parasitology team. Additionally, I was responsible for the planning, organisation and execution of the field work in relation to snail control and for collection of snail samples.

Abbreviations

AEs	Adverse Events
AIDS	Acquired Immunodeficiency Syndrome
ALB	Albendazole
BM	Behavioural Modification
bp	Base Pair
BZ	Benzimidazole
CDD	Community Drug Distributor
CI	Confidence Interval
CR	Cure Rate
CWT	Community Wide Treatment
DALYs	Disability-Adjusted Life Years
df	Degrees of Freedom
DNA	Deoxyribonuccleic Acid
epg	Eggs Per Gram
ERR	Egg Reduction Rate
GPELF	Global Programme on Elimination of Lymphatic Filariasis
Hb	Haemoglobin
HIV	Human Immunodeficiency Virus
IFN -γ	Interferon Gamma
Ig	Immunoglobulin
IL	Interleukin
IVM	Ivermectin
LF	Lymphatic Filariasis
MBZ	Mebendazole
MDA	Mass Drug Administration
NHM	Natural History Museum
NJ	Neighbourhood Joining
NPV	Negative Predictive Value
NTDs	Neglected Tropical Diseases
OR	Odds Ratio
PC	Preventive Chemotherapy
PCR	Polymerase Chain Reaction
PHL-IdC	Public Health Laboratory-Ivo de Carneri
PPV	Positive Predictive Value
PZQ	Praziquantel
SB	School Based
SBT	School Based Treatment
SCI	Schistosomiasis Control Initiative
SCORE	Schistosomiasis Consortium for Operational Research and Evaluation
SD	Standard Deviation
STH	Soil Transmitted Helminths

TE buffer	Tris-Ethylene diamine tetra-acetic acid buffer
Th	T-helper
UNICEF	United Nation's International Children's Emergency Fund
WHA	World Health Assembly
WHO	World Health Organization
ZEST	Zanzibar Elimination of Schistosomiasis Transmission
ZHCP	Zanzibar Helminth Control Programme
ZNCP	Zanzibar National Control Programme

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Chapter 1: Introduction

1.1 General introduction

Infections with schistosomes (the main three human species being *Schistosoma mansoni, S. haematobium* and *S. japonicum*) and Soil Transmitted Helminths (STH), *Ascaris lumbricoides, Trichuris trichiura* and hookworms (*Ancylostoma duodenale* and *Necator americanus*) pose a major public health problem in tropical and subtropical countries (de Silva et al., 2003). These infections are estimated to afflict 2 billion people (Hotez et al., 2003) resulting in disability-adjusted life-years (DALYs) of 5.18 million for STH (Pullan et al., 2014)) and 70 million for schistosomiasis (Hotez and Fenwick, 2009). In endemic areas, infection with the STHs, *A. lumbricoides* and *T. trichiura*, and with schistosomiasis can be acquired during infancy (Goodman et al., 2007).

With STHs, prevalence tends to increase with age to adulthood whilst schistosome prevalence often peaks in the mid teenage years (Figure 1.). However, intensity of both STHs and schistosomes is high in school-aged children between 5-15yrs (Figure 1), who are often the most infected population and harbour a high number of parasites (Hotez et al., 2006b). Albeit, the egg pattern in stool samples is influenced by the diagnostic technique to be used and sometimes multiple samples would be needed to improve the diagnosis (Knopp et al., 2008). However, hookworm infection exhibits a different pattern by often being more common in older people (Hotez et al., 2003). Both males and females are infected although in most studies males are mostly infected particularly in the case of schistosomiasis (Guerra-Silveira and Abad-Franch, 2013). For example, in Egypt, it has been reported that males are five times more commonly infected with *S. haematobium* than females (Hicks, 1983). A number of factors are implicated in the acquisition and transmission of schistosomiasis and STH infections such as: 1) environment 2) socio-economic status 3) access to safe water supply and 4) sanitation. It has been shown that increasing frequency of water contact, in particular, increases the risk of acquisition of schistosomiasis (Sow et al., 2011).

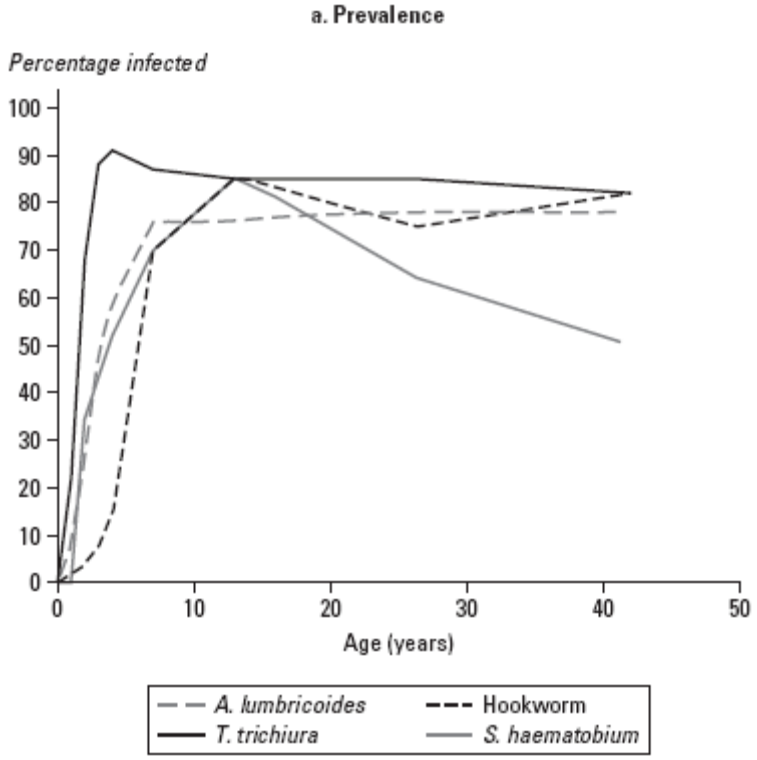


Figure 1: Distribution of prevalence *A. lumbricoides, T. trichiura*, hookworms and *S. haematobium* in different age groups in the population.

Source: (Hotez et al., 2006b)

1.2 Life cycle for common STHs

Following ingestion of infective eggs of *A. lumbricoides* or *T. trichiura,* these hatch to liberate larvae which find their preferential sites. The larvae of *A. lumbricoides* penetrate the blood stream and move through a heart- lung migration, breaking out of the lung capillaries into the alveoli. Upon reaching to the upper respiratory tract, the larvae can be swallowed back to the small intestine where adult parasite stages reside. The larvae of *T. trichiura* move directly to the large intestine. For hookworms, soon after penetrating the human skin, the larvae follow a similar route to that of *Ascaris* and eventually live in the small intestine. The adult worms reproduce sexually and produce eggs, which are passed in human faeces and deposited in the external environment, for example soil. Adult worms survive for several years and produce large numbers of eggs or larvae after 4–6 weeks. Geohelminth eggs or larvae need a period of maturation in

the soil to become infective. The eggs can remain viable in the soil for several months or years (*T. trichiura* and *A. lumbricoides*) and larvae a few weeks (hookworms), depending on existing environmental conditions such as warmth and moisture (Brooker et al., 2006b).

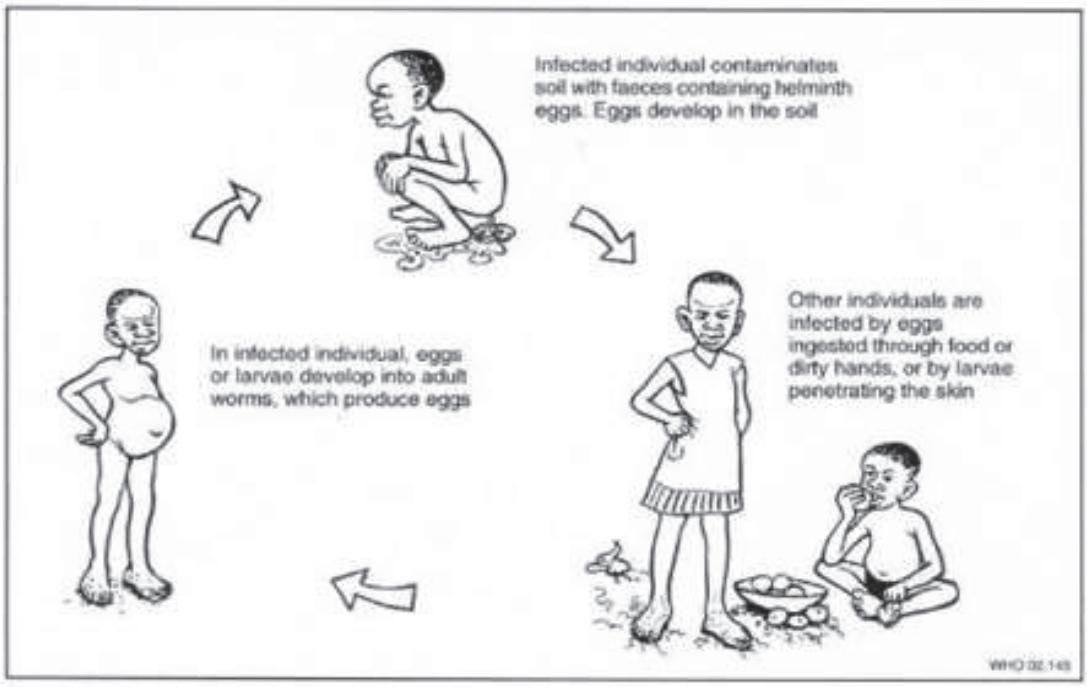


Figure 2: Life cycle of the common soil transmitted Helminth, *Ascaris*, *Trichuris* and hookworms

(Source: http://www.who.int/wormcontrol/statistics/en/cycle.jpg)

1.3 Life cycle of *S. haematobium*

S. haematobium is the only schistosome species transmitted in Pemba and so description is focused on this species. Acquisition of urogenital schistosomiasis, caused by *S. haematobium*, requires contact with freshwater infested with aquatic snail hosts of the genus *Bulinus*. Infected individuals contaminate (during micturition) freshwater with infective eggs which immediately hatch to produce ciliated miracidia. These free-swimming larvae cannot survive longer than a few hours unless they find their suitable intermediate host, a *Bulinus* snail. Within the snail tissue, a miracidium undergoes several developmental stages and eventually produce cercariae, the human-infective stage. Infected snails release cercariae which penetrate human host skin, transform to

schistosomula and enter the blood stream. After an intravascular lung migration a proportion reaches the liver where they mature in a few weeks and pair up. Males and females (mature schistosomes) remain paired and are estimated to live between 5-20 years. The pairs migrate against the portal blood flow to reach the venous plexus of the urinary bladder. The gravid female moves to venules in the wall of the urinary bladder for deposition of eggs which pass through the bladder wall and are passed in the urine (Shebel et al., 2012).

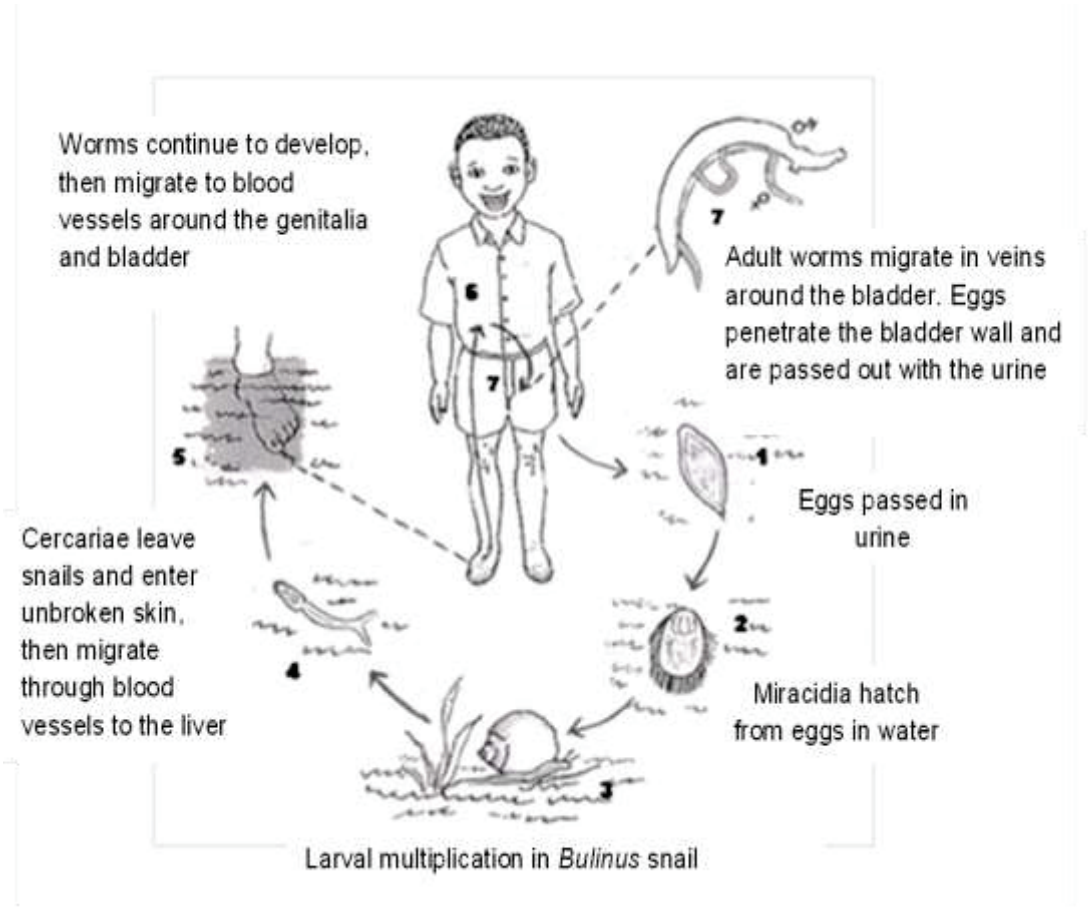


Figure 3: Diagrammatic representation of the life cycle of *S. haematobium*.

Source: http://www.who.int/wormcontrol/statistics/en/cycle.jpg

1.4 Pathogenesis of schistosomiasis

The consequences of *Schistosoma* infections are dependent on the stages of maturation and on the migration of the parasite in human tissues and organs. Following penetration of schistosome cercariae into human skin, micro-papular dermatitis, sometimes referred as swimmer's itch, can be noticed but the symptoms are transient and self-healing

(Corachan, 2002). Acute schistosomiasis, or Katayama fever, is most commonly seen following initial infection in travellers and is believed to be caused by serum sickness associated with antibody complexes with antigen derived from the developing worms or eggs. This condition may require medical attention and treatment. Generally, infection with these parasites tends to be chronic, building up over a period of years and to have detrimental effects to human host depending largely on their intensity (Hicks, 1983).

Schistosomes like most helminths show an over-dispersed distribution i.e. many people are infected by light infections but some people get heavy infection and more severe disease. In Africa, each year, 300,000 people are estimated to lose their lives as a result of schistosomiasis (Hotez and Fenwick, 2009). There are two clinical types of schistosomiasis (i) intestinal/hepatosplenic caused principally by *S. mansoni* and (ii) urogenital schistosomiasis caused solely by *S. haematobium.* The chronic stage of urogenital schistosomiasis, caused by *S. haematobium*, is associated with obstructive uropathy, hydronephrosis, calcified fibrotic bladder and/or ureter, carcinoma of the urinary bladder (Hicks, 1983) and even infertility (Kjetland et al., 1996, Balasch et al., 1995, Swai et al., 2006). In schistosomiasis endemic areas, the incidence of bladder carcinoma occurs at a younger age (~ 46yrs) compared to non-endemic areas (60yrs) (Hicks, 1983).

The pathogenesis of schistosomiasis is caused by the eggs trapped in the tissues which become surrounded by a host granulomatous response (Pearce and MacDonald, 2002) and there is a clear association of the presence of infection and the development of pathologies (Forsyth and Macdonald, 1965). In *S.mansoni* and *S. japonicum* infections it is the eggs carried to the liver in the portal blood which most commonly give rise to serious hepatosplenic disease. The eggs become lodged in the liver sinusoids and this begins to block the portal blood flow. Potent antigens (soluble egg antigen, SEA) leak out from the living miracidium larva through micropores in the egg shell and stimulate formation of the granuloma. The schistosome egg granuloma is made up of lymphocytes, macrophages, epithelioid cells, giant cells, eosinophils and fibroblasts although the relative proportions of these cell types changes as the granuloma ages. The miracidium eventually dies (or is killed by the immune cells) and the old granuloma consists of the empty egg shell surrounded by a small fibrotic reaction. In longstanding infections with constant egg deposition extensive fibrosis occurs and may give rise to

irreversible calcification of the portal vessels. In *S. haematobium* infections the granulomatous response results in fibrosis and later to calcification of the bladder and ureters. Studies in mice have shown the granuloma to be a T helper-cell dependent immune response caused by the eggs inducing strong Th2 responses. Of particular importance for the pathogenesis is IL-13 which is responsible for the fibrosis (Chiaramonte et al., 1999).

1.5 Pathogenesis of STHs

During the larval stage of *A. lumbricoides* and hookworms, when the parasites enter the blood circulation and migrate through heart, lung, upper respiratory tract and eventual return to the small intestine, they cause symptoms which can resemble asthma, cough, eosinophilic pneumonitis, nausea and sometimes vomiting. The different STHs when developed into adult worms cause different pathologies. Hookworms are the most important in terms of public health since they cause blood loss by biting onto the mucosa of the small intestine pumping blood through their intestines and also leaving bleeding haemorrhages. Where iron intake is low this causes hypochromic microcytic iron-deficiency anaemia (Pearson et al., 2012). *A. lumbricoides* which lives in the lumen of the small intestine, often cause malabsorption and abdominal discomfort but in heavy infections can cause life threatening intestinal obstruction in infants and ectopic infections in various locations. *T. trichiura* living embedded in the mucosa of the large intestine causes colitis and in heavy infection, dysentery and/or rectal prolapse (Cooper et al., 1992, Genta, 1993). Overall, chronic infections with STHs are associated with malnutrition, iron deficiency anaemia, growth stunting during childhood, impaired physical and cognitive capacity, and intestinal obstruction (Musgrove and Hotez, 2009) impeding working capacity resulting to economic loss (Bethony et al., 2006, Hotez et al., 2009) and sometimes, but uncommonly, causing death (van Riet et al., 2007).

1.6 Immunity and immune evasion in schistosomiasis

As also reviewed by (Pearce and MacDonald, 2002), adults in endemic areas show lower levels of re-infection following treatment, and this is not entirely explained by lower water contact in adults than in children as it is also seen in fishing communities with high adult water contact (Kabatereine et al., 1999). This resistance to reinfection of humans post puberty correlates with elevated schistosome specific IgE and with

eosinophilia and it is suggested that these immune components may kill skin stage schistosomula in resistant humans as they do *in vitro*. Irrespective of the mechanism this strong age-dependent resistance has a significant limiting effect on the disease. There is also some evidence of Th2-mediated immunity to STH (Jackson et al., 2004) but there is little evidence of immunity developing to hookworm infections which continue to build up in adulthood.

Schistosomes and STHs have mechanisms to help evade the human host immune system allowing them to initiate chronic infection and persist for many years, up to 30 years in the case of schistosome and 10 years for hookworms (Kamal and El Sayed Khalifa, 2006) although they use different strategies. Schistosomes absorb alpha (α) -2 macroglobulin, red blood cell and blood group determinants of the human host (van Riet et al., 2007) and thus may disguise their recognition by host's immune response. There is also constant membrane repair and turnover. A tough, weakly antigenic outer cuticle together with molecular mimicry are mechanisms used by STHs to establish persistent human infections (van Riet et al., 2007). During the acute phases of schistosomiasis and STH infection the host immune response is usually characterized by a T helper (h) 1 response (Maizels and Yazdanbakhsh, 2003) with production of interferon gamma (IFN γ) and Interleukin (IL)- 2. But as the diseases progress toward chronic stages, there is a switch to a induction of potent Th2 responses with abundant production of IL-4, IL-5, IL-10 and IL-13 which in turns activates B-lymphocytes to produce immunoglobulin (IgE), development of eosinophilia and mast cell responses (van Riet et al., 2007, Kamal and El Sayed Khalifa, 2006).

1.7 Effects of worm infections on concomitant immune responses

The skewing of Th1 to Th2 responses observed during the course of schistosomiasis and STH infection can influence ongoing immune responses in the host and so modify clinical presentation of concomitant diseases (Harris et al., 2009) or hamper efficacy of vaccination targeted against bacterial infections and viral diseases (van Riet et al., 2007). This Th2 response is also associated with induction of immunomodulatory and immunoregulatory mechanisms which can lead to hyporesponsiveness and anergy (Kamal and El Sayed Khalifa, 2006). As a consequence, it has been postulated that helminth infections could interfere with efficacy of vaccines due to switching of Th1 to

Th2 responses and that this could be reversed by anthelminthic treatment (van Riet et al., 2007, Cooper et al., 2000). Thus, Cooper et al., (2000) have demonstrated remarkably increased levels of vibriocidal antibodies and sero-conversion post vaccination with the oral, live attenuated cholera vaccine CVD 103-HgR in albendazole-treated individuals bearing STH than in the untreated control group. Similarly individuals co-infected with cholera and STH infection mounted poor mucosal immune responses to the sub-unit B cholera toxin (CTB) (Harris et al., 2009). The impairment of vaccine efficacy observed in STH or schistosomiasis infected individuals is not limited to oral vaccines. Studies show individuals infected with schistosomiasis had reduced IFN-γ responses to tetanus toxoid (TT) as compared to controls following tetanus vaccination (van Riet et al., 2007). In another study, it has been shown that individuals with helminth infection failed to elicit immune response to tuberculin purified derivative (PPD) when subjected to *Mycobacterium tuberculosis* or vaccinated with Bacille Calmette-Guĕrin (BCG) (Borkow and Bentwich, 2004).

It has been suggested that schistosomiasis and STH infection influence susceptibility to other microorganisms such as malaria, tuberculosis (TB) and HIV/AIDS (van Riet et al., 2007). However, the published data is inconsistent. For example in relation to malaria (Nacher et al., 2000) reported that people infected with *A. lumbricoides* were protected against cerebral malaria but other workers indicated that *A. lumbricoides* infection was associated with development of severe malaria (Le Hesran et al., 2004). Moreover, (Shapiro et al., 2005) found no association of helminth infection and development of malaria. Likewise, some studies have reported negative interactions between schistosomiasis and control of HIV-1 (Secor, 2006). Helminths are also able to affect responses in autoimmune diseases. *T. trichiura*, in particular, has been reported to be beneficial in modulating inflammatory bowel disease (Broadhurst et al., 2012, Broadhurst et al., 2010)

1.8 Control of STH and schistosomiasis

Like the other parasitic diseases of humans, there is, as yet, no effective vaccine against STH infection and schistosomiasis (Liu and Weller, 1996, Fenwick et al., 2006b) despite intensive effort in developing those vaccines in experimental animals. The hookworm vaccine candidate recombinant secreted protein 2 (Na-ASP-2) has produced

partial protection in experimental animals (Xiao et al., 2008, Bethony et al., 2008) but phase 1 human trials using recombinant *Na* ASP-2 in alum were terminated because of allergic reactions in sensitized people. A number of vaccine candidates for schistosomiasis have also shown promise in experimental animals but no vaccines are available yet. Thus effective control measures for all of these parasites require integration of various approaches i.e. improved sanitation, personal hygiene, accessibility to safe water supplies and, currently most importantly, chemotherapy with PZQ for schistosomiasis and Benzimidazoles (BZ) for STHs (Gabrielli et al., 2011, WHO, 2006, Utzinger et al., 2003), behavioural changes (Fenwick et al., 2006b, Sturrock, 2001) and health education. Combination of safe water supply, improved sanitation and chemotherapy can reduce *A. lumbricoides* infection markedly more effectively than water supply and sanitation alone (Esrey et al., 1991). Similarly chemotherapy in conjunction with provision of safe water had a higher impact on reduction of prevalence of schistosomiasis than safe water provision alone (Esrey et al., 1991). In reality, for many reasons, a number of these approaches are difficult to achieve in many developing countries (Sturrock, 2001) where about 90% of schistosomiasis exists. It has been noted that sanitation alone is a slow method which requires a wide coverage (Warren, 1981). Furthermore, health education has a minimal benefit in preventing contamination or exposure if provision of safe water supply and proper ways of disposing excreta are not available (Sturrock, 2001).

Controlling snails through environmental modification and application of molluscicide, notably niclosamide, has proved very effective in some areas but the use of niclosamide has raised concerns over the environmental effect (Smits, 2009). Therefore chemotherapy has become the mainstay of controlling STH infection and schistosomiasis because of 1) low cost/donation of the drugs and safety records and 2) the rapid results which can be obtained (Fenwick and Webster, 2006). It has been shown that the cost of treating a single school-child with albendazole ranges from US$ 0.063-0.105 and the distribution costs were between US$ 0.04- 0.08 in different districts in Uganda (Kabatereine et al., 2005). Similar costs for drug distribution were also reported in Ghana, Tanzania (Kabatereine et al., 2005) and Myanmar (Montresor et al., 2004). Mebendazole and albendazole belonging to the benzimidazole class are cheap, safe and well tolerated drugs with broad-spectrum activity, that the World Health Organisation (WHO) has advocated for use in the control of morbidity of STH infection

among the high risk population where the problem is endemic (WHO, 2006). The drugs are frequently targeted to children in schools (school-based treatment [SBT]) and to women and preschool children attending health clinics. However, the strategies for drug provision vary with prevalence (WHO, 2006). The drugs can also be distributed by community mass drug administration[1] (MDA) at a single dose of 400 mg or 500 mg for albendazole and mebendazole respectively (Geary et al., 2009, WHO, 2006) . Similarly, since its discovery in 1970s, Praziquantel (PZQ) has become the mainstay for the treatment as well as control of morbidity due to schistosomiasis and therefore WHO and other United Nation agencies have suggested it for that purpose (WHO, 2006). The drug is given as a single dose of 40 mg/kg body weight (WHO, 2006). There is now overwhelming evidence on the safety of PZQ in pregnancy due to the fact that many pregnant and lactating women have been treated with PZQ in mass campaigns without developing adverse events (Fenwick and Webster, 2006). In addition, in a recent study conducted in Zanzibar to assess the safety of co-administration of triple therapy, albendazole, praziquantel and ivermectin in MDA, no major untoward effects were observed (Mohammed et al., 2008). Based on this report, the three drugs can be simultaneously administered in areas where lymphatic filariasis (LF), schistosomiasis and STH infection co-exist. In endemic areas with no previous treatment, the triple therapy co-administration is subject to further investigation. However, effectiveness of MDA largely depends on the coverage (Smits, 2009). In China, MDAs using PZQ treatment integrated with other control measures managed to reduce schistosomiasis infection by 52.2% over ten years (1992-2001) (Xianyi et al., 2005a). Similarly a tremendous reduction of schistosomiasis, from 6.2% to 0.3%, was demonstrated in Morocco following provision of PZQ combined with health education and snails control, for a period of 14yrs (Laamrani et al., 2000b).

1.9 Resistance to mebendazole/albendazole

BZs have been extensively used for the treatment of parasitic diseases of animals since their discovery in 1960s. Later the drugs were also proven to be highly effective against

[1] Mass drug administration (MDA) or sometimes knowns as community wide treatment (CWT) is a term used to denote distribution of drug(s) to all eligible members in a community irrespective of infection status.

a variety of human parasitic infections such as gastrointestinal nematodes, cestodes, tissue nematodes and intestinal protozoa (Morgan et al., 1993, Robinson et al., 2004, Prichard, 2007). Functionally, the drugs are claimed to inhibit polymerisation of beta (β)-tubulin, a component of cytoskeletal structure, or affect neuromuscular function of the parasites (Liu and Weller, 1996) resulting in disruption of the parasite homeostasis and eventually parasite death. Thus, parasite populations with altered β-tubulin structure are potentially resistant to BZs drugs. So far, a single nucleotide polymorphism (SNP) – phenylalanine to tyrosine or histidine substitution, at the codon position 167 or 200, of the isotype-1 β-tubulin gene of helminths has been associated with the development of resistance of nematodes of veterinary importance to BZs drugs (Schwenkenbecher et al., 2007, Robinson et al., 2004). A prolonged and frequent use of monotherapy for the treatment of infectious diseases triggers selective pressure to micro-organisms and hence leads to the development of drugs resistance. Improper use and rapid metabolism of drugs are among factors considered to associate with selection for drug resistance (Sangster and Gill, 1999). To date, BZ resistance is well documented in animal helminthiasis in many parts around the World (Sangster and Gill, 1999, Chandrawathani et al., 1999) and has raised a great concern for the economy of the countries. Although, several factors may play a role in the selection of resistance in animal helminthiasis it is generally suggested that parasites with short life cycles are prone to contract resistance earlier (Sangster and Gill, 1999). As a lesson from animal infections, the emergence of BZ resistance in human STH infections should not be a surprising occurrence and the increase in targeted and mass treatments will increase selection pressures for resistance. In Pemba, Zanzibar, targeted and mass BZ treatments for STHs have been applied over a number of years and this led to investigation of the drug sensitivity of hookworms to these drugs. It was shown that hookworms from Pemba showed somewhat lower sensitivity than a strain from Mafia island where periodic treatment was not practiced (Albonico et al., 2005) but there was no evidence of the mutations typically associated with benzimidazole selection in animal helminths (Albonico et al., 2004c, Diawara et al., 2009). However, the fact that neither albendazole nor mebendazole are completely effective in curing hookworm and *T. trichiura* infections raises the risk of selection of resistance (Geary et al., 2009, Albonico et al., 1994). These concerns highlight the importance of monitoring drug efficacy in ongoing treatment campaigns (Albonico et al., 2004a).

1.10 Resistance to Praziquantel

It is about 40yrs since the discovery of praziquantel, the drug which has potent killing action against a number of trematodes and cestodes. Since then, praziquantel has been the only drug widely used for the treatment of schistosomiasis at the individual level and also for the control of morbidity in high risk populations. Despite its widespread use, the exact killing mechanism of this drug is poorly elucidated (Kasinathan et al., 2010). Nevertheless, it is thought that the drug kills susceptible parasites by disruption of the tegument as well as imposing muscular contraction probably through interference with calcium ion (Ca^{2+}) homeostasis (Greenberg, 2005, Kasinathan et al., 2010). This mechanism has been postulated from the fact that muscular contraction and tegument disruption are not apparent in the absence of Ca^{2+} (Greenberg, 2005). It has also been proposed that changes in membrane phospholipids and membrane fluidity may play a role in PZQ action (Greenberg, 2005). Moreover, the production of anti-schistosomal antibodies elicited during the course of the infection accelerate the killing effect of praziquantel as the disrupted membrane makes schistosome antigen accessible to antibodies (Liu and Weller, 1996). The current over-reliance on a single drug poses a threat for the potential development of resistant parasites which would pose a great challenge for schistosomiasis control. Some studies that assessed the efficacy of PZQ in treatment of schistosomiasis have raised fears about the possibilities of the emergency of drug resistance among schistosome populations. For instance, in Egypt, it was demonstrated that 2.4% of the individuals infected with *S. mansoni* were not cured following administration of three doses (40 mg/kg [twice] and 60 mg/kg [once]) of PZQ (Ismail et al., 1996). Similarly, in Senegal, a remarkably low cure rate (CR) (18%) was detected following administration of 40 mg/kg of PZQ (Gryseels et al., 2001b). However, it was later concluded that the low cure rates were due to the presence of immature worms and subsequent studies, using *S. mansoni* isolates collected from Senegal, did not demonstrate the emergence of praziquantel resistance (Gryseels et al., 2001a). However, strains of schistosomes isolated from some such cases in Senegal and Egypt showed lower susceptibility to PZQ experimentally as do strains selected by sub-optimal PZQ exposure during mouse passage (Cioli et al., 2004). Nevertheless there is no convincing evidence yet of development and selection of genetically-based resistance following repeated rounds of treatment (Guidi et al., 2010, Botros et al.,

2005, Black et al., 2009). Nevertheless, the increasing number of countries applying repeated annual treatment programmes, increased frequency and coverage of treatment (e.g. the new National Control Programme in Pemba involves biannual treatments through implementation of whole community- based MDA rather than school-based programmes (Fenwick et al., 2009) and the proposal to extend treatment to younger (>2year old) children (Sousa-Figueiredo et al., 2010c) will apply selection pressure to a larger proportion of the gene pool and may facilitate emergence of resistance. Therefore, a strong monitoring system for PZQ efficacy is needed in control programmes based on preventive chemotherapy so that any signs of emergence of resistance in the schistosome population can be detected at an early stage.

1.11 Control efforts for schistosomiasis and STH in Zanzibar: historical perspectives

In 1986 the Zanzibar Schistosomiasis Control Programme was initiated and implemented periodic selective chemotherapy (i.e. treatment of individuals diagnosed as infected) to successfully control morbidity due to schistosomiasis (Savioli and Mott, 1989, Savioli et al., 1990). In the early 1990s this control initiative with PZQ was progressively integrated with other major disease control strategies including STH with BZ and lymphatic filariasis (LF) with ivermectin as part of the National Control programmes (Mohammed et al., 2006). However, due to shortage of drugs, the chemotherapy campaigns between 1999 and 2004 were less consistent. Starting in 2001 the Global Programme for Elimination of Lymphatic Filariasis (GPELF) carried out 6 rounds of MDA with provision of ivermectin and albendazole. This strategy was successfully implemented with good drug coverage (>80%) and led the prevalence of microfilariaemia dropping to <1%, a threshold for considering discontinuation of MDA treatment campaigns for LF. From 2004 to 2006 the Schistosomiasis Control Initiative (SCI) supported the MDA with PZQ on a community basis using the delivery system utilised by the GPELF, and in 2006 the strategy became SBT.

Since December 2006, when the last MDA campaigns was carried out with triple therapy regimen (Mohammed et al., 2008) no community–based treatment has been delivered (Figure 6). Since then no documented control effort has been implemented against schistosomiasis or STHs. Consequently, it was planned that from 2010 the

distribution of PZQ and ABZ would be reintroduced and again targeted to school-children.. This package was in synergy and coordination with distribution of antimalaria, bed nets, vitamin A, immunizations and other health interventions and planned to be delivered through the reinforcement of the current network of peripheral health centres in order to constitute an innovative and efficient system for delivery of preventive chemotherapy packages.

This phase of NTD control in Zanzibar was envisaged to be maintained year on year and to progress towards control of STH as a public health problem and towards elimination of schistosomiasis. As there had been only sporadic evaluation of the impact of the various previous interventions, the work proposed for this PhD programme has been partially designed to carefully evaluate the impact of control or elimination efforts in Pemba. In 2010 baseline surveys were undertaken and follow-ups carried out in 2011 after the single annual SBT with a further follow-up planned for 2012 again following the 2011 school based treatments. However, in 2010 the Ministry of Health (MoH) Zanzibar revised the strategy for control of STH and schistosomiasis for Zanzibar, recommending increasing the frequency of drug delivery (from single annual to biannual). Furthermore, in 2010, Zanzibar was selected by SCORE (Schistosomiasis Consortium for Operational Research and Evaluation) for trialling additional interventions, molluscicide and behavioural change for progressing towards elimination of schistosomiasis. An alliance was formed including various partners (e.g. WHO Headquarters, WHO AFRO, Ivo de Carneri Foundation- (IdCF), SCI, Swiss Tropical and Public Health Institute (STPHI), Natural History Museum (NHM), London, and Zanzibar Ministries) and called the Zanzibar Elimination of Schistosomiasis Transmission (ZEST) to be implemented in 2012. The aim of ZEST was to assess the impact of three parallel control intervention strategies in Pemba:- (i) The National Control Programme (NCP) alone based on PZQ treatment and health education, ii) NCP plus molluscicide and iii) NCP plus behavioural modification. Fifteen *Shehias* out of a total of 45 were randomised to each arm. The studies planned for this PhD programme were extended in light of these developments. They were designed:- to inform the establishment and conduct of ZEST; to evaluate, compare and contrast the impacts of the various different intervention strategies on prevalence and intensity of schistosomiasis and STH; to evaluate the delivery of the MDA and any changes in PZQ efficacy over the course of the intensive intervention period; to assess

the *Bulinus* snail populations and their infection status. The earlier parasitological monitoring during the SBTs of 2010-2012 would provide baseline data to compare with the impact of the more intensive control measures. It was hypothesised that combining schistosomiasis interventions (Snail control plus MDA or Behavioural changes plus MDA) would produce substantial impact in reducing transmission and consequently lead to elimination.

1.12 Aims and objectives

Aims and Objectives of the experimental chapters:

Chapter 3

Aim 1: To assess the impact of reintroduction of single annual, School-based treatment (SBT) with Praziquantel (PZQ) in Pemba for control of urogenital schistosomiasis

Objectives:

[a] To recruit a cohort of 24 schools across different areas of Pemba in order to undertake a survey of infection levels in Standard (Std)-1 and Std-3 children

[b] To determine the prevalence and intensity of *S. haematobium* infection in the Std-1 (new school entry) children by urine filtration at baseline.

[c] To determine microhaematuria using Heamastix

[d] To determine the prevalence and intensity of *S. haematobium* infection and prevalence of haematuria in Std-1 and St-3 children during 2 annual rounds of PZQ treatment

Aim 2: To assess the implementation of MDA±snail control or human behavioural modification on urogenital schistosomiasis.

Objectives:

[a] To recruit a cohort of 45 schools across the whole of Pemba in order to undertake a baseline survey of infection levels in Std -3 and Std -4 children.

[b] To randomly allocate 15 of the 45 schools to each of three different intervention arms: (MDA alone, MDA + Snail control, MDA + behavioural modification)

[c] To compare the prevalence and intensity of *S. haematobium* infection in the different intervention arms over 3years

Chapter 4

Aim1: To assess the impact of reintroduction of single annual, SBT using Albendazole (ALB) in Pemba for control of soil transmitted helminthiasis (STHs:-*Ascaris lumbricoides, Trichuris trichiura* and hookworm)

Objectives:

[a] To determine the prevalence and intensity of the STHs in Std-1 children in the cohort of 24 schools.

[b] To determine haemoglobin concentration in the Std-1 children by Hemocue

[c] To determine the prevalence and intensity of infection and haemoglobin concentration during 2 annual rounds of ALB treatment

Aim 2: To assess the impact of reintroduction of mass drug administration (MDA) with ALB in Pemba for control of STHs.

Objectives:

[a] To determine the prevalence and intensity of the common STHs in Std-1 children in the cohort of 24 schools

[b] To determine level of haemoglobin concentration in the Std-1 children by Hemocue

[c] To determine the prevalence and intensity of *S. haematobium* infection and haemoglobin concentration after implementation of two years of biannual MDA with ALB.

Chapter 5

Aim: To assess drug coverage during the MDA and factors associated with its compliance

Objectives:

[a] To assess drug (ALB and PZQ) distribution coverage among the communities

[b] To determine the level of ALB and PZQ compliance among the community members

[c] To assess knowledge and attitudes of community members in relation to compliance to ALB and PZQ treatment

Chapter 6

Aim: To investigate *S. haematobium* infection in children <6yrs

Objectives:

[a] To assess the prevalence and intensity of *S. haematobium* infection in pre-school aged children <6yrs

[b] To assess risk factors associated with transmission of *S. haematobium* infection in pre-school aged children through questionnaire to mothers/guardians

Chapter 7

Aim: To investigate transmission dynamics of *S. haematobium* in Pemba

Objectives:

[a] To evaluate the snail populations in *S. haematobium* transmission sites

[b] To assess physical and chemical characteristics of the snail habitats

[c] To assess presence of *S. haematobium* in the snails

[d] To characterize the snail intermediate hosts responsible for transmission of *S. haematobium* infection

Chapter 8

Aim: To determine efficacy and safety of praziquantel and its impact on *S. haematobium* genetic diversity

Objectives:

[a] Using the cohort of 24 schools, to collect miracidia during the surveys pre- and post- PZQ SBT and to store them in FTA™ cards for molecular analysis

[b] To assess efficacy and safety of praziquantel for the treatment of urogenital schistosomiasis in school-aged children during SBT

[c] To establish the efficacy of PZQ after the SCORE elimination programme by follow-up diagnosis at 4 weeks or 7 weeks after the 2013 treatments

[d] Using the miracidial DNA, to assess genetic diversity of *S. haematobium* pre-and post- treatment

Chapter 2 Materials and Methods

2.1 Study site and population

Pemba is the smaller of the two main islands of Zanzibar off the east cost of mainland Tanzania. The Island had an estimated population at 362,166 inhabitants with a growth rate of about 3.1% (census 2002) with 80% living in small villages and making their living from subsistence agriculture, fishing, tourism (which is becoming more important due to the fall of the clove price in the World market) and cultivation of cloves and seaweeds for export. Administratively, Pemba is divided into two regions with four districts (two districts per region). Within each district there are several *shehias*[2]. The average rainfall in Zanzibar is 1500 mm. The big rains ("Masika") occur between April and June and the small rains ("Vuli") between November and December. The hottest weather occurs from December to the end of March. During this time usually there is little rainfall and thus most ponds and streams become dry or have insufficient water for domestic (washing of utensils, clothes) and recreational (swimming, bathing) use. In Zanzibar water infrastructures are widespread even in remote areas but safe water supply is not fully available (Mshindo–personal communication).

In Pemba, STH infection and urogenital schistosomiasis are currently among the major public health problems. *Bulinus globosus* snails are intermediate hosts for *S. haematobium* in this area (Stothard et al., 2002). In Zanzibar there has been intensive control of malaria using combination of interventions (Indoor Residual Spray [IRS], Long-Lasting Insecticide Nets [LLIN] and Artesunate–Amodiaquine) which resulted to a remarkable decrease in malaria cases such that it is not now considered a public health problem.

2.2 Collection and examination of urine samples

Urine samples were collected in mid-morning from children. Each child was given a plastic container and asked to provide about 20ml of urine. The samples were

[2] shehia is the lowest level of local administration which is led by a *Sheha* -who is appointed by the District commissioner.

transported immediately to the Public Health Laboratory-Ivo de Carneri (PHL-IdC) for processing and examination. Each sample was thoroughly mixed and 10ml was transferred in a 10ml syringe. The sample was passed through a filter holder housing a polycarbonate filter membrane (Vesteraard Frandsen, Denmark). The filter was then placed on a clean grease-free microscope slide and one drop of normal saline was added to avoid drying. The slide was examined using a compound microscope with a low power (x10) objective. The number of eggs was counted and reported per 10ml. Those samples with egg count ≤49/10ml of urine were considered as light infection and the ones with egg count ≥50/10ml of urine were considered as heavy infection (WHO, 1983, Ahmed et al., 2012).

2.3 Assessment of macro and micro-haematuria

For macro-haematuria, the appearance of each urine sample was compared to the colour chart developed by the WHO (see Appendix 2.1). The urine colour was scored as 1 (clear urine sample) through 6 (bloody urine sample) depending on the colour perceived by the observer.

Micro-haematuria was assessed by using Hemastix® (Siemens Healthcare Diagnostics Inc, USA). The procedure was carried out as indicated by the manufacturer's instruction. Briefly, the haemastix strip was immersed into the urine sample, immediately removed and compared with the colour chart indicated on the bottle within 60 seconds. In some studies the results were recorded as positive or negative (any sample recorded as a trace response through to positive +++ was considered as positive). In other studies the graded score was recorded (nil, trace, +, ++, +++)

2.4 Assessment of Praziquantel efficacy

After urine samples had been collected, each child was given praziquantel tablets (40mg/kg) regardless of his/her infection status as part of the schistosomiasis control programme campaigns using height pole or as sometimes known as dose pole). Thereafter, all children who were found positive with schistosomiasis at baseline were followed up at the 4^{th} and 7^{th} week post treatment. During each visit, all children who were still found positive with schistosomiasis were asked to provide another urine

sample which was assessed for the presence of *S. haematobium* eggs as described above. At the end of the assessment, any infected children were re-treated with praziquantel (40mg /kg).

In addition, miracidia were collected from *Schistosoma* eggs recovered before and after treatment, for DNA sequencing and PCR analysis. *S. haematobium* eggs were filtered through a pitchford™ filter, consisting of two meshes: the inner mesh of 200 µm allowed eggs to pass through but collected any larger debris and the outer mesh of 40 µm collected the eggs. The filter was then washed and left in water at room temperature in sunlight for ~4-5 hours. The presence of miracidia was assessed using a dissecting microscope (CETI, Twin Z, UK). Individual miracidia were captured with ~3µl of mineral water (drop) and transferred onto Whatman FTA® cards (Whatman™, GE Healthcare, UK). The cards were allowed to air dry and stored at room temperature until ready for use.

2.5 Collection and examination of stool samples

Each child was provided with a stool collection container (without preservative) and asked to bring a stool sample of at least two grams the next morning. The samples were collected at the same time as for urine samples. The samples were then sent to the Public Health Laboratory-Ivo de Carneri (PHL-IdC) for processing and examination. The samples were assessed for the presence of intestinal nematode eggs using the Kato-Katz method as recommended by the World Health Organization (WHO, 1994). The 41.7mg templates were used for preparation of stool smears. Slides were examined within 30 minutes of the slide preparation to avoid clearing of hookworm eggs. Intensity results were expressed as eggs per gram faeces by multiplying with a factor of 24.

2.6 Assessment of haemoglobin level

For each child, a capillary blood sample was obtained by finger prick using a standard disposable capillary lancet. The first drop of blood to appear was wiped off; the second was collected into a special microcuvette holder and analysed immediately by HemoCue (HemoCue, Denmark) in accordance with the manufacturer's instructions.

Determination of Hb level was performed within 2 to 3 minutes with the same HemoCue hemoglobinometer that was calibrated daily.

2.7 Administration of questionnaire for the infant infection rate study

The mothers of enrolled children were asked to participate in the interview to assess their knowledge, attitude and practice towards schistosomiasis. This was done using a structured questionnaire (Appendix 6.1).

2.8 Preservation of snails for molecular studies

All snails (whether shed or unshed) were placed in 20-100ml tubes containing water collected from their original sites. The container was placed at 4^{o}C for 20 minutes. After this incubation period, the snails were blotted dry and individually placed in 20ml tubes containing absolute ethanol. The tubes were placed at room temperature until ready for transport to Natural History Museum (NHM) for further analysis.

2.9 Extraction of DNA from FTA cards

An individual FTA card was placed on a clean cutting mat and the 0.2mm discs that contained the miracidia DNA were removed using a Harris-Micro-Punch (VWR, UK) and placed individually into 0.2ml Eppendorf tubes. The punch and the pad were cleaned with absolute ethanol between each sample. The individual punches were washed in 200µl of FTA purification reagent and incubated at room temperature (RT) for 5 minutes when the purification reagent was removed and the process repeated for a total of 3 times. Then 200µl of TE buffer was added to each punch and incubated for 5 minutes. After this incubation period the Tris-Ethylene Diamine Tetra Acetic acid (EDTA) (TE) buffer was removed and the TE wash repeated. The TE buffer was removed and the tube was left open and placed in a PCR machine at 56^{o}C for 1hr to completely dry the disc.

2.10 Amplification of the Cox-1 gene from miracidia for bar-coding

The amplification of the Cox-1 gene was carried out using PCR beads (IllustraTM puRETaq Ready-To-Go PCR beads (GE Healthcare, UK). The bead was directly added to the dried punch and then 1µl (10 pmol) of both the forward primer (Schisto_Cox1_5') and the reverse primer (Schisto_Cox1_3') added along with 23µl of DNAse free water. (The primer sequences used 5′-TCT TTR[3] GAT CAT AAG CG-3′for forward primer and 5′-TAA TGC ATM[4] GGA AAA AAA CA-3′ for reverse primer). For the positive control 2µl of the DNA extract (DNA extract of *S. haematobium* worms from Zanzibar from the NHM SCAN collection), 1 µl of each forward and reverse primers and 21µl of DNAse free water were added to the bead. For the negative control 2µl of DNA extract and 23 µl were added to the bead. The mixture was briefly centrifuged then loaded into a Thermal cycler (Gene Amp, PCR system 9700 Applied Biosystems, UK). The PCR reaction was carried out by initial denaturation at 94^{o}C for 5minutes - one cycle, then denatured at 95^{o}C for 15 seconds, annealed at 40^{o}C for 30 seconds and extended at 72^{o}C for 45 seconds, this cycle being repeated 40 times followed by final extension and holding at 72^{o}C for 7 minutes. 4ul of the PCR amplicons were run on 0.8% gel red agarose gels together with Hyperladder IV. The PCR amplicons were visualized using an ultra-violet (UV) transilluminator. Positive amplicons were identified and the size of the amplicon was checked (expected ~1000 base pair [bp]).

2.11 Purification of the PCR product, sequencing and analysis of Cox-1 gene from miracidia

Only amplicons from positive reactions were purified and sequenced. Efforts were made to ensure that DNA sequences were obtained from a similar number of miracidia from each child. However, for each child (n=5), a minimum of 8 miracidia were obtained. Overall, a total of 89 miracidia (44 collected pre-treatment and 45 post-treatment) from which the cox-1 gene had been amplified were included in the analysis. Four µl of each amplicon was visualised on a 0.8% gel-red agarose gel and positive PCRs products were purified using the QIAquick PCR purification Kit (Qiagen Ltd, UK) and then

[3] R represents any nucleotide (A, T, G or C)
[4] M also represents any nucleotide just like R above

sequenced on an 3730XL 96 capillary automated sequencer (Applied Biosystems, UK) in both directions using 1.6pmol dilutions of the original PCR primers and an Applied Biosystems Big Dye Kit (version 1.1).

For the purpose of construction of phylogenic tree, all sequences for the different (Cox1 and nad-1 [nicotinamide adenine dinucleotide-1]) datasets were assembled and manually edited using Sequencher v.4.6 (http://genecodes.com) to remove any ambiguities between forward and reverse strands. Data from the two mitochondrial genes (cox-1 and nad-1) was used to analyse any differences in the phylogenic signal from the two mitochondrial regions. For each sample consensus sequences were aligned in Sequencher and polymorphic positions observed between individuals were checked and confirmed by visualisation of the original sequence chromatograms. The identity (species and gene) of the sequence was also confirmed using the Basic Local Alignment Search Tool (NCBI-Blast). Data analysis was carried out as explained by (Webster et al., 2012a). Briefly the assessments of the genealogical relationship amongst different mitochondrial gene region sequences were aligned in MacClade 4.05. The spanning network was created in the program TCS as described (Templeton et al., 1992) (http://darwin.uvigo.es/software/tcs.html). The evolutionary relationships between the haplotypes were inferred using Neighbour-Joining Minimum Evolution and Maximum Parsimony method using the Kimura 2-parameter model (K2P) in Mega V5. Analyses were subjected to 1000 bootstraps to evaluate the consistency of the branches of the trees. The topologies were rooted by the closely related species *S. bovis*.

2.12 Selection and identification of transmission sites and collections of snails

Potential *S. haematobium* transmission sites were selected for the assessment of *Bulinus* populations. The selection of these sites was based on the prevalence of *S. haematobium* infection in school children in the 24 schools study during the survey in January 2010 (chapter 3; survey_1 cohort 24). Additionally, the accessibility of the site was considered. This survey of water bodies in the study area was made possible through the cooperation of local community members. Each site was visited once a month for the period of one year from October 2010 to September 2011. The nature of water body i.e. pond or stream, and type of vegetation present was also recorded. Two experienced malacology technicians helped search for snails over a distance of 15 meters from the

initial contact point, for a maximum of 15 minutes. *Bulinus* snails were collected using metal scoops (Figure 2.1) or picked up using forceps, where possible, and taken to PHL-IdC for processing and assessment of cercarial shedding as described below. In July 2011, with the assistance of, Linzy Elton, an MSc project student from the LSHTM, additional sites from 15 shehias, allocated for snail control under the SCORE programme, were visited. In those 15 shehias an intensive search of water bodies was carried out and *Bulinus* spp. snails collected as described above. Similarly, chemical characteristics of water from the sites were assessed as described below.

Figure 4: Photograph of the author searching for snails in one of the visited sites.
The arrow head indicates a dish immersed in water to allow easy washing.

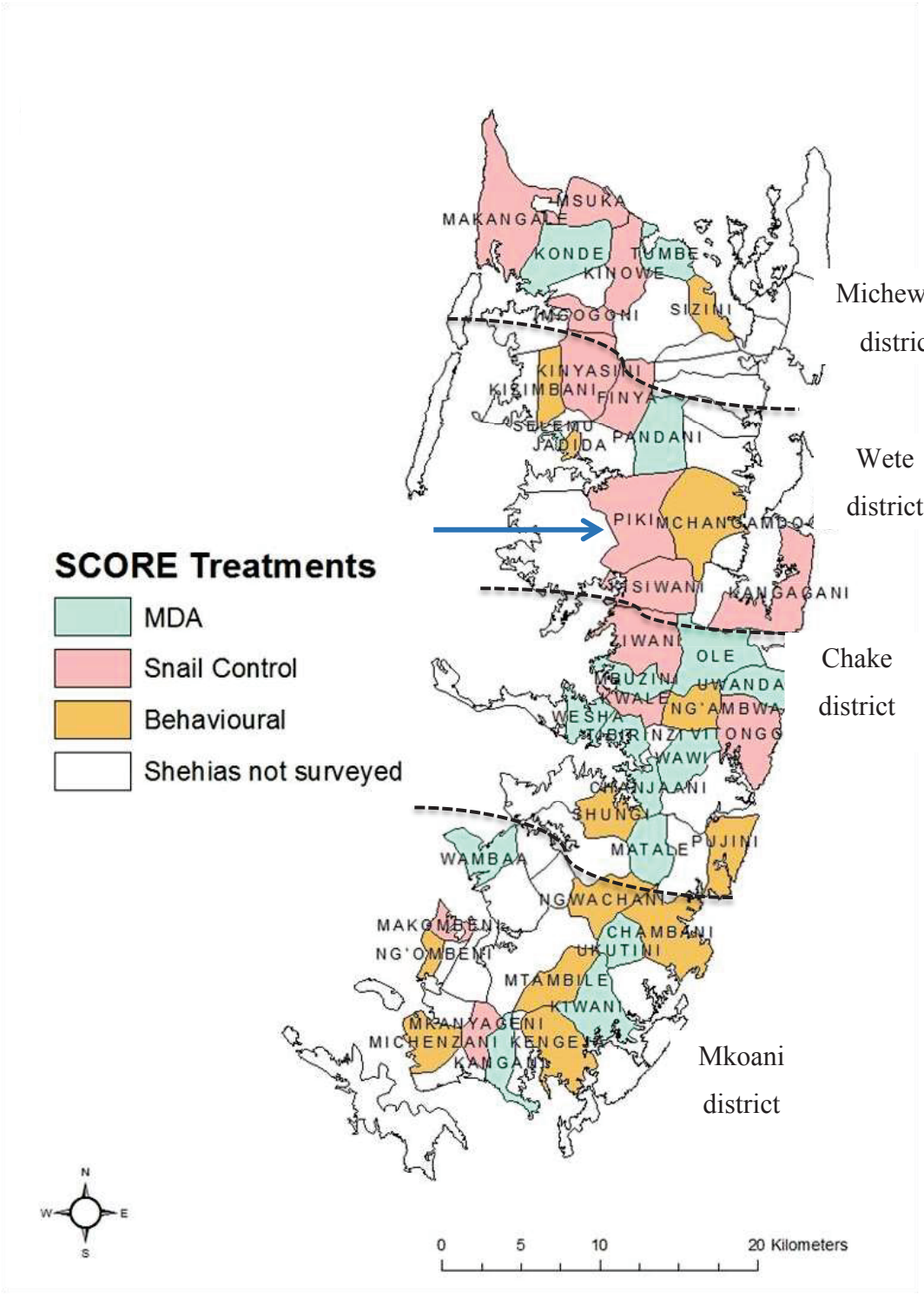


Figure 5: Map of Pemba Island showing snail sampled shehias and other interventions planned for SCORE.

2.13 Chemical characterisation of water from snail transmission sites

A small amount of water (just enough to ensure the electrode can be submerged) from the source was collected in a container and a dual water meter (HACH *sension5*, USA) electrode was immersed and the parameters of interest (salinity, total dissolved salt

(TDS), temperature and conductivity) were recorded. A separate electrode was used to measure pH.

2.14 Assessment of cercarial shedding from field collected snails

Collected snails from the field were taken to the PHL-IdC to observe cercarial shedding. Whenever possible, shedding was carried out on the same day as the snail collection. Otherwise, the snails were kept in a bowl containing water from their source with dried cabbage leaves to feed them. To induce shedding, individual snails were placed into 24-well culture plates containing 2ml of bottled drinking water (Figure 2.3). The plates were then exposed to sunlight and the cercarial shedding was assessed after every hour. This was performed by looking in each well using a dissecting microscope (SZ-ST, Olympus, Japan) and cercarial production was recorded. When snails did not shed cercariae on the first attempt they were kept in a bowl containing water from the source and the shedding process was repeated for a maximum of three consecutive days. Any snail shedding cercariae was removed and stored as described below. Also, some of the cercariae were collected and stored on FTA card (Whatman™, GE Healthcare, UK) for DNA isolation and determination of *S. haematobium* genetic variability. This was carried out at the NHM, London.

2.15 Detection of patent and prepatent *S. haematobium* in snails: dissection of snail tissue and DNA extraction

Due to limited resources available only a small proportion of the snails collected were processed for this experiment. However, all wild caught snails shedding cercariae were included.

Individual snails were dissected using a dissecting microscope (LEICA MZ6). The snail tissues were briefly re-hydrated in millique water for about 5-10 minutes and stored at -20^{o}C until ready to use.

For DNA extraction, snail tissue was chopped up using a scalpel and placed in a 1.5 ml Eppendorf tube. The scalpel and slide were wiped with absolute ethanol between each

sample. The DNA extraction was done using a DNeasy Blood and Tissues kit (Qiagen) as described in the protocol. Briefly, this was done as follows:

To each sample, 360[5] µl of ATL (tissue lysis buffer) buffer and 40 µl of proteinase K were added and the mixture was incubated at 56^{o}C overnight on a rocking platform for digestion. After this incubation period, the mixture was vortexed and 400 µl of AL (lysis buffer) buffer/ absolute ethanol was added and mixed by vortex. The tubes were centrifuged at 3000 RPM for 1 minute. For each sample, 500 µl of the lysate was removed and placed into a separate well of the 96 DNeasy plate. The plate was sealed with air pore tape and centrifuged at 6000 RPM for 10 minutes. The tape was removed and 500 µl of ethanol-AW1 buffer was added to each sample. Then the plate was sealed with air pore tape and centrifuged at 6000 RPM for 5 minutes. Thereafter tape was removed and 500 µl of ethanol-AW2 buffer was added to each sample. Then the plate was centrifuged at 6000 RPM for 15 minutes. For DNA elution, the plate was transferred into a new rack without affecting the orientation and 200 µl of Tri-Acetate EDTA (TAE [AE]) buffer was added to each sample. The plate was sealed with air pore tape and incubated at room temperature for 1 minute. After this incubation period the plate was centrifuged at 6000 RPM for 2 minutes. DNA samples were stored at -20^{o}C until when they were ready for use. The quantity of DNA extracted was measured in a *NanoDrop*™ (ND-1000) spectrophotometer at 260 nm wave length.

2. 16 Detection of patent and prepatent *S. haematobium* in snails: amplification of the Dra-1 gene from snail tissue

The genomic DNA samples were removed from the freezer and left at room temperature for a minimum of 30 minutes, for thawing. The stock solutions were diluted or suspended using sterile millique water (Fisher Scientific). The master mix solution was prepared by mixing 2.5µl of PCR buffer (containing 20mM magnesium chloride [$MgCl_2$]), 2.0 µl each for (2.5µM) forward and reverse primer, 2.0 µl (2.5µM) dNTPs, 0.25µl of DreamTaq™ Green Taq DNA polymerase (Fermentas, Life Sciences) and 15.25µl of distilled water were added. The primer sequences used were 5´GAT CTC

[5] In some cases the volumes of ATL buffer, Proteinase K, AL buffer and Ethanol were doubled due to the size of the snail tissues

ACC TAT CAG ACG AAAC3′ and 5′TCA CAA CGA TAC GAC CAAC3′ (Hamburger et al., 2004) for forward and reverse primer, respectively (Sigma Aldrich). The PCR reaction was set by mixing 24µl of master mix solution and 1µl of the genomic DNA sample. The reaction was carried out on a Thermal cycler (Gene Amp, PCR system 9700 Applied Biosystems, UK). The PCR reaction was carried out by initial denaturation at 95^{o}C for 5 minutes, then denatured at 95^{o}C for 1 minute, annealed at 60^{o}C for 1 minute and extended at 72^{o}C for 30 seconds, the cycles being repeated for 35 times with final extension and holding at 72^{o}C for 10 minutes. The PCR product was loaded into a 1.5% agarose gel in 1X TAE buffer and run at 100V for 20-25 minutes together with ladder – HyperLadder IV (Bioline). The gel was stained with gel-red dye. The DNA bands were visualized using an ultra violet (UV) trans-illuminator. The size of the DNA expected was 121bp.

2.17 Amplification of the cox-1 (Asmit-1 gene): detection of *Bulinus* species

The total genomic DNA samples extracted previously were used for amplification of the cox-1 gene. The samples were removed from the freezer and left at room temperature for a minimum of 30 minutes, for thawing. The master mix was made based on Illustra™ pureTaq Ready-To-Go PCR Beads (GE Healthcare, UK) procedure. Briefly for each reaction, 1µl (10pmol) each of forward and reverse primers, 22µl of DNase free water, 1µl DNA sample (pre-diluted 1:10) and 1 PCR bead were added. At the volume of 25µl, the bead yielded a final concentration of 200 µM of each dNTP, 10 mM Tris-HCL, 50 mM KCl and 1.5 mM of $MgCl_2$. The primer sequences used 5′TTT TTT GGG CAT CCT GAG GTT TAT3′for forward primer (Asmit1) and 5′TAA AGA AAG AAC ATA ATG AAA ATG3′ for reverse primer (Asmit2) (Kane et al., 2013). The reaction was carried out on a Thermal cycler (Gene Amp, PCR system 9700 Applied Biosystems, UK). The PCR reaction was carried out by initial denaturation at 94^{o}C for 5 minutes -one cycle, then denatured at 94^{o}C for 15 seconds, annealed at 40^{o}C for 30 seconds and extended at 72^{o}C for 45 seconds, the cycles being repeated for 45 times with final extension and holding at 72^{o}C for 7 minutes. The PCR product was loaded into a 1.5% agarose gel in 1X TAE buffer and run at 100V for 20-25 minutes together with positive control and DNA markers. The gel was stained with gel-red dye.

The DNA bands were visualized using ultra violet (UV) transilluminator. The size of the DNA expected was 300 bp.

2.18 Purification of the PCR products: Qiagen PCR Purification (single columns)

Since the PCR product that has been obtained following amplification of genomic DNA may contain other products it was necessary to purify the product before sequencing as those products might interfere with sequencing. The QIAquick purification (Qiagen, UK) method was used and carried out as follows:

One hundred and five microlitre (105µl) of PBI buffer was added to each of the PCR products. The mixture was transferred into a QIAquick spin column, placed in a 2ml collection tube and centrifuged for 1min at 13000rpm. Following this brief centrifugation, the flow through was discarded and the column returned into the same tube. The tube was washed and 750µl of PE Buffer was added and centrifuged for 1 min at 13000rpm. The flow through was discarded. The column was returned to the collection tube and centrifuged for 1 min at 13000 rpm (to remove any residual ethanol). The column was placed in 1.5m Eppendorf tube for DNA elution. 30µl of elution (EB) buffer was added and left to incubate for one minute at room temperature. The column was centrifuged for 1 min at 13000rpm. The purified DNA product was transferred into the new tube and stored at -20^{o}C until used for sequencing.

2.19 Sequencing of Cox-1 gene from snails

Due to limited resources available it was not possible to process all the samples but effort was made to ensure a sub-sample from each selected site was sequenced. This allowed assessing distribution of snails from various sites and their potential role in transmitting schistosomiasis. Thirteen samples were randomly selected from the pool of 74 snails in which the cox-1 gene had been demonstrated but which had not produced cercariae in the field. In addition almost all samples that had shed cercariae were included in the analysis. For each reaction mixture, 3µl of buffer, 3µl of sterile distilled water, 1µl of *Big* dye, 1µl of each forward and reverse primer (1.6pmol) and 2µl purified PCR product were added. This was carried out using fluorescent dye terminator (Applied Biosystems, UK) as described previously by other workers (Stothard and

Rollinson, 1997b, Kane et al., 2008). The chromatograms produced were manually edited and used to perform BLAST search using National Centre for Biotechnology Information against GenBank.

2.20 Organisation of the MDA and Assessment of drug coverage

2.20.1 Selection of shehia

Administratively Zanzibar is divided into regions, districts and shehias. A shehia, being the lowest administrative unit, consists of one or several villages depending on some special situations. In Pemba there are 2 regions, 4 districts and 122 shehias -which are not equally distributed among the districts. For the purpose of drug distribution, *special units* (prisons, military camps) are regarded as one shehia -which is included in this number. So for the drug coverage studies, all shehias were included and the drugs were administered as described below.

2.20.2 Selection and recruitment of community drug distributors (CDDs)

During the implementation of GPELF, a combination of CDDs[6], health workers and school teachers, across Pemba Island were involved in the distribution of drugs in the communities. This composition of the CDDs was intended to be maintained in the subsequent MDA. However, with the passage of time and due to limited financial resources, the number of school teachers involved in later MDAs declined and was replaced by secondary school leavers unemployed in formal sectors. In each shehia, the Sheha (local leader in a shehia) was asked to provide the names of secondary school leavers who could be involved in the drug distribution. So in the later MDAs the CDDs were mostly health workers and secondary school leavers.

2.20.3 Drug delivery

Before implementation of each MDA, CDDs were trained by health professionals in drug delivery and record keeping. Prior to drug delivery the CDDs made a visit to each

[6] Community drug distributors is a general term used to indicate diverse group of individuals (health professionals and non-professionals) who are responsible for drugs distribution in their respective communities.

household within their communities to determine the number of household members. Drugs were delivered house-to-house to all eligible people mentioned above and were administered using the modified dose pole (Sousa-Figueiredo et al., 2010a) in the case of PZQ. In addition one tablet of ALB (400 mg) was administered to each eligible individual. The CDDs recorded the numbers of individual treated, the amount of drugs given and the reasons for not taking the drugs. Each CDD was assigned ~50 households. Ideally CDDs were required to observe treatment but in some occasions this was impossible.

2.20.4 Assessment of compliance and of side effect

Selection of shehias for participation in the assessment of compliance has been described above. However, due to difficulties in accessing the so called *special units* they were excluded in the randomisation process for participation in the compliance assessment. The remaining shehias were randomly selected to get a total of 15 shehias to participate. For the purpose of this exercise only subjects aged ≥10yrs were selected to participate in the study as they were perceived to be able to answer interview questions and also recall the amount of drugs given. One week post MDA administration a structured questionnaire (annex 5.1) was completed with each eligible participant. Only individuals who were given the drugs were allowed to participate in the survey. The participants were assessed on their knowledge about schistosomiasis, and the number of tablets given and taken was recorded. In addition the participants were asked if they experienced any side effect following consumption of the drugs. For the compliance assessment, any participant who did not take the drugs at all or took less than the actual number provided was considered as non-compliant.

2.21 Data management and analysis

All data were entered in EpiInfo version 3.4.3 and later exported to Microsoft Excel for cleaning. Cleaned data were then transferred in STATA 10.0 (College Station, Texas, USA) for analysis. Arithmetic means were used to categorize the infection intensity for STH infections, *A. lumbricoides*, hookworms and *T. trichiura* according to the thresholds set by the WHO (Montresor et al., 1998) and revised by (Albonico et al.,

2003). *S. haematobium* ova counts were quantified and classified as light infection (<50 ova/10 ml) or heavy infection (≥50 ova/10 ml) according to WHO standards (Ahmed et al., 2012, WHO, 1983). The Chi Square test (χ^2) was used to assess association of categorical data and the probability of ≤0.05 was considered as significant. McNamara χ^2 was used to determine the association of paired data. For comparison between data from multiple schools e.g. prevalence between one year and another or between different classes in the school, multivariate analysis was applied using the Mantel-Haenszel test. The Mantel-Haenszel test was also used to assess the trend of infection over the years. The proportion test (z-test) was used to compare the differences between two proportions in two time points (Kirkwood and Sterne, 2003). Haemoglobin level (anaemia) was classified according WHO (WHO, 2015, WHO, 2001) haemoglobin concentration of ≥11.5 g/dl was considered as normal (i.e. non-anaemic), 7.1-10.4 g/dl was regarded as moderate anaemia and ≤7.0 g/dl was considered as severe anaemia. Efficacy of PZQ was judged by assessing cure rate (CR) and egg reduction rate. The latter was calculated as follows:

Egg reduction rate was calculated using the formula:

$$ERR = \frac{arithmetic\ mean\ egg\ count\ at\ baseline - arithmetic\ mean\ egg\ count\ at\ followup}{arithmetic\ mean\ egg\ count\ at\ baseline} x\ 100$$

The general estimation of the prevalence of (STH) any worm was calculated using the formula:

$$P_{ath} = \frac{(a + t + h) - (a\ x\ t + a\ x\ h + t\ x\ h) + (a\ x\ t\ x\ h)}{1.06}$$

Where:

a = proportion of *Ascaris* infection; t = proportion of *Trichuris* infection and h= proportion of hookworm infection (WHO, 2011b).

Chapter 3 Impact of control interventions on *S. haematobium* infections in Pemba

3.1 Introduction

Attempts to control schistosomiasis in Pemba started in 1986 (Savioli and Mott, 1989) with the ultimate objective of reducing schistosomiasis-related morbidities through focusing on health care delivery systems and specifically on case detection and treatment. This effort led to a remarkable reduction in the prevalence of both visual and micro-haematuria (Savioli et al., 1989a). Furthermore, this effort promoted subsequent schistosomiasis control programs in the area although these have been intermittent and varied in approach to control the disease.

The various interventions applied are summarised in Figure 6 which is based on earlier analysis carried out in collaboration with Dr Marco Albonico and in consultation with the helminth control program manager in Pemba, Dr Khalfan Mohammed and with Mr. Kassim Shimely. It represents a review of the literature on activities and studies carried out in Pemba since the establishment of schistosomiasis control initiatives and the later integration of Soil Transmitted Helminth (STH) and Lymphatic Filariasis (LF) control strategies. Figure 6 makes reference to the integrated helminth control programmes carried out and the various anthelminthics employed i.e. the Zanzibar Helminth Control Programme (ZHCP) which was responsible for controlling morbidity of schistosomiasis and STH infections; and the Global Programme for Elimination of Lymphatic Filariasis (GPELF) aimed at eliminating lymphatic filariasis due to *Wuchereria bancrofti*. Along the top of the Figure are the values for prevalence of *S. haematobium* recorded in publications/records. During the early years of schistosomiasis control, the impact of the control measures had relied on the use of haemastix on urine for monitoring infection as suggested by microhaematuria, rather than on parasitology. The control of STH was steadily merged into the existing schistosomiasis programme and subsequently integrated with LF so that different drugs/drug combinations were administered during the course of implementation of the control strategies. Initially PZQ was used alone to control schistosomiasis and later combined with mebendazole (MBZ) (500mg) or albendazole (ALB) (400mg) to control STH. The control of transmission of LF employed a combination of ivermectin (IVM) and ALB. Following revival of the schistosomiasis control in 2004, PZQ was distributed in communities by MDA and later

in schools by SBT. At the late stage (in 2006) of the transmission control of LF, IVM combined with PZQ and ALB was distributed by ration (MDA), as part of an operational community-based trial to assess the side effects following administration of those three drugs together (Mohammed et al., 2008). In this MDA each individual in the communities aged ≥5ys, excluding those with chronic diseases or conditions (hypertension or diabetes), pregnant women and lactating mothers at first week, was given that drug combination. The drugs were distributed by house to house visit through community drug distributors (CDDs) from their respective *shehias*.

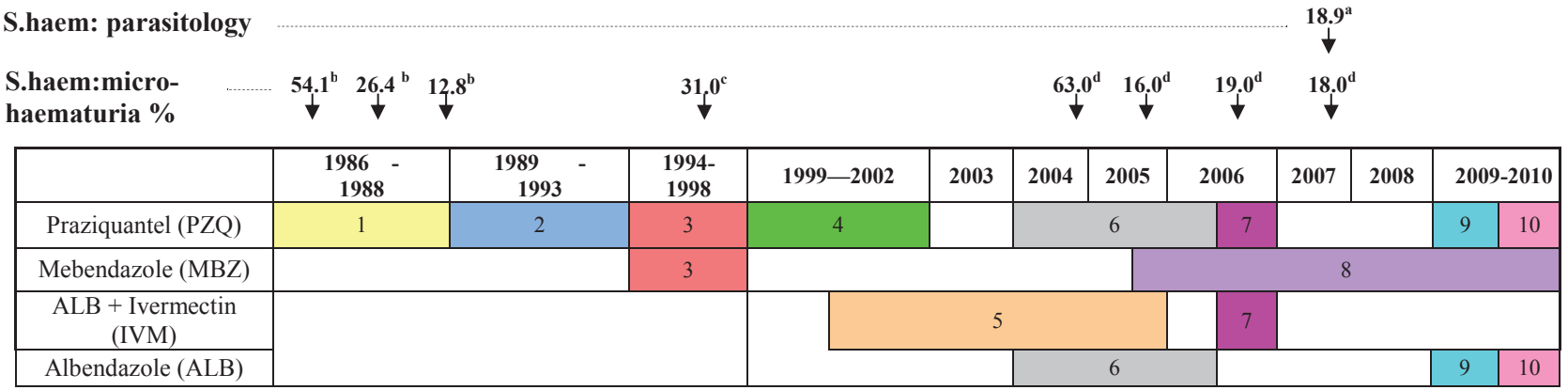
As shown in Figure 6 the intensive annual selective treatment[7] targeted at school-children (1986-1988) in which school-children aged between 5-19yrs were diagnosed and those found positive were treated, led to a marked decline in prevalence of haematuria from the baseline of 54.1% to around 13% (Savioli and Mott, 1989). After this there was a period (1989-1998) of school-based treatments[8] (SBT), twice a year but later, due to the high cost and limited availability of praziquantel, only once a year and restricted to high prevalence schools was delivered between the years 1994-2002. After this period the prevalence had risen to 31% and following subsequent sporadic provision of SBT prevalence had rebounded by 2004 to 63% among the school-children and 37% in the community (Mr Haji, Unpublished data). Then in 2004-2006 reintroduction of intense treatment through CWT-MDA supported by the Schistosomiasis Control Initiative (SCI) again led to marked reductions in prevalence which in 2007 had fallen to 18% based both on haemastix testing and egg detection. After this there was minimal consistent intervention or monitoring until the initiation of this study and the implementation of the National Plan for Pemba in 2010 which initially involved annual SBT with ALB and PZQ.

In 2001, the WHO passed resolution WHA 54.19 which urges schistosomiasis endemic countries to have essential drugs for schistosomiasis and to reach to at least 75% of school-children and those at risk (WHO, 2006). The resolution emphasized the use of chemotherapy to control schistosomiasis morbidity. Additionally, following lessons learnt from other schistosomiasis endemic countries such as China and Morocco, which

[7] Selective treatment is a term used to indicate treatment only of those individuals found to be infected following diagnosis.
[8] School based treatment (SBT): treatment targeted to school aged children and delivered through the schools.

have successfully eliminated the disease (Engels et al., 2002) it was thought feasible for other countries to eliminate schistosomiasis. Thus, the WHO has endorsed resolution WHA 65.21 which encourages schistosomiasis-endemic countries to change their control strategies and focus on elimination (www.who.int/schistosomiasis/strategy/en/)

Figure 6: Review of Schistosomiasis control in Pemba



[a](Guidi et al., 2010)
[b](Savioli and Mott, 1989)
c(Albonico et al., 1997)
[d]Haji– unpublished SCI data

All based on school-children

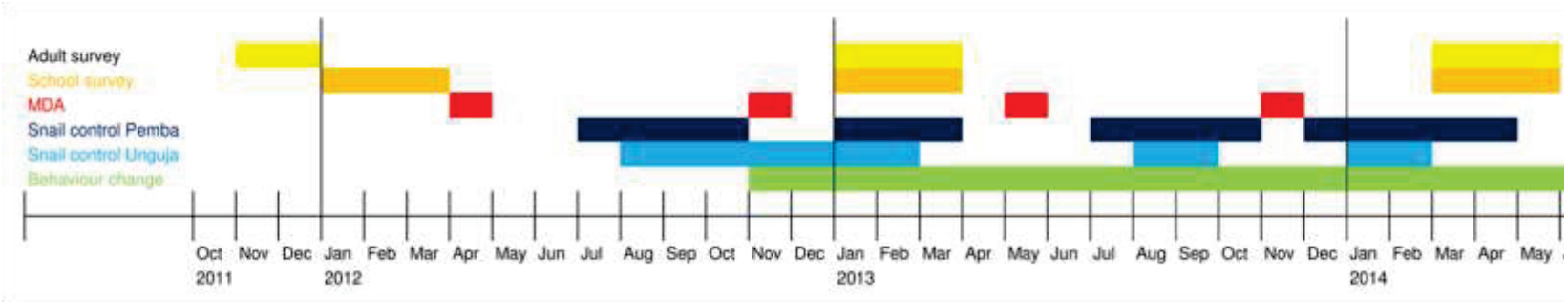
1	Selective chemotherapy to children and adults - test and treat campaign, twice a year
2	School targeted treatment, twice a year
3	ZHCP: Initiated PZQ for school targeted treatment, only in high prevalence schools MBZ for school targeted treatment, twice a year
4	Irregular distribution
5	GPELF: Community MDA (> 5 years of age), once a year . In 2006 PZQ was also included in the package and the three drugs were delivered together in the communities in MDA.
6	ZHCP ,> 5 years of age, once a year 2004 - 2005, Community MDA in Pemba (supported by SCI - Schistosomiasis Control Initiative) 2006, School based treatment in both Islands
7	Integrated Triple Drug Administration, Community MDA, only once
8	Preschool targeted treatment (12 - 59 months), twice a year, supported by UNICEF
9	School based treatment, once a year: (The treatment was partially implemented), no PZQ distributed
10	School based treatment (SBT), once a year (during the National Baseline Survey). SBT was followed by MDA from 2012 to 2018

In 2010 annual SBT was re-introduced with the intention of it being run continuously and the work in this chapter was initiated to monitor and assess the impact of this more consistent approach. However, in 2012 the Zanzibar National Neglected Tropical Diseases (NTDs) control program (ZNCP) decided to push for elimination of urogenital schistosomiasis, in an initiative called Zanzibar Elimination of Schistosomiasis Transmission (ZEST[9]) and so it was deemed necessary to change the treatment approach to introduce more frequent and widespread treatments. Furthermore, the Schistosomiasis Consortium for Operational Research and Evaluation (SCORE[6]) initiative chose Zanzibar (Unguja and Pemba) as one of the locations to assess the impact of alternative supplementary control measures on schistosomiasis. So the work in this chapter which started looking at the SBT programme applied in 2010 became concerned with establishing and then comparing and monitoring these new approaches in Pemba. For the SCORE initiative 45 local administrative units (shehias) in each Island (Unguja and Pemba) were sub-divided into three interventions arms and assigned either to receive MDA alone, MDA plus snail control or MDA plus behavioural changes intervention.

The timeline for these more intensive interventions is shown in Figure 7.

[9] ZEST is an alliance of different institutions and international organisations (Natural History Museum, Ministry of Health- Zanzibar, WHO and SCORE, SCI) with common interest of eliminating schistosomiasis in Zanzibar.
SCORE is housed at the University of Georgia, United States of America and is funded by the Bill and Melinda Gates Foundation. http://www.score.uga.edu

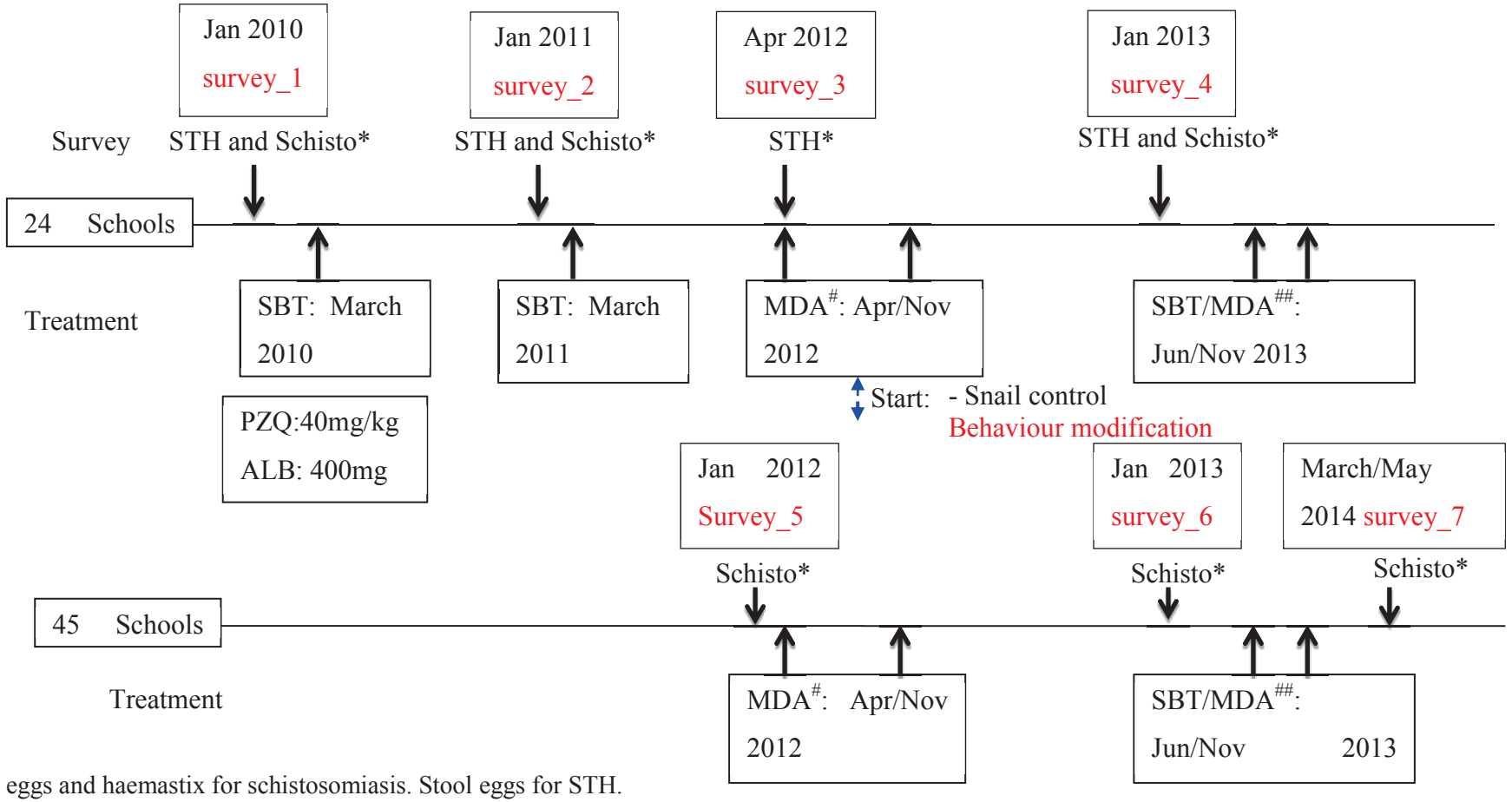
Figure 7: Timeline indicating implementation of different SCORE interventions for controlling schistosomiasis in Zanzibar



As detailed below (section 3.3.1.2), at the outset of ZEST in 2012 the overall baseline prevalence in Pemba was 10.1% and the programme started by introducing biannual MDA of praziquantel to all eligible individuals aged ≥3yrs in 2012, the initial treatment round being in April and the second one in November of 2012. This MDA treatment campaign was delivered through house-to-house approach. However, the subsequent parasitological survey carried out one year later revealed a minimal reduction of the disease prevalence (8.2%). Thus during the SCORE annual general meeting to evaluate the project progress it was proposed that the existing drug delivery strategy may not have been implemented effectively to reach the desired coverage rate. So it was decided to change the drug delivery approach with school-aged children being treated at school and adults and preschool children (aged 3-5yrs) being treated in the community by house to house drug administration. This regimen (referred to as "SBT/MDA") was first implemented in November, 2013.

The surveys summarized in Figure 10 (below) started with establishing the baseline prevalence at the beginning of this project work in 2010 and subsequently assessed the impact of the successive control strategies. School-based treatment was reintroduced in Pemba in 2010. The impact of these SBTs was assessed by three surveys during 2010 – 2012 which are described below. In 2012 MDA was implemented twice yearly supplemented in defined areas with either molluscicide or behavioural change initiatives, supported by the SCORE programme. The data described serves to compare these approaches. In the surveys parasitological diagnosis based on microscopic examination of filtered urine was carried out alongside use of haemastix for blood in the urine which had been used traditionally for prevalence assessments in Pemba (see Figure 6). The analysis described in this chapter deals principally with the use of the parasitological data to define impact of control on prevalence.

Figure 8: Timeline for STH and schistosomiasis surveys and treatment strategic approach



* Urine eggs and haemastix for schistosomiasis. Stool eggs for STH.

** Included 17 out of the 24 Schools cohort; # MDA: community-based, house to house treatment to> 3yrs.

3.2 Results

3.3. Outline of the various surveys

N.B. Surveys_1, 2 and 5 are described first since they deal with the pre-MDA period when only SBT had been applied.

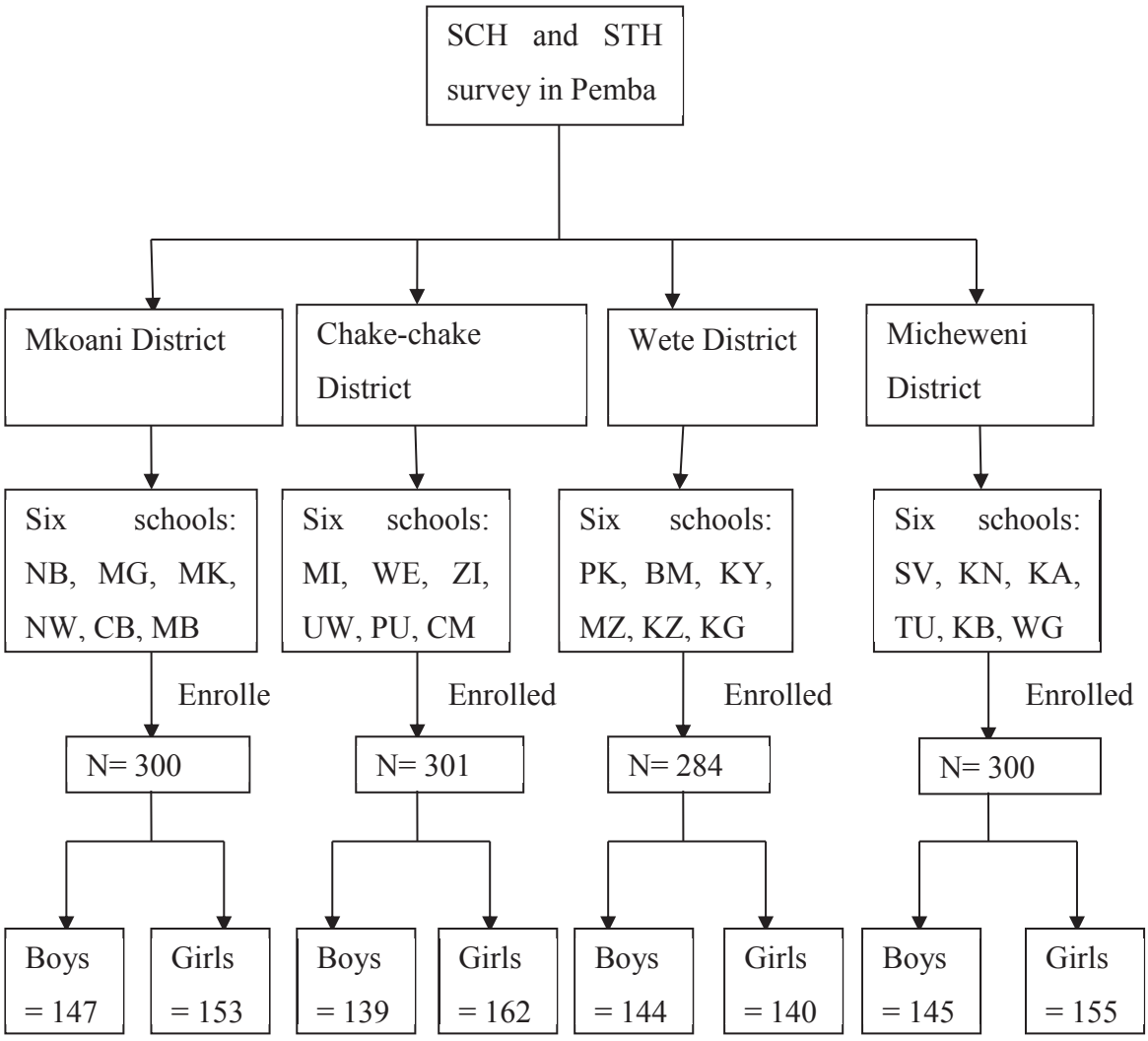
3.3.1 Survey_1 of baseline prevalence and intensity of S. haematobium *infections in schoolchildren in Jan 2010*

3.3.1.1 Study population:

During January 2010 a survey was carried out in 24 primary schools in Pemba Island (6 primary schools in each of the four districts), referred to as "Cohort 24". The schools were selected to cover both urban vs. rural locations although the whole Pemba may be regarded as rural (Stoltzfus et al., 2000).

A total of 1185 standard-1 (Std-1) school-children were enrolled in the study. The children had a mean age of 7.3yr (± SD 0.7). Figure 9 shows the profile of the children enrolled. Of the children enrolled, 610 (51.5%) were girls and 575 (48.5%) were boys.

Figure 9: Outline of the districts and Cohort 24 schools surveyed for schistosomiasis and soil transmitted helminthiasis**.**



NB =Ng'ombeni, MG= Mizingani, MK= Mkanyageni, NW=Ngwachani, CB=Chambani, MT=Mtambile, MI=Michakaini, WE=Wesha, ZI, Ziwani, UW= Uwandani, PU= Pujini, CM=Chanjamjawiri, PK=Piki, BM=Bagamoyo, KY=Kiuyu Minungwini, MZ=Mzambarauni, KZ=Kizimbani, KG=Kangagani, SV=Sumba Viamboni, KN=Kinowe, KA= Konde Msingi A, KB= Konde Msingi B, TU=Tumbe, WG=Wingwi

3.3.2 Survey _2 in Jan 2011 after one annual SB treatment

3.3.2.1 Study population

This survey of Cohort 24 schools was to determine any effects of this single annual school-based treatment on helminth infection rates in the new Std-1 cohort recruited a year later. In addition, Std-3 children were evaluated since this was the age group, along

with Std-4, selected for monitoring during the proposed future National Plan and SCORE activities. Parallel sampling of infection in the Std-3 children also allowed comparison of their levels of infection after having been treated in 2010 with that in the previously untreated Std-1 children. A total of 2382 children were enrolled. Of these, 2372 (99.6%) produced enough urine sample for processing.

3.3.3 Survey _5 in Jan 2012 after two annual SB treatments

3.3.3.1 Study population

Prior to the implementation of the SCORE initiatives themselves, a new baseline survey to assess the prevalence of schistosomiasis across Pemba Island was conducted between January–March, 2012. Forty five primary schools ("Score Cohort 45") were selected and 15 of these allocated to the snail control arm, 15 to the behavioural change arm and 15 to the MDA alone arm. The list of the 45 schools is shown in appendix 3.1. Of these, 17 were from the Cohort 24 schools used in the previous surveys (2010-2011) during the implementation of single annual school-based treatment. For some of the analysis described later these 17 schools will be considered separately under "Cohort 24/17"as they allowed comparison of the trend of schistosomiasis in Pemba following the different control strategies. Table 1 shows the allocation of schools between the Cohort 24, the Score Cohort 45 and the three different arms of the SCORE intervention.

A total of 7977 school children were enrolled in Survey_5 of which 7834 (98.2%) had complete records of urine samples. Of these 3497 (44.6%), 2243 (28.6%), 2094 (26.7%) were in Std-1, Std-3 and Std-4 respectively. Overall the children had a mean age of 9.4yrs (± SD 1.9), Std-1 children having a mean age of 7.7yrs (± SD 0.9), Std-3 children had a mean age of 10.2yrs (± SD 1.1) and Std-4 had a mean age of 11.4yrs (± SD 1.0). In all standards the number of girls was slightly higher than that of boys (52.8%, 51.9% and 55% for Std-1, Std-3 and Std-4 respectively).

3.3.4 Survey _4 (Cohort 24 schools)

This survey was carried out in 2013 to assess the trends of schistosomiasis (and also STH) in the Std-1 children from the Cohort 24 schools that had been surveyed annually

since 2010 and so allowed comparison of the effects of the SBT with the ZEST/SCORE MDA and MDA supplemented interventions. This was necessary because the SCORE surveys (survey_6 and survey_7) only sampled Std-3 and Std-4 children.

Survey_4 was carried out between January-March, 2013 and involved a total of 2309 Std-1 school-children (52% girls and 48% boys). The children had a mean age of 7.3yrs (± SD 0.7).

3.3.5 Survey_6

This was carried out along with Survey_4 to evaluate the impact of SCORE interventions in Std-3 and Std-4 children following one year of ZEST/SCORE implementation.

In this survey a total of 5632 children were enrolled of whom 4862 (83.1%) gave consent and also provided urine samples. Of these 54.6% were girls. The children had a mean age of 10.6yrs (± SD 0.95).

3.3.6 Survey_7

This was carried out as a routine programme monitoring to assess the effectiveness of the ongoing ZEST/SCORE strategies after 2years of their implementation. In this survey a total of 5684 school children were enrolled. Among those 51.0% were girls. The children had a mean age of 10.6yrs (± SD 1.1).

Table 1: Relationship between the "Cohort 24" schools and the "Score cohort 45" schools involved in each of the surveys.

	Survey_ 1	Survey_ 2	Survey_ 5	SCORE ARM	Survey_ 4	Survey_ 6	Survey_ 7
24 school cohort	24*	24	7 (N.D.)#		7*		
			17	Behaviour	6	6	6
				Snail	6	6	6
				MDA	5*	5*	5*
45 school cohort			28	Behaviour	N.D.#	9	9
				Snail		9	9
				MDA		10	10
School Standard sampled	1	1 &3	1,3,4		1	3&4	3&4

* Underlined numbers show the schools from the original 24 which were just subject to the chemotherapy interventions.

ND – not done in this survey

Shaded area – the 45 schools included in the SCORE study (which includes 17 from the original 24 school cohort).

3.4 Monitoring of School Based Treatment (SBT) control implemented 2010 -2012 (Cohort 24).

3.4.1 Assessment of prevalence

3.4.1.1 Baseline prevalence Jan 2010 (Survey_1)

Overall, *S. haematobium* infections as defined by single urine filtration were found in 112 (9.5%) (95% CI = 7.8-11.1) children. Of these, 44 (39.3%) were girls. Further analysis showed a significant association of infection with sex (χ^2 = 7.4; df = 1; p = 0.007), boys being more frequently infected.

The prevalence in each of the 24 schools in the four districts of Pemba is shown in Figure 10 and the mean for the schools in each of the 4 districts in Figure 11.

There was marked variation in schistosomiasis prevalence in schools both within and between the 4 districts ranging from 0-30% for individual schools, The mean prevalences for schools in the 4 districts (Figure 11) were significantly different (χ2 = 32; df = 3; p = 0.0001 for the districts). The differences in prevalence both within and between districts are likely to be due to ecological differences between the areas (districts). For example Mkoani district has more hills and valleys with abundant water bodies which increase the potential transmission of schistosomiasis, while Micheweni district is more flat and sandy with relatively fewer water bodies. The presence of hills and valleys extends from Mkoani district to the northern west of Pemba Island. Similar factors are likely to underlie the differences seen within districts e.g. Ng'ombeni (2% prevalence) and Chambani (30% prevalence), are both in Mkoani district, but there are more water bodies in Chambani than in Ng'ombeni.

Figure 10: Prevalence for each of the Cohort 24 schools by district

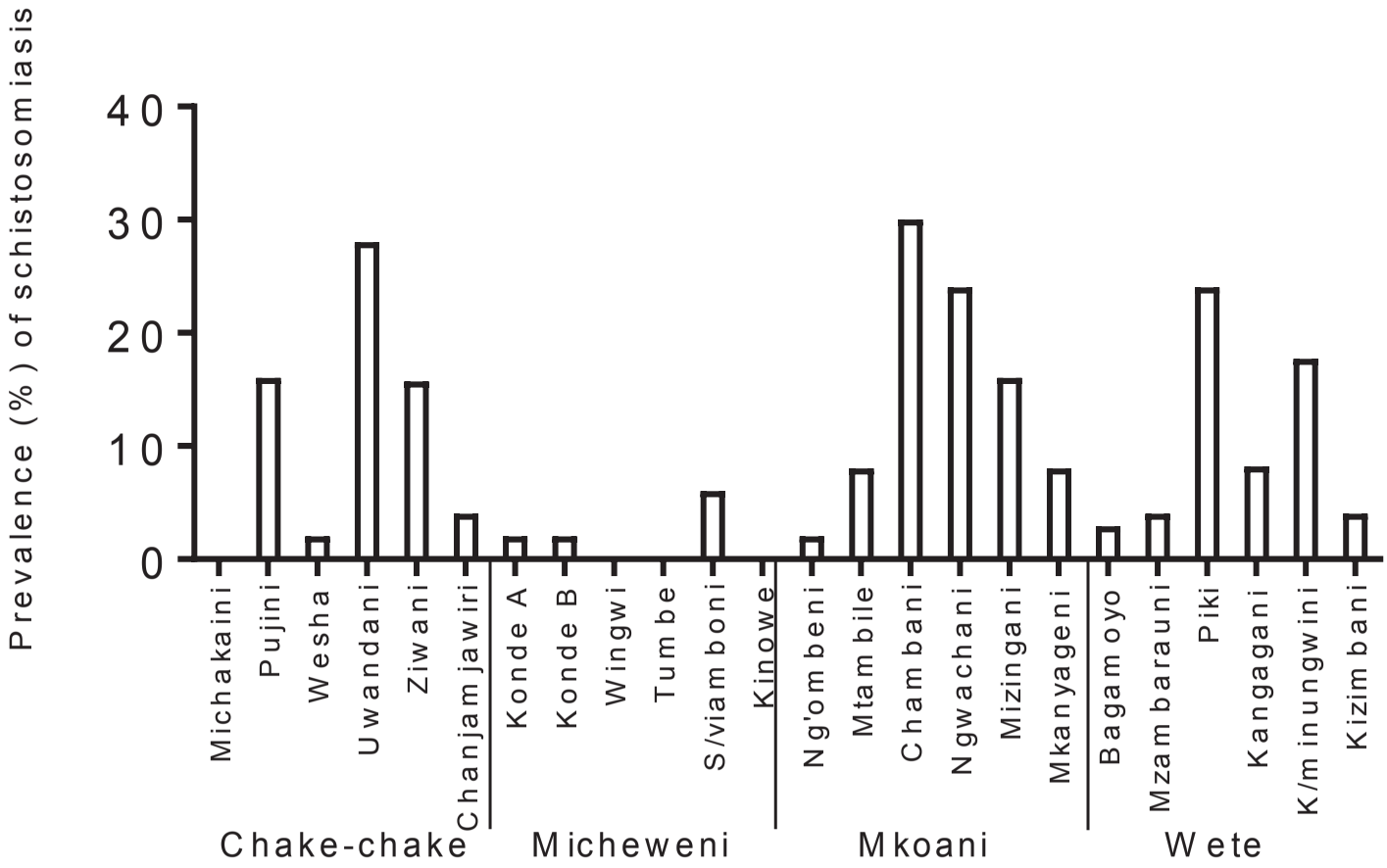


Figure 10 Prevalence for each of the Cohort 24 schools by district

Figure 11: Overall (mean) prevalence of schistosomiasis among districts in Pemba in 2010

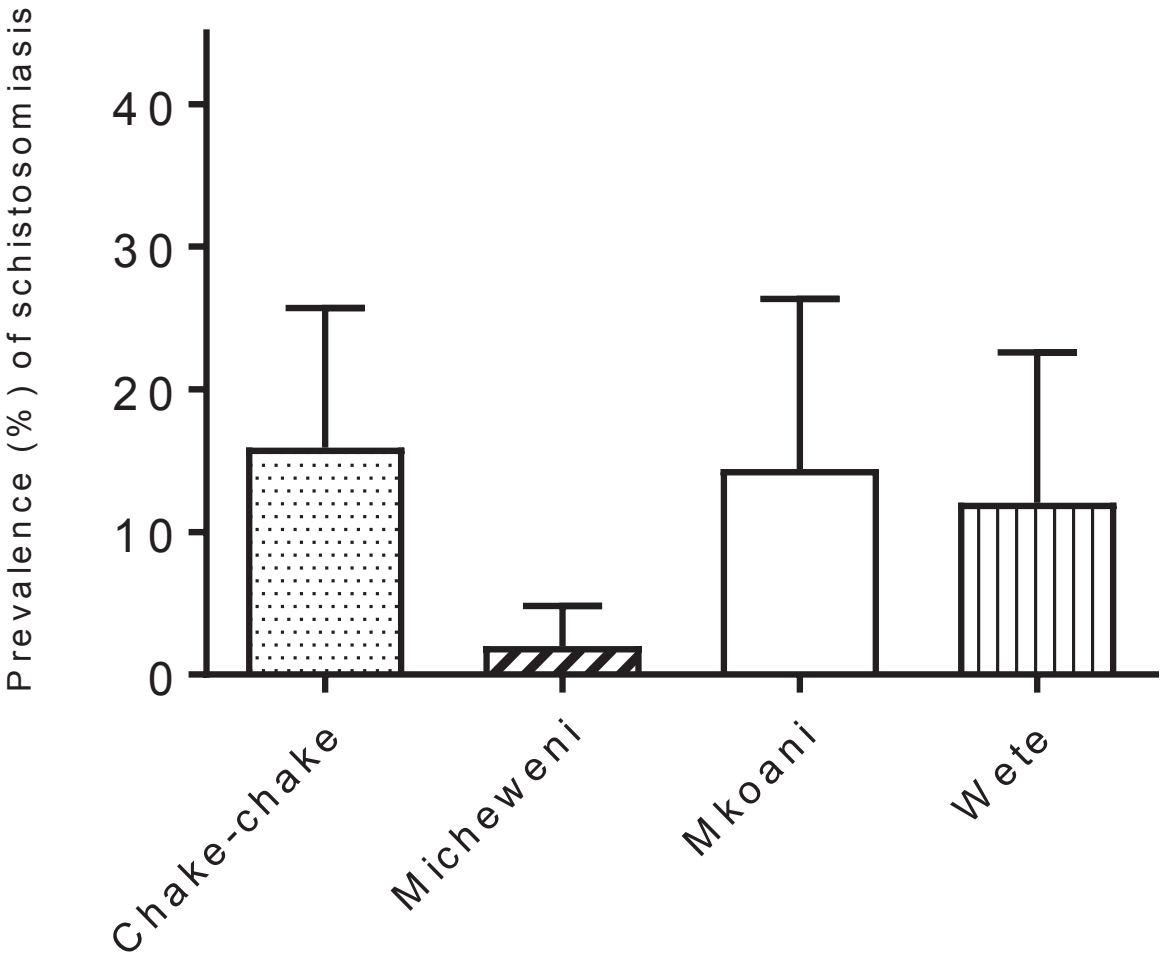


Figure 11 Mean prevalence (+S.D.) for schools in each of the four districts in 2010

3.4.1.2 Comparison of prevalence in the Cohort 24 schools in Jan 2010 (Survey_1), Jan 2011 (Survey_2) and Jan 2012 (survey_5).

The prevalences in the 24 schools (surveys_1 and 2) and the subset of 17 from the original 24 (survey_5) are aligned for comparison in Figure 12. NB Data from the Std-4 children in survey_5 is not shown here since this age group were not sampled in earlier rounds.

Figure 12: Comparison of prevalence of schistosomiasis in Cohort 24 schools (2010-2012)

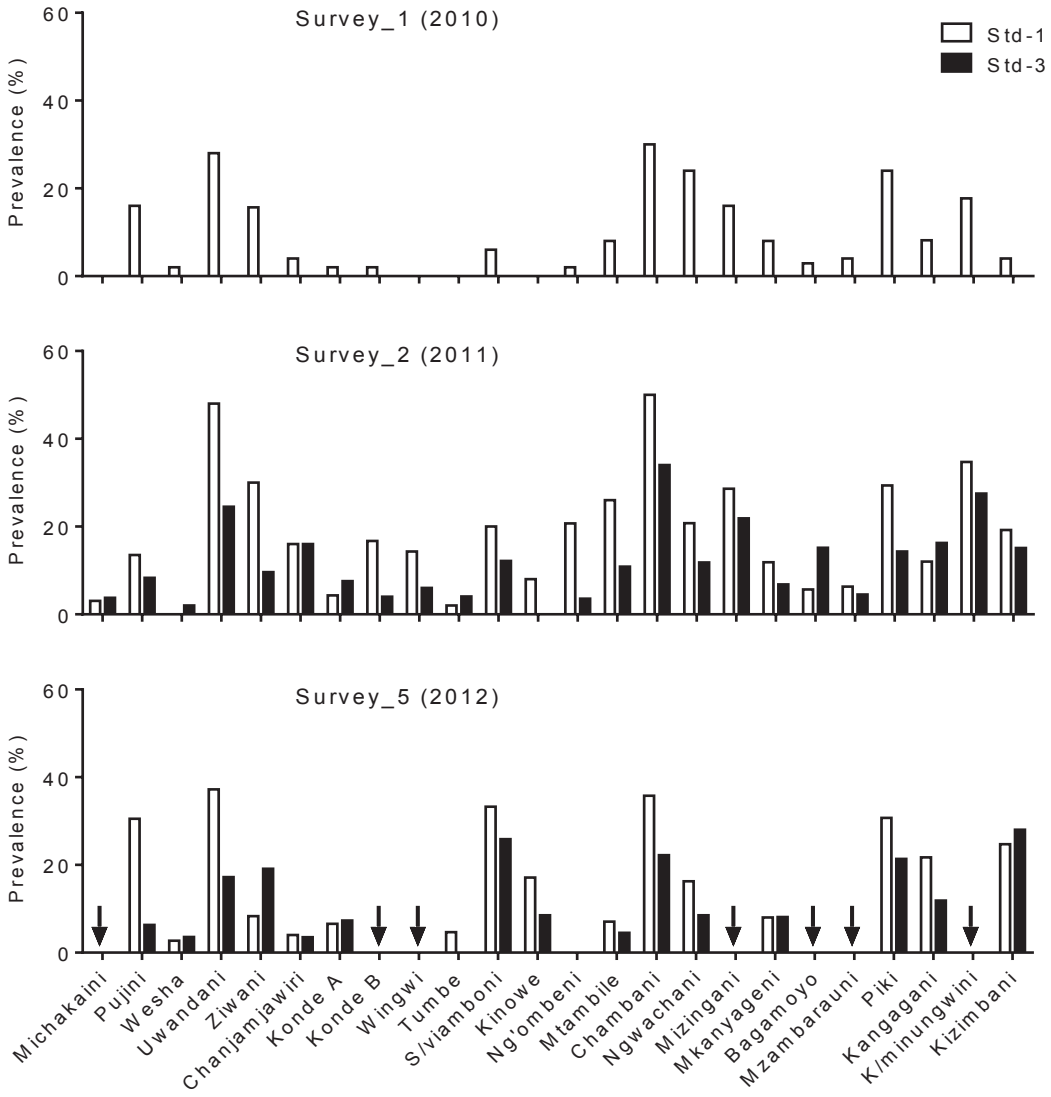


Figure 12. Prevalence in the individual schools in 2010-2012.
Arrows show the schools of Cohort 24 not included in Survey_5.

For a clearer comparison of the prevalence by year the data for the Std-1 children in the individual Cohort 24/17schools studied in all three surveys is also represented in Figure 13.

Figure 13: Prevalence of schistosomiasis by year for each of the Cohort 24/17 schools.

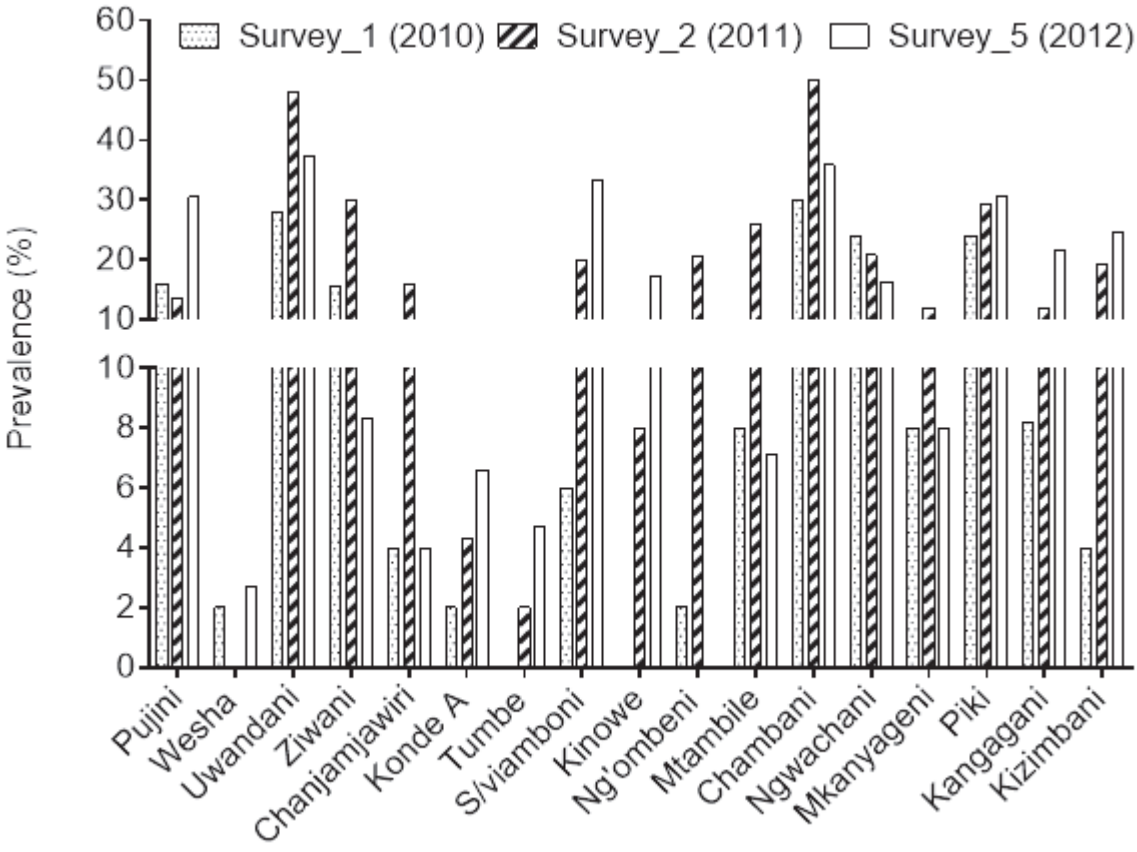


Figure 13: Prevalence in years 2010, 2011 and 2012 for the Std-1 Children in the Cohort 24/17 schools.

From Figures 12 and 13, it is clear that the pattern of infection levels across the schools was essentially similar in 2010, 2011 and 2012 e.g. Chambani and Uwandani Schools consistently had the highest level of infection as compared to other schools.

Somewhat unexpectedly, as more clearly seen in Figure 14, the overall mean prevalences, for the Std-1 children in the 17 schools, of 19.6% (95% CI = 16.9-22.3) and 17.6% (95% CI = 15.6-19.6) in 2011 and 2012 respectively, were noticeably higher than that recorded in the same age group in 2010 (10.7% [95%CI = 8.6-12.8]) ($z = 5.1$; $p = 0.0001$ for the levels observed between 2010 and 2011]). The slight decrease in mean prevalence between 2011 and 2012 was not significant ($z = 1.2$; $p = 0.2$). Mantel-Haenszel analysis was also used to assess the significance of this difference in prevalence. Mantel-Haenszel analysis is able to assess the overall odds ratio (OR) for the change in prevalence between two time points e.g. 2010 and 2011 by integration of

the individual odds ratios for the prevalence change in each of the 24 schools individually. Applying Mantel-Haenszel analysis to the changes in prevalence between 2010 and 2011 showed there was a highly significant OR for prevalence being higher across the schools in 2011 (MH_{OR} = 2.3 [95%CI = 1.81-3.04]; p = 0.0001), (see appendix 3.5A). Similarly, applying Mantel-Haenszel analysis to all individual schools to estimate the trend of infection over the three year (2010-2012) period, there was a significant OR for infection increase (MH_{OR} = 1.2 [95% CI = 1.1 -1.3], p = 0.003).

Figure 14: Mean prevalence for the Cohort 24/17 sampled 2010-2012

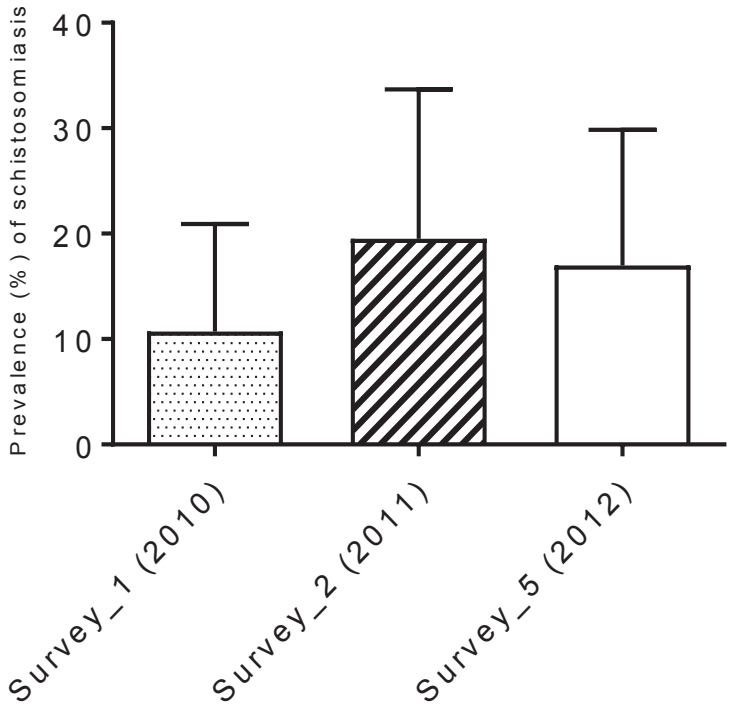


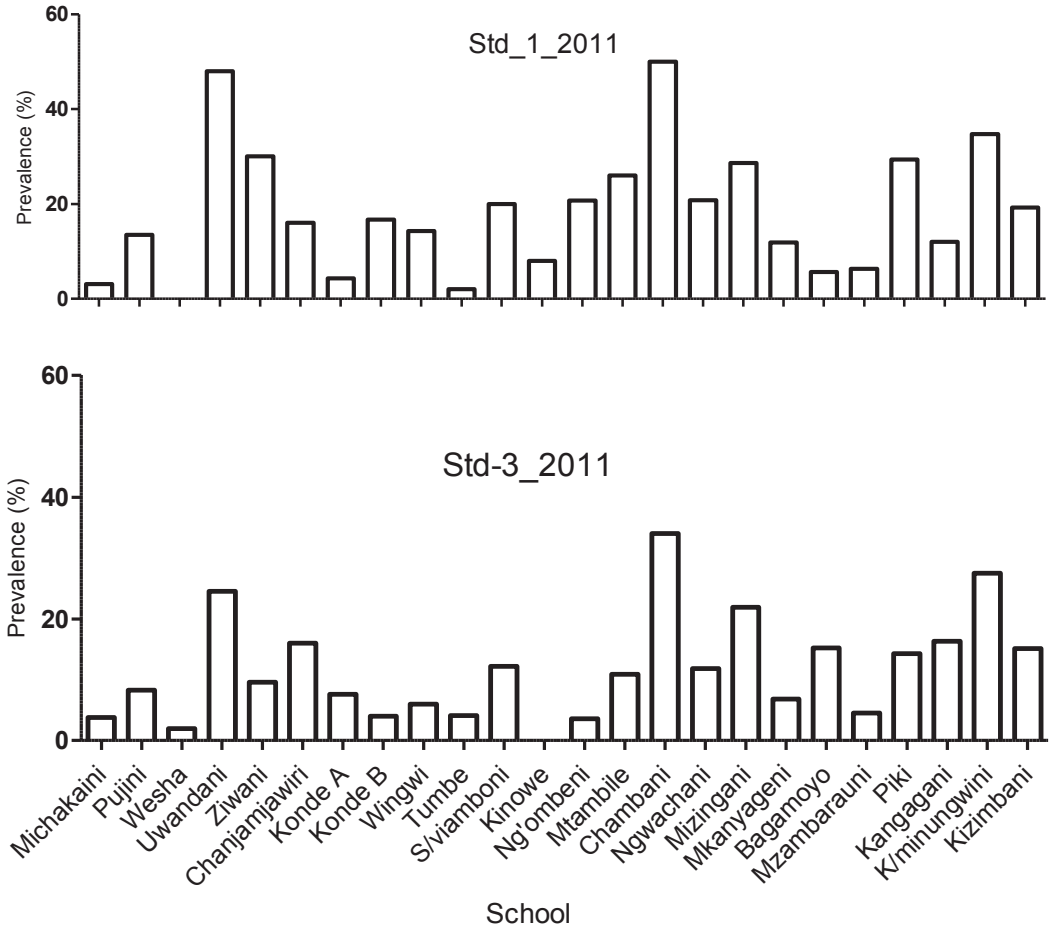
Figure 14: Mean (± SD) percentage prevalence for the Standard 1 children in the 17 schools tested in each of years 2010, 2011 and 2012. Error bars = standard deviation.

The higher overall prevalence in 2011 compared with 2010 was reflected in higher prevalences in 19 out of the 24 schools and across the different districts. Of the Std-1 children, there were significantly more boys infected than girls in 2010 (overall prevalences 58.2 vs. 41.8% respectively; χ^2 = 3.5; p = 0.06) and also in 2011 (61.8 vs. 38.2% for boys and girls respectively; χ^2 = 15.3; p = 0.0001) but not in 2012 (47.7% for boys vs. 52.3% for girls; χ^2 = 0.4; p = 0.4). It is generally observed, in settings like Pemba, that boys usually engage more in risk behaviours for transmission of

schistosomiasis than girls which in turn increase the potential of acquiring the infection. So the finding of higher prevalence of schistosomiasis in girls in 2012, albeit non-significant, was unexpected. However, this pattern was not observed for Std-3 children tested in 2011 and 2012. On both of these occasions: boys were more infected than girls (69.4% vs. 30.6% for boys and girls respectively in 2011; $\chi^2 = 19.3$; $p = 0.0001$) and 66.7% for boys vs. 33.3 for girls in 2012; $\chi^2 = 16.5$; $p = 0.0001$) in 2012.

The inclusion of Std-3 children in the surveys in 2011 (to conform to the newly planned ZEST/SCORE programme) allowed a comparison of the prevalence in Std-1 and St-3 children. Std-1 children had not been previously treated and so their level of infection represents cumulative infection over preschool years. In contrast the Std-3 children sampled in 2011 had been treated via SBT once in 2010 and those sampled in 2012 would have been treated twice (annually in 2010 and 2011). So differences between the Std-1 and Std-3 levels of infection is likely to reflect the impact of elimination of the preschool worm burden but may also be affected by changing exposure levels or development of immunity. In 2011, 359 (15.1% [95%CI = 13.7- 16.6%]) of the children were infected, with Std-1 children showing a significantly higher prevalence (18.6% [95%CI = 16.6-21.0], 226 infected) than Std-3 (11.3% [95%CI = 9.5-13.2], 133 infected) ($\chi^2 = 26$; $p = 0.0001$). Furthermore, the prevalence in individual schools (Figure 15.) showed marked variation between the schools for both Std-1 and Std-3 children with many schools showing prevalences ≥20% for Std-1 classes. Interestingly the pattern of infection was somewhat similar within the schools for the two classes. In 2012, after two rounds of annual SBT, the situation was similar with a higher prevalence of infection in Std-1 (17.6% [95%CI = 15.6-19.6%]) than Std-3 (11.6% [95%CI = 9.6-13.6%]; $\chi^2 = 15.\ 8$; $p = 0.0001$). Applying Mantel-Haenszel analysis to all the individual schools showed a highly significant odds ratio for prevalence being lower in Std-3 children ($MH_{OR} = 0.56$ [95%CI = 0.44-0.2%], $p = 0.0001$).

Figure 15: Comparison of prevalence of schistosomiasis in Std-1 and Std-3 in 2011 (Survey_2: Cohort 24 school) in Pemba



3.2.1.3. Comparison of overall prevalence by district 2010-2012.

The data for mean prevalence by district for 2010-2012 is shown in Figure 16. As can be seen, the above mentioned increase in overall prevalence between 2010 and 2011 was seen in all four of the districts with the regional patterns of prevalence being similar between 2010 and 2011. In 2012 the pattern was somewhat different. Overall, the data indicate a progressive increase in prevalence in Micheweni and Wete, the levels of infection being significantly

Figure 16: Mean prevalence of schistosomiasis by district 2010-2012

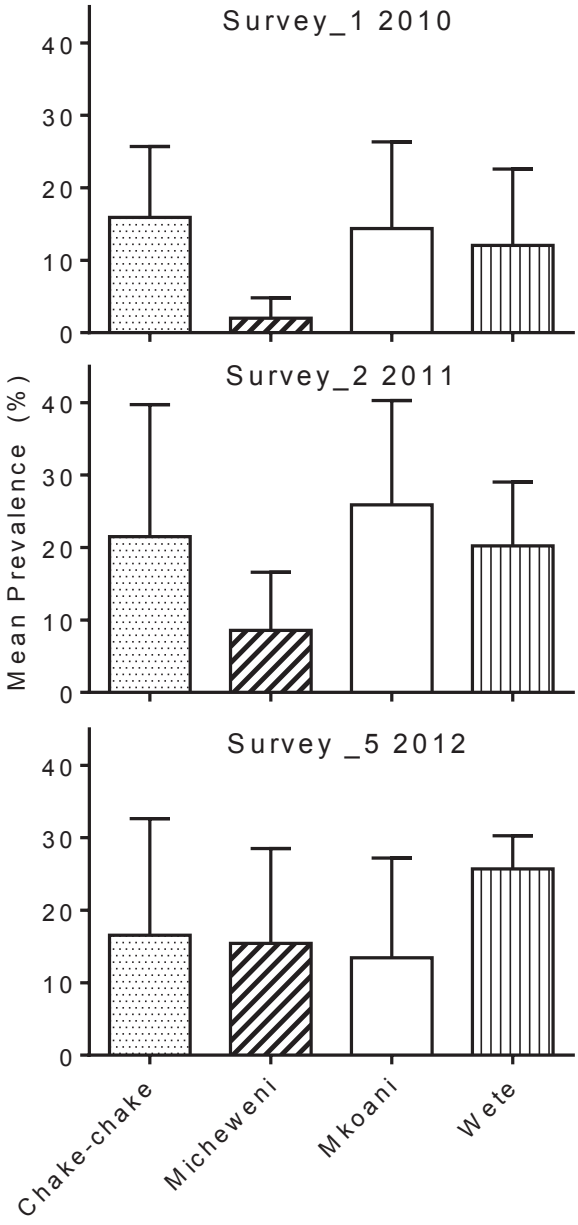


Figure 16 Mean (+SD) prevalence of schistosomiasis in Pemban districts in 2010-2012

increased over the years from 2% (95%CI = 0.6-3.9) and 12.1% (95%CI = 6.9-17.3) respectively at the baseline (2010) to reach 16.2% (95%CI =12.0-20.3% [z -5.1; p = 0.00001]) and 25.4% (95%CI = 20.0-30.8% [z -3.2; p = 0.002]) respectively in 2012. The prevalence of 16.2% observed at Micheweni district was surprisingly high as compared to the baseline 2%. The reason for this marked increase of schistosomiasis

infection was not apparent as there had been no any environmental changes that would favour transmission.

Overall this data provides no evidence that the one or two rounds of annual SBT had any impact on prevalence of schistosomiasis. Indeed prevalence was seen to increase significantly in 2010-2011 the reasons for which are unknown. The difference in prevalence demonstrated between Std-1 and Std-3 is likely to reflect the impact of elimination of the preschool worm burden but may also be affected by changing exposure levels or development of immunity

3.4.2. *Assessment of intensity*

3.4.2.1. Comparison of arithmetic mean Intensities by School (2010-2012)

This analysis involved only Cohort 24/17 schools for which there was complete data. The overall mean intensities in the different individual schools and standards and the means across all children are shown in Figures 3.2.8 and 3.2.9. As described above, at baseline, only Std-1 children were sampled and their overall mean intensity ± standard deviation was 9.1± 65 eggs/10 ml of urine. Some schools especially Pujini had somewhat higher mean intensities i.e. (53.8 ±204.7). Although the patterns varied, the overall pattern indicates stable or increasing intensities in the majority of schools.

Figure 17: Intensity of infection 2010-2012

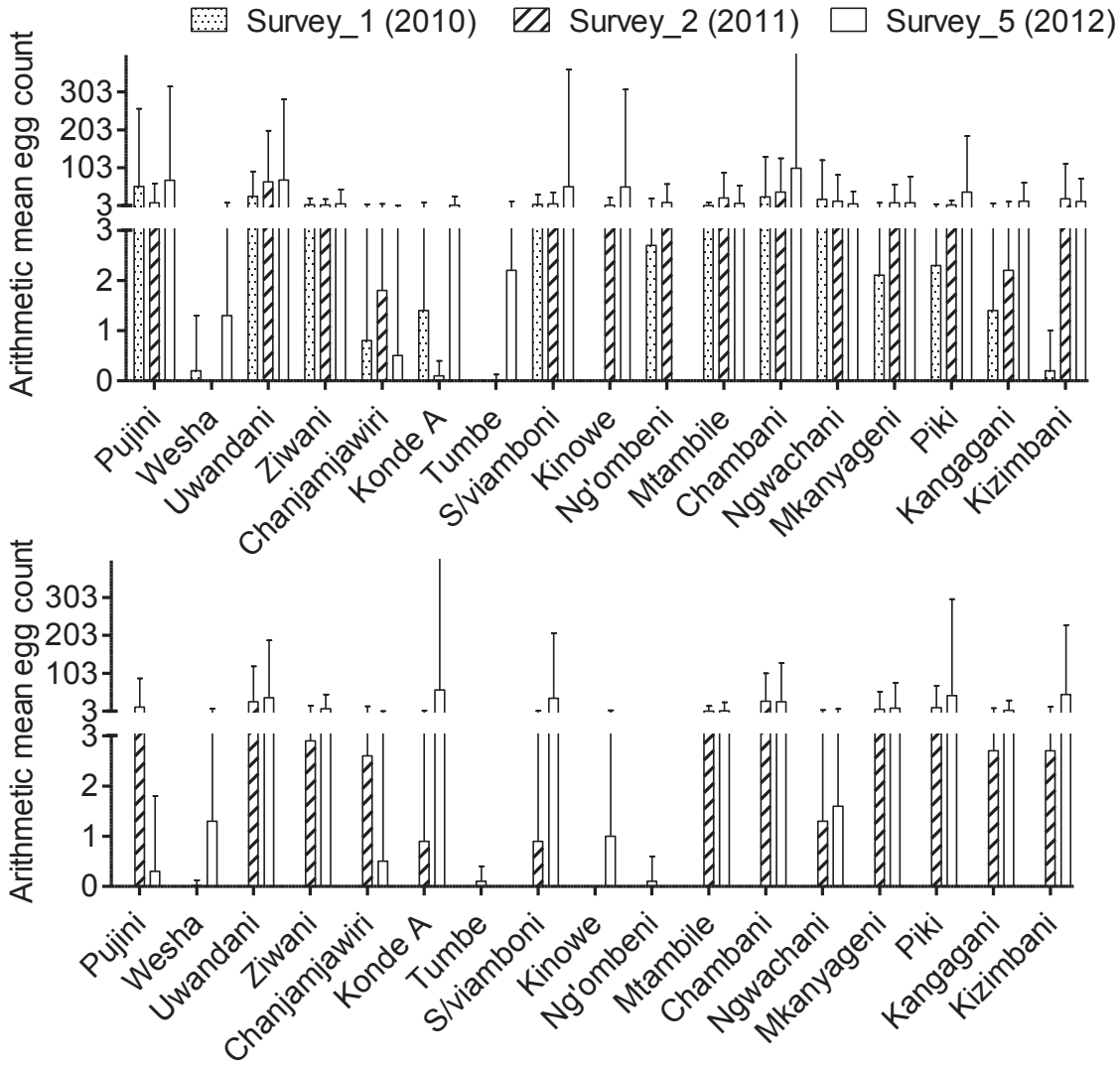


Figure 17 Arithmetic mean Intensity (SD) of infection 2010-2012. Top graph Std-1 and bottom Std-3

Arithmetic mean egg intensities derived from the individual child egg counts from all schools are shown in Figure 18. (Standard error bars are shown in this graph due to the large variance in egg counts). These were progressively increased as compared to baseline for both Std-1 and St-3. The mean intensities ± S.D. were 9.069 ± 65.0, 13.21 ±57.8, 28.31 ±161.6 in 2010, 2011 and 2012, respectively in Std-1 and 6.259 ±39.3 and 17.05 ±135.4 in 2011 and 2012 respectively, for Std-3. Since the egg count data was not normally distributed the non-parametric Mann-Whitney statistical test was used. This showed a significant difference for the Std-1 children between the Survey _1 and

Survey _5 values ($p = 0.0001$). However, the values for both Std-1 and Std-3 children in 2011 compared with 2012 were not significantly different.

Figure 18: Arithmetic mean intensity 2010-2012

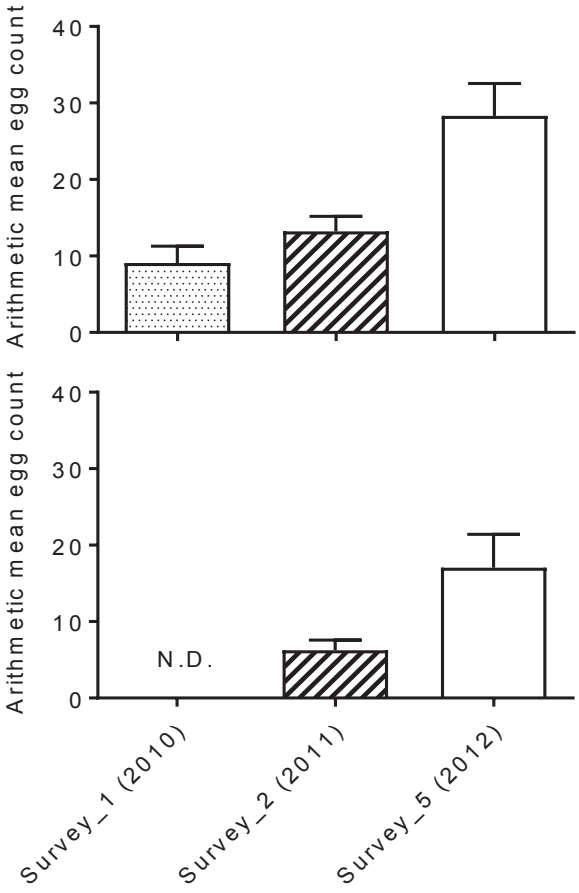


Figure 18 Arithmetic mean intensity (+SE) 2010-2012 based on the mean values from all individual children in all Cohort 24/17 schools. Top graph Std 1, bottom graph Std 3.

3.4.2.2. Comparison of Intensity by WHO designation (heavy [≥ 50 eggs/10ml urine] and light [≤49 eggs/10ml urine](Cohorts 24 and 24/17: Survey_1, 2 and 5)

This analysis concerned the data from Cohort 24 and its sub-set of 17 schools collected from 2010 to 2012 from Std-1 children prior to, and at the start, of the shift of treatment approach from annually SBT to SBT/MDA. The sampling of Std-1 children in 2012 in the ZEST/SCORE program allowed further comparison of the trends of both prevalence and intensity.

At baseline, (survey_1, 2010) the intensity of infection in Cohort 24 was 7.4 eggs/ 10ml of urine ± 56.0. Among the infected children, 76 (67.9%) had light infections and 36 (32.1%) heavy infections, which were more common in boys (26 [38.2%]) than girls (10 [22%]) although this was not statistically significant ($\chi^2 = 2.94$; df 1; $p = 0.086$). The children classified with light schistosomiasis infection had an arithmetic mean egg count of 15.2 (± SD 10.8) /10ml of urine with a range of 2-44/10ml of urine while those with heavy infection had a mean count of 210.4 ± SD 248.6/10ml of urine and a range of 50-994/10ml of urine.

For survey_2 (2011), Std-1 children in Cohort 24 had a mean intensity of 12.3 eggs/10ml of urine ± 53.7. Of the infected children (n=226), 153 (67.7%) had light intensity and 73 (32.3%) had heavy intensity. However, the proportions of boys (27.97%) and girls (27.6%) with heavy intensities were very similar. The children identified with light infection had an arithmetic mean egg count of 11.7 (± SD 12.2) /10ml of urine with a range of 1-49eggs/10ml of urine whilst those with heavy intensity had an arithmetic mean count of 177.68 (± SD 133.87) /10ml of urine with a range of 50-653 eggs/10ml of urine. So comparison between the two years (2010-2011), for cohort 24 schools only, clearly showed that the overall mean egg count was significantly higher ($t = -2.18$; $p = 0.02$) in 2011 but the proportions of light or heavy intensities were similar between the years (proportion of light intensities: 67.9% v.s 67.7% for 2010 and 2011 respectively).

Considering Std-1 children in Cohort 24/17 schools between 2010 -2012 (surveys_1, _2 and _5), 62 (68.1%) had light infection at baseline compared with 115 (69.7%) in survey_2 and 138 (53.9%) in survey_5. Further analysis revealed that lightly infected children had overall mean intensities of 15.2 ± 11.1 eggs/10ml at baseline, 11.9 ± 12.2 in survey_2 and 14.3 ± 12.7 in survey_5 whilst those with heavy infections had mean intensities of 233.3 ± 271 eggs/10ml, 195.4 ± 144.2 in survey _2 and 333.3 ± 472 in survey _5. The differences in these intensities between successive years were not statistically significant. As in the Cohort 24 data, a somewhat higher percentage of the infections were deemed heavy in boys compared with girls (Table 2) in each survey.

Table 2: Proportion of infected Std-1 children in relation to sex with heavy infections from 2010-2012 for cohort 24/17

	Sex	
	Boys	Girls
2010	37.7	23.7
2011	33.3	25.4
2012	48.6	44.0

3.4.3. Schistosomiasis and anaemia (Cohort 24, survey_1 and survey_4)

It was intended at the start of this project to assess the impact of the newly reintroduced SBT on the haemoglobin (Hb) concentration in school-children. Therefore, this was assessed in the baseline survey of Cohort 24 in 2010 (survey_1) but the monitoring scheme for the ZEST/SCORE programme did not involve Hb assessment and so this assessment was not continued. However, to provide some insight into any impact on anaemia of the interventions, Hb was assessed in the 2013 survey_4 of Cohort 24 schools. This was after two annual rounds of SBT and the two rounds of MDA in 2012 for the ZEST/SCORE interventions.

Among the children enrolled at baseline, the mean haemoglobin concentration was 11.3 ± 1.7 g/dl. Of the 1185 children, 514 (43.8%), 665 (56.1%) and 6 (0.5%) respectively had normal (≥ 11.5 g/dl), moderately reduced (7.01-11.4g/dl), and severely reduced (≤ 7 g/dl) haemoglobin levels and so the overall prevalence of anaemia was 56.6%. Generally, the prevalence of anaemia (moderate or severe) was not significantly associated with *S. haematobium* infection status (58.0% in infected and 56.5% in non-infected children (χ^2 = 0.1; p = 0.75). Moreover, schistosomiasis was not statistically associated with severity of anaemia (Fisher exact test: 0.6). However, most of the children with anaemia, 232 (34.6%), were from Mkoani district which had the highest prevalences of both schistosomiasis and STH.

Further analysis of anaemia in relation to sex revealed that the proportions of girls and boys with or without anaemia that were either infected with schistosomiasis or not were

comparable. For instance, 56.6% of the boys and 56.4% of the girls who were not infected with schistosomiasis had anaemia and 55.9% and 61.4% respectively of boys and girls infected with schistosomiasis had anaemia.

At the subsequent survey_4, the mean Hb for the children was 11.9±1.8 g/dl and the prevalence of anaemia was 38.2% (95%CI = 36.2-40.2) which was significantly (z = 7.2; p = 0.0001) lower compared to baseline (56.6% [95%CI = 53.8-59.4]). Of these anaemic children, only 5 (0.6%) had severe anaemia (Hb ≤ 7.0 g/dl); thus most of the children had moderate anaemia 866 (99.4%). Of the *S. haematobium* infected children, 68 (42.8%), had anaemia but as at baseline the anaemia was not statistically associated with schistosomiasis (χ^2 = 1.5; p = 0.21). Furthermore, schistosomiasis was not statistically associated with severity of anaemia (Fisher exact test: 1.0).

With regards to anaemia in relation to schistosomiasis in the different sexes, it was found that the proportion of girls (47.1%) who were infected with schistosomiasis and who were anaemic was slightly higher than that of boys (39.6%) but this was non-significant (χ^2 = 0.9; p = 0.34). The proportions of girls (35.7%) who were not infected with schistosomiasis but were anaemic was lower than that of boys (40.1%) and this was statistically significant (χ^2 = 4.1; p = 0.04).

Comparison of anaemia relative to schistosomiasis infection at baseline between 2010 and 2013 showed that at baseline the mean Hb in egg positive children was lower (11.2 g/dl [95%CI = 10.9-11.3]) than that observed three years later (11.7 g/dl [95%CI = 11.5 -11.9]) following implementation of SBT and MDA. The increase in Hb concentration was statistical significantly (t-test = -2.6; p = 0.01). Similarly, the proportion of *S. haematobium* infected children who were anaemic at baseline (58.0% [95%CI= 48.9-67.1]) was also significantly (z = 2.46; p = 0.01) reduced to 42.8% (95%CI = 35.1-50.5) in 2013.

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3.5 Monitoring of the impact of introduction of the ZNCP and SCORE interventions

The SCORE programme introduced in 2012 consisted of a comparison of (i) more intensive (biannual) PZQ administration to all ≥3yrs old (MDA) (ii) MDA plus additional snail control (SC) or (iii) MDA plus behavioural modification (BM). These

methods were introduced into 45 defined areas, 15 of each randomized for each intervention strategy.

By the end of the study period covered in this thesis there had been 6 rounds of MDA and 2 follow-up surveys of prevalence and intensity (Surveys_ 6 and _7) for this 45 school cohort (see Figures 3.2 and 3.3) which is referred to as "Score cohort 45". Of these 45 there were 5 schools which were included in the original 24 schools sampled during the SBT period (2010-2012) (surveys_1, 2 and 5) which were included in the MDA (only) arm of MDA/SCORE and so subject to the more intensive MDA after MDA/SCORE introduction. To assess impact of the switch from SBT to MDA data for these 5 schools ("Score cohort 5") is analysed separately. In addition, all of the original 24 schools were also sampled during the SBT (2010-12, surveys_1 and _2) and once after the first year of MDA (survey_4). This data is analysed under "Cohort 24").

3.5.1 Analysis of "Score cohort 45" - PREVALENCE

3.5.1.1 Baseline prevalence for the ZNCP/SCORE control initiative Jan 2012 (Survey_5)

The baseline ZNCP/SCORE data involved sampling of Std-1, 3 and 4 children (n = 3556, 2290 and 2131 for Stds-1, 3 and 4 respectively). N.B. the inclusion of the Std-1 children in Survey_5 was to provide another data point for the earlier SBT evaluation (described above) and Std-1 children were not included in the subsequent ZNCP/SCORE monitoring. So data for Std-1 children are only included in Figure 19.

Overall, schistosomiasis was found in 793 (10.1% [95% CI= 9.4-10.8]) of the children. Figure 19 shows the distribution of schistosomiasis among the 45 schools. The prevalence of schistosomiasis varied markedly between the schools (0-32.5%) but only 4 schools: Daya, Madungu, Makombeni and Ng'ombeni had no cases of infection.

3.5.1.2 Overall impact of SCORE interventions on the whole "Score cohort 45".

Figure 24 shows the combined prevalence data from the three arms for all 45 schools tested at baseline (Survey _5) and at subsequent follow-ups in 2013 and 2014 (Surveys _6 and 7 respectively). The data is presented as the mean prevalence for the 45 schools.

For both Std-3 and Std-4 the patterns were generally similar showing little difference between 2012 and 2013 indicating that the two MDAs in April and November of 2012 had no discernible effect on prevalence in 2013. This led to the change in strategy with the introduction of SBT/MDA in 2013 as described above (see Figure 8). As seen in Figure 24 there was a more marked drop in prevalence between 2013 and 2014. For Std-3 the values for each successive year were 7.91 % (95%CI = 5.29-10.53); 6.92 % (95%CI = 4.65-9.2); 5.34% (3.20-7.48). This equates to 32.5% reduction 2012-2014. For Std-4 the values were 8.57% (95%CI = 5.90-11.23); 8.38% (95%CI= 5.02-11.74); 5.27% (95%CI=3.41-7.14) giving a 38.5% reduction 2012-2014. The combined prevalence of infection for Std-3 and Std-4 children were 8.24% (95%CI=6.41-10.07); 7.66% (95%CI = 5.66-9.66); 5.31% (95%CI = 3.92-6.70) giving a reduction 2012-2014 of 35.6%. Applying Mantel-Haenszel analysis to estimate the trend for the odds of infection (2012-2014), revealed significant declining odds ratios over the years for both Std-3 (MH_{OR} = 0.82 [95%CI = 0.73-0.92]; p =0.001), Std- 4 (MH_{OR} = 0.78 [95%CI = 0.71-0.87; p = 0.0001]) and for the combined data for Std-3 and Std-4 (MH_{OR} = 0.8 [95%CI = 0.74-0.87]; (p = 0.0001]).

Figure 19: Baseline prevalence of schistosomiasis in each of the 45 schools in 2012

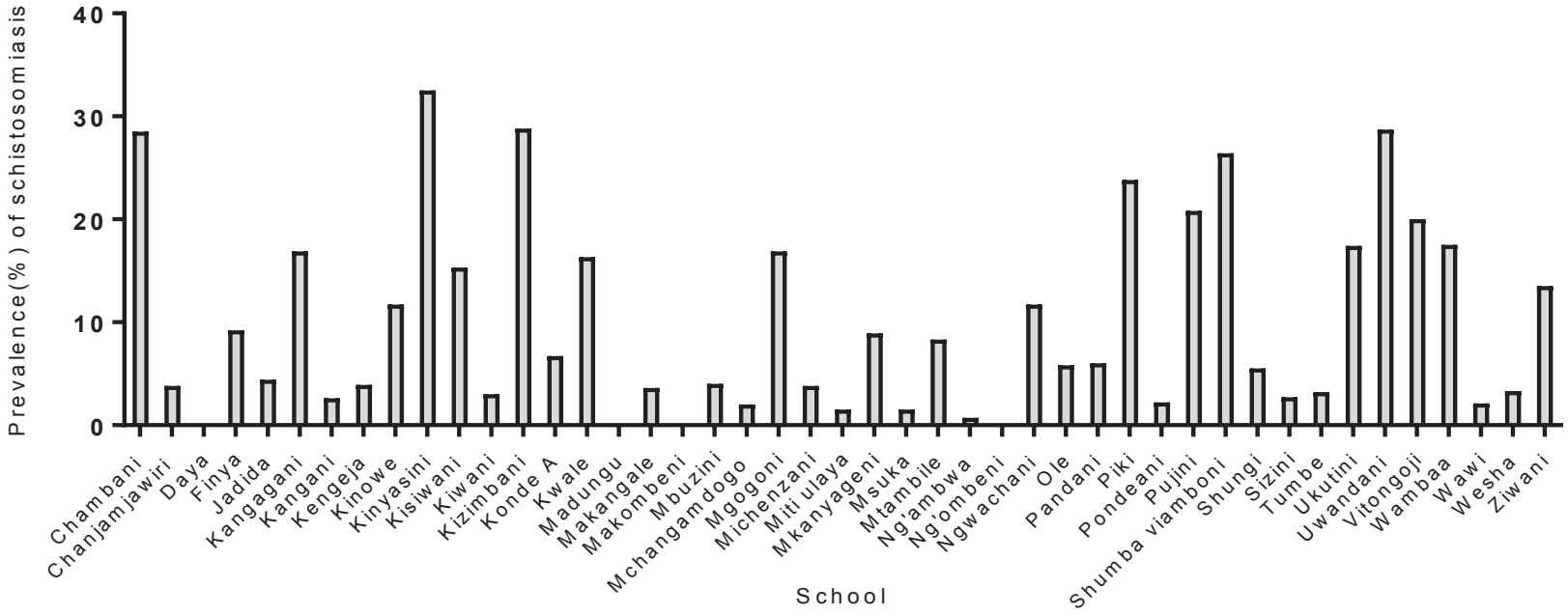


Figure 19. Baseline prevalence for the 45 schools in the ZNCP/SCORE interventions (Survey_5).

Figure 20: Mean prevalences of schistosomiasis from all 45 schools

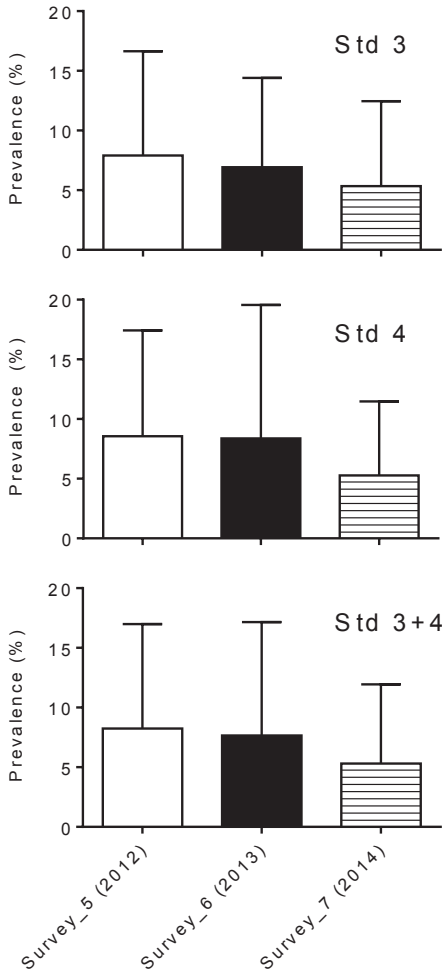


Figure 20 Mean ± S.D. of prevalences in all 45 schools by standard in 2012, 2013 and 2014.

3.5.1.3 Impact of the three different SCORE initiatives on prevalence in each of the 45 schools

As seen in Figures 21 and 22, the prevalence of infection in Std-3 and Std-4 relative to interventions varied between and within the schools over the years (3yrs period). Over the two year period a similar trend of declining prevalence in the majority of schools was seen in both the Std-3 and Std-4 groups for the three different interventions. The

Std-3 data showed: for MDA:- a decline in 47% of schools and an increase in 33%; for Behaviour modification (BM):- a decline in 60% and an increase in 27%; for SNAIL control:- a decline in 60% and an increase in 27%. The Std-4 data showed: for MDA:- a decline in 73% and an increase in 13%; for BM:- a decline of 80% and in increase in 0%; for SNAIL control:- a decline in 67% and an increase in 27%. Of the schools with prevalence of >10%, 24 (83%) showed a decline and 17% and increase. For some schools marked declines in prevalence were noted e.g. in Kwale (34.2→1.7% [Std-3]; 34.2→1.2% [Std-4]) although in other areas prevalences increased to >10% e.g. in Kinowe (5.1→14.3% [Std-3 and Std-4]). Analysis of the possible reasons for, and persistence of, such marked and varied changes in the different areas will be of great interest.

Considering the impact of the BM intervention which required individual behaviour modification there was no obvious marked difference in the trend for prevalence in 2012-2014 in the (older) Std-4 children compared with the Std-3 children i.e. there was no suggestion that the older children responded more effectively than the younger ones.

It should also be pointed out that when the prevalence changed between 2012 and 2014 the corresponding values for 2013 did not consistently show an intermediate value so monitoring over successive years will be essential to determine if the trend is stable.

In the analysis below the mean prevalence values for the schools in each arm are considered allowing statistical analysis of the trends suggested above.

Figure 21: Prevalences for the 45 schools according to intervention strategy – Standard 3

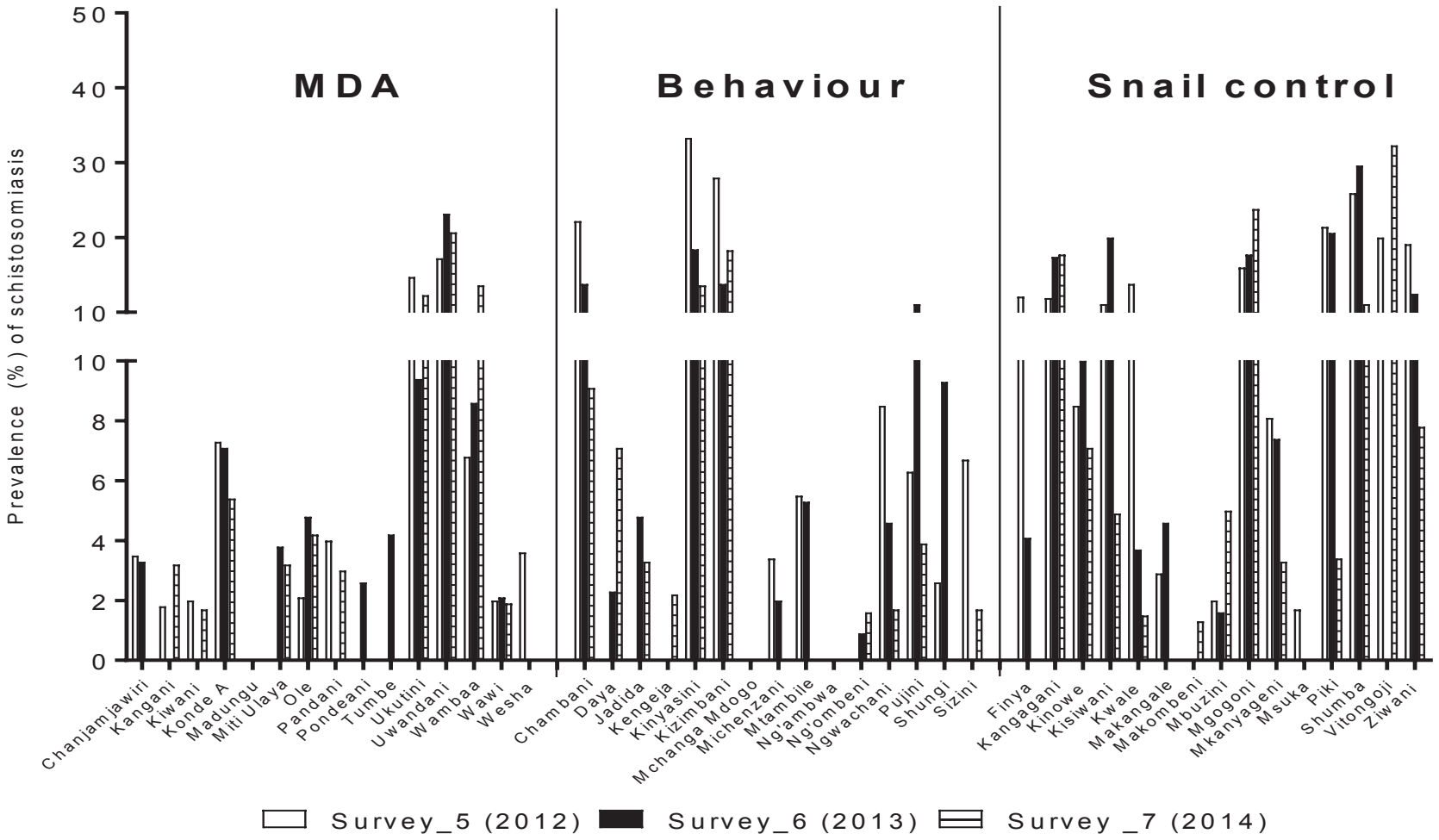
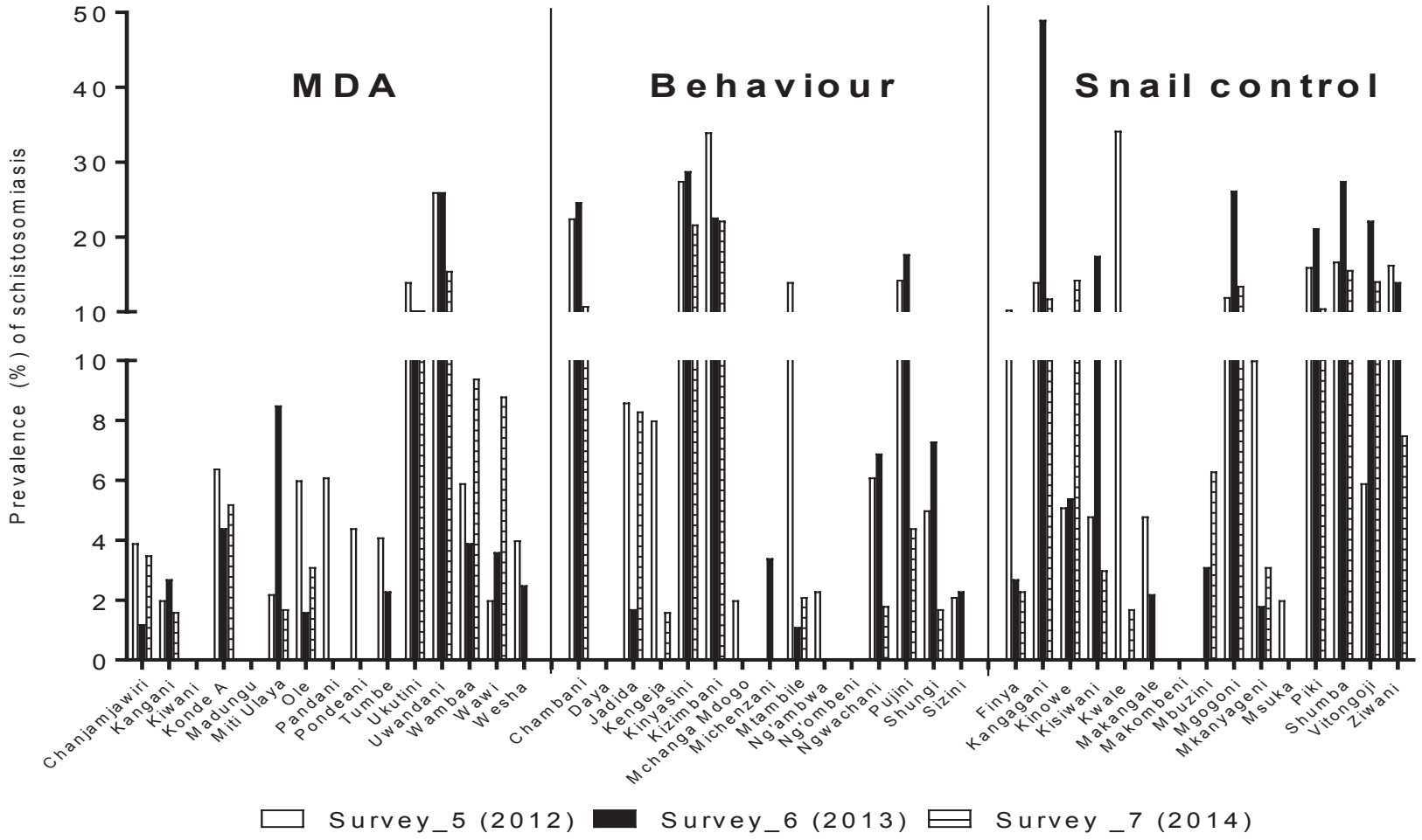


Figure 22: Prevalences for the 45 schools according to intervention strategy – Standard 4



3.5.1.4 Impact of the individual SCORE interventions on mean prevalences for the 15 schools in each of the intervention arms

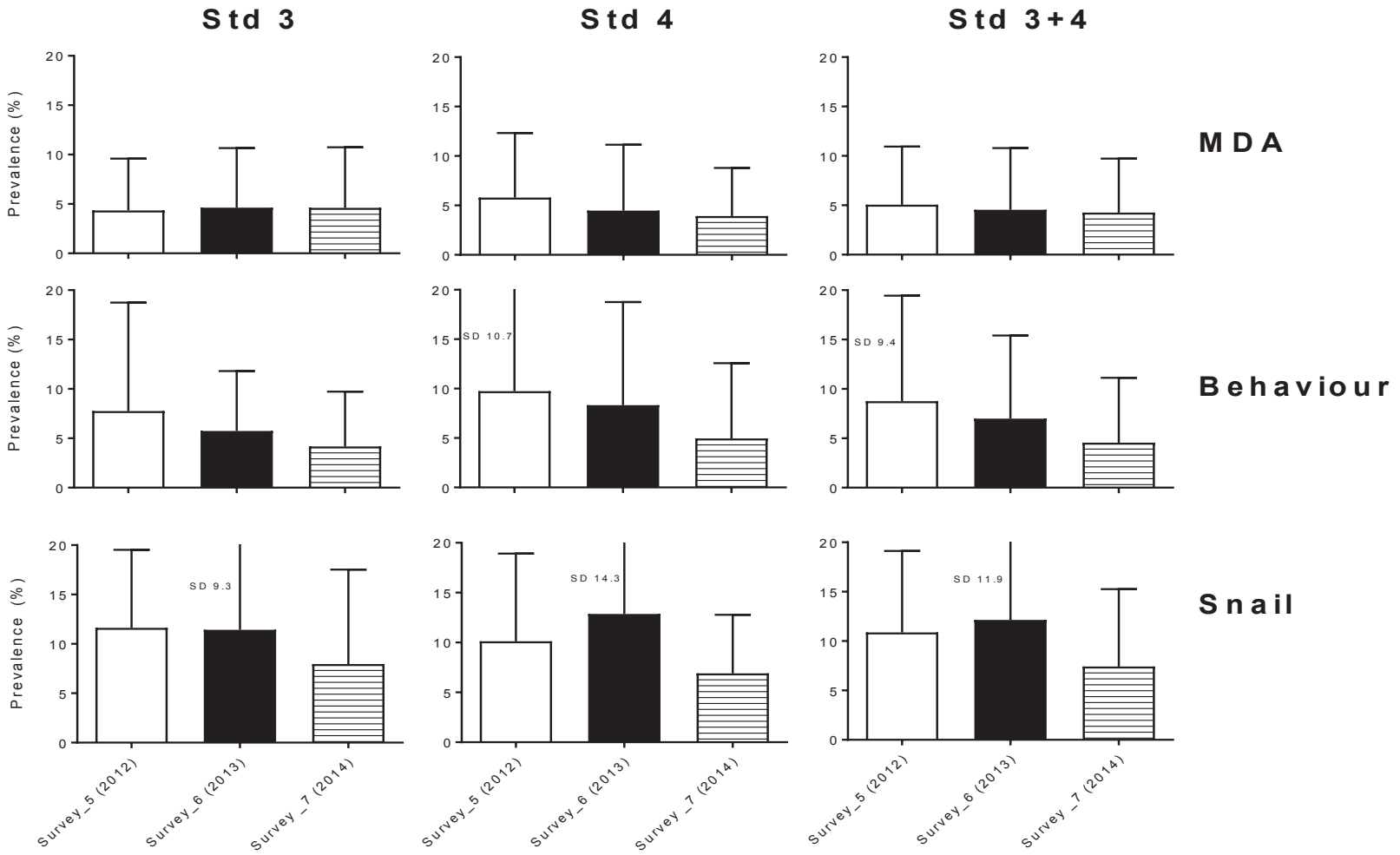
The mean prevalences from the 15 schools in each intervention arm of SCORE are shown in Figure 23. The mean prevalences at baseline for the schools randomized into the three intervention arms differed for Std3, Sd4 and the combined Std-3 and Std-4 values (which represent the combined prevalences for both Standards from each school and not the mean prevalences for the two Standards).

Std-3 cohort

The Std-3 children placed in the MDA arm had a baseline (2012) prevalence of 4.33% (95%CI =2.9-5.7) that was slightly increased to 4.61% (95% CI= 3.0-6.1) in 2013, although, the difference was non-significant (z = -0.28, p = 0.7; MH-$_{OR}$ 1.23 [95%CI = 0.76-1.99; p = 0.39]). For 2014, the prevalence was unchanged (4.60% [95%CI = 3.2-6.0]). The Mantel-Haenszel score test for trend of infection for Std-3 children in MDA arm when controlled by intervention and standard showed an equal odds ratio (MH-$_{OR}$ 1.0 [95%CI = 0.8-1.3]) i.e. the MDA had no significant effect on prevalence in this 3yr period (2012-14).

For the Behavioural Change intervention, the baseline (2012) prevalence of infection for the Std-3 children was 7.75% [95%CI = 5.82-9.67]. In the succeeding year (2013) this reduced to 5.74% (95%CI = 3.96-7.51) a difference which was not significant by z test (z = 1.49, p = 0.13) but the Mantel-Haenszel test was significant at the <5% level (MH-$_{OR}$ 0.56 [95%CI = 0.35-0.88; p = 0.01]). The trend for reduction of prevalence was also apparent in 2014 (4.16% [95%CI = 2.77-5.54]) but this was not significantly lower by either test (z = 1.39, p = 0.16; MH-$_{OR}$ 0.84 [95% = 0.50-1.40; p = 0.51]). The Mantel-Haenszel score test for trend of infection (2012-2014) when controlled by intervention and standard revealed a significantly lower odds ratio (MH-$_{OR}$ 0.68 [95%CI = 0.54-0.84; p = 0.0006].

Figure 23: Mean prevalences of schistosomiasis 2012-2014 for the 15 schools in each of the SCORE interventions



For the Snail Control arm, the Std-3 children had the highest baseline prevalence (11.62% [95%CI = 9.46-14.17]) relative to the other interventions. This prevalence was not significantly reduced in the succeeding year (10.62% [95%CI = 8.23-13.04]; z = 0.57, *p*=0.56; MH-$_{OR}$ 0.75 [95%CI = 0.52-1.06; *p*=0.11]). Prevalence declined a little more by 2014 (7.95% [95%CI = 6.1-9.7], although, the decline was not significant by z-score (z = 1.78; *p* = 0.07), or Mantel-Haenszel analysis (MH-$_{OR}$ 0.84 [95%CI = 0.58-1.21; *p* = 0.35]). However, the Mantel-Haenszel score test for trend of infection over the 3yr period (2012-14) revealed a lower odds ratio that was significant (MH-$_{OR}$ 0.79 [95%CI = 0.67-0.93; *p*= 0.007])

Std-4 cohort

At baseline (2012), the Std-4 children allocated to the MDA arm had a prevalence of infection of 5.79% (95%CI= 4.1-7.75) that was reduced to 4.46% (95%CI = 3.16-5.75) in the succeeding year (2013) but this was not significantly lower (z = 1.24, *p* = 0.2; MH-$_{OR}$ 0.67 [95%CI = 0.43-1.05; *p* = 0.08]). By 2014 prevalence was again lower at 3.92% (95%CI = 2.63-5.20) the reduction again being non-significant (z = 0.57, *p* = 0.56; MH-$_{OR}$ 0.99 [95%CI = 0.62-1.58]). Moreover, the MH score test for trend of infection for the Std-4 children allocated in the MDA group over the 3yr period (2012-14), when controlled with intervention and standard, revealed lower, but not significantly lower, odds ratio (MH-$_{OR}$ 0.81 [95%CI = 0.64-1.02; *p* = 0.08]).

For the Behavioural Change intervention, the Std-4 children had a baseline (2012) prevalence of infection of 9.74% (95%CI = 7.53-11.94). This prevalence was non-significantly reduced by 2013 to 8.32% (95%CI = 6.52-10.1) (z = 0.98; *p* = 0.32; MH-$_{OR}$ 0.98 [95%CI = 0.71-1.36; *p* = 0.91]). However, in 2014 the prevalence of infection was reduced to 4.96% (95%CI= 3.5-6.41), a significant difference by both statistical analyses (z = 2.8, *p* = 0.004; MH-$_{OR}$ 0.48 [95%CI = 0.33-0.70; *p* = 0.0001]). The Mantel-Haenszel score test for trend of infection for the period 2012-14 when controlled by intervention and standard also showed a significantly reduced odds ratio (MH-$_{OR}$ 0.71 [95%CI = 0.59-.0.85; *p* = 0.0002].

The Std-4 children in the Snail Control group had a high baseline prevalence (10.13% [95%CI = 7.83-12.42]) of infection which was, in fact slightly increased the following year (2013) (12.86% [95%CI = 10.75-14.96]; z = -1.69; *p* = 0.09; MH-$_{OR}$ 1.47 [95%CI

= 1.08-2.0; p = 0.01]). In the subsequent year (2014), the prevalence of infection was reduced to 6.91% (95%CI = 5.2-8.61) which, compared to the prevalence observed in 2013, was significantly lower (z= 4.2, p = 0.0001; MH-$_{OR}$ 0.44 [95%CI = 0.32-0.60; p = 0.00001]). Also the Mantel-Haenszel score test for trend of infection showed a significantly reduced odds ratio (MH-$_{OR}$ 0.81 [95%CI = 0.69-0.96; p = 0.01]) when controlled for intervention and standard for the period 2012-2014. See appendix 3.4A

Combined Std-3&4 cohort

For the Std-3&4 group, the baseline (2012) prevalence in the MDA arm 5.06% ([95%CI = 3.96-6.17]) was only slightly and non-significantly reduced to 4.54% [95%CI = 3.54-5.53;] (z = 0.7, p = 0.5; MH-$_{OR}$ 0.89 [95%CI = 0.64-1.23; p = 0.49] in 2013 and to 4.26% [95%CI = 3.3-5.2] (z = 0.3, p = 0.4; MH-$_{OR}$ 0.95 [95%CI = 0.68-1.31; p = 0.76]) in 2014. Furthermore, the score test for trend for the overall period 2012-2014 was also not significant (MH-$_{OR}$ 0.92 [95%CI = 0.78-1.08; p = 0.33]).

For the Behavioural Change intervention, the baseline prevalence for the Std-3&4 group was 8.75% (95%CI = 7.28-10.21) which was reduced to 6.99% (95%CI =5.72-8.25) in 2013. This change was non-significant (z = 1.3; p = 0.2; MH-$_{OR}$ 0.85 [95%CI = 0.65-1.10; p = 0.22]). In the subsequent year (2014) the prevalence was further reduced to 4.56% (95%CI =3.55-5.56) and this reduction in the prevalence was significant (z = 2.96, p=0.003; MH-$_{OR}$ 0.56 [95%CI = 0.42-0.76; p = 0.0001]). Overall the prevalence was 48% lower and the score test for trend of infection for the combined data was also significant (MH-$_{OR}$ 0.7 [95%CI = 0.61-0.81; p = 0.00001]) for a period of three years (2012-2014).

For the snail control arm, at baseline (2012), the prevalence was 10.88% [95%CI = 9.24-12.51] which subsequently increased to 12.14 (95%CI = 10.54-13.73) in 2013. However, the increase was non-significant (z = -1.07, p = 0.28; MH-$_{OR}$ 1.13 [95%CI = 0.91-1.42; p = 0.25]). Nevertheless, for 2014, the prevalence was significantly reduced (7.43% [95%CI = 6.20-8.65]; z = 4.60; p = 0.0001 (MH-$_{OR}$ 0.56 [95%CI = 0.44-0.71; p = 0.00001]). Moreover, the score test for trend of infection over the period 2012-14 showed significantly reduced odds ratio (MH-$_{OR}$ 0.8 [95%CI = 0.71-0.90; p = 0.0003]). See appendix 3.4B

Overall, the data indicates that the MDA alone had little impact on prevalence following either the 2012/2013 or the enhanced 2013/2014 interventions. However, it appeared that the Behaviour (plus MDA) interventions led to progressive reductions following each of the annual interventions. With the Snail control (plus MDA) interventions there was no sign of impact 2012-2013 but a more marked decline between 2013-2014.

3.5.2 Analysis of "Score cohort 45" - INTENSITY

3.5.2.1 Overall impact of the SCORE intervention on intensity

The simple arithmetic mean intensity is shown in Figure 24. The data shows the trend in mean egg count to be downwards over the study period. However, because the distribution of egg counts was highly skewed due to the high percentage of zero values in all of the data sets no statistical analysis is appropriate.

3.5.2.2 Intensity according to WHO classification.

When the *S. haematobium* infection intensity was classified into light (<50 eggs/10 ml of urine) and heavy (≥50 eggs/10 ml of urine), as defined by the World Health Organization (WHO), the percentages of heavy infections across all children for both Std-3 and Std-4 were 2.71, 1.83 and 1.71 for surveys 5, 6 and 7 respectively. But considering only those infected, the proportions of heavy infections were 32.4% at baseline, 22.3% for survey_6 carried out a year later and 31.4% for survey_7 carried out two years after baseline. Furthermore, looking at the proportions of heavy infection for Std-3 and Std-4 separately, these were comparable between the Stds and between the years.

Figure 24: Arithmetic mean intensity for children in all intervention arms

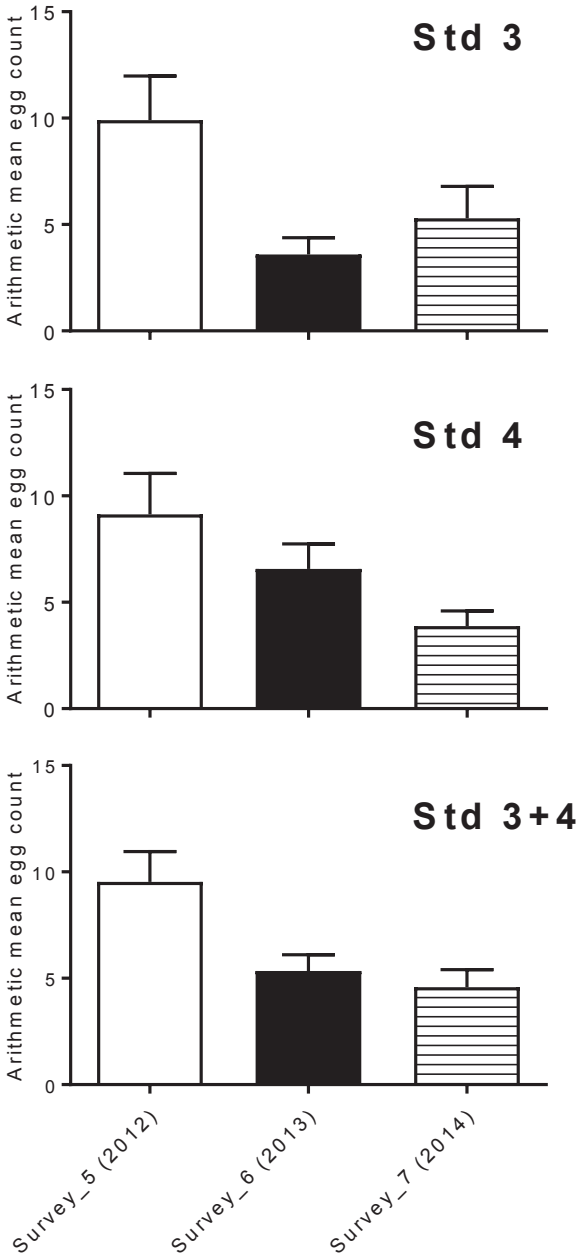


Figure 24 Graph shows arithmetic mean (±SE) intensity (eggs/10ml urine)

3.5.2.3 Overall impact of the SCORE intervention on intensity: data from the three individual arms for Std-3 and Std-4 children for the individual 45 schools

The mean intensities for the individual schools for Std-3 and Std-4 children across the three years are shown in Figures 25 and 26 respectively. The mean values were very low and the standard deviations high. Also the trends in mean intensities across the three years were variable amongst the schools and in some cases divergent between St-3 and Std-4 groups within the same school (e.g. for Konde A the intensity dropped in 2013 for Std-4 but not for Std-3 and for Pujini the intensity increased progressively in the Std-3 cohort but decreased in the Std-4). Regarding the diversity across schools, some showed consistently higher values in all years e.g. Ukutini, Uwandani, Chambani and Kangagani, others showed consistently low values e.g. Chanjamjawiri, Kangani, Kiwani, Tumbe. In other schools the intensities rose or fell in individual years. Overall though there was no obvious consistent trend seen for either a rise or fall in intensity.

Figure 25: Mean (±SD) intensity of infection from the Std-3 children for the individual 45 schools

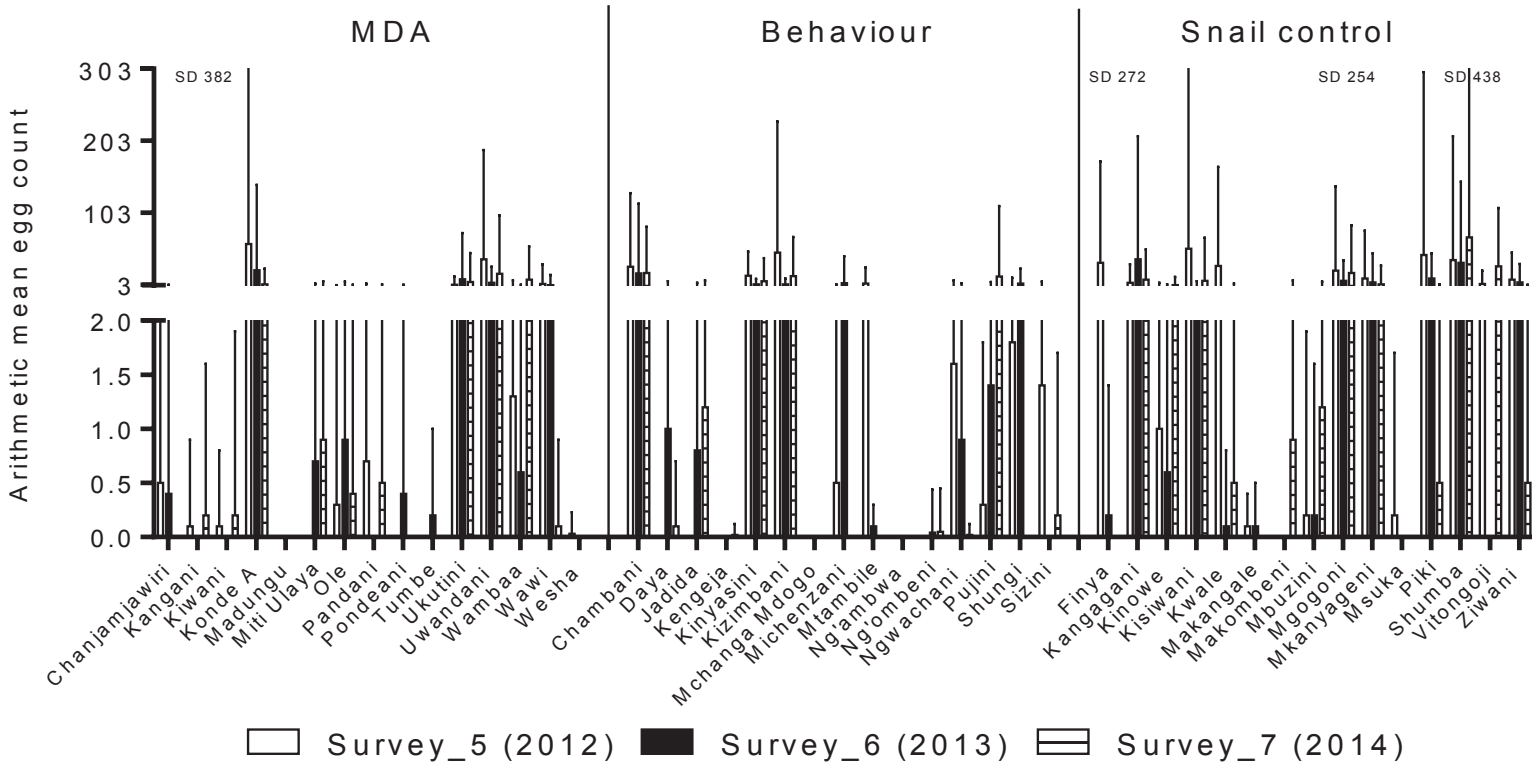
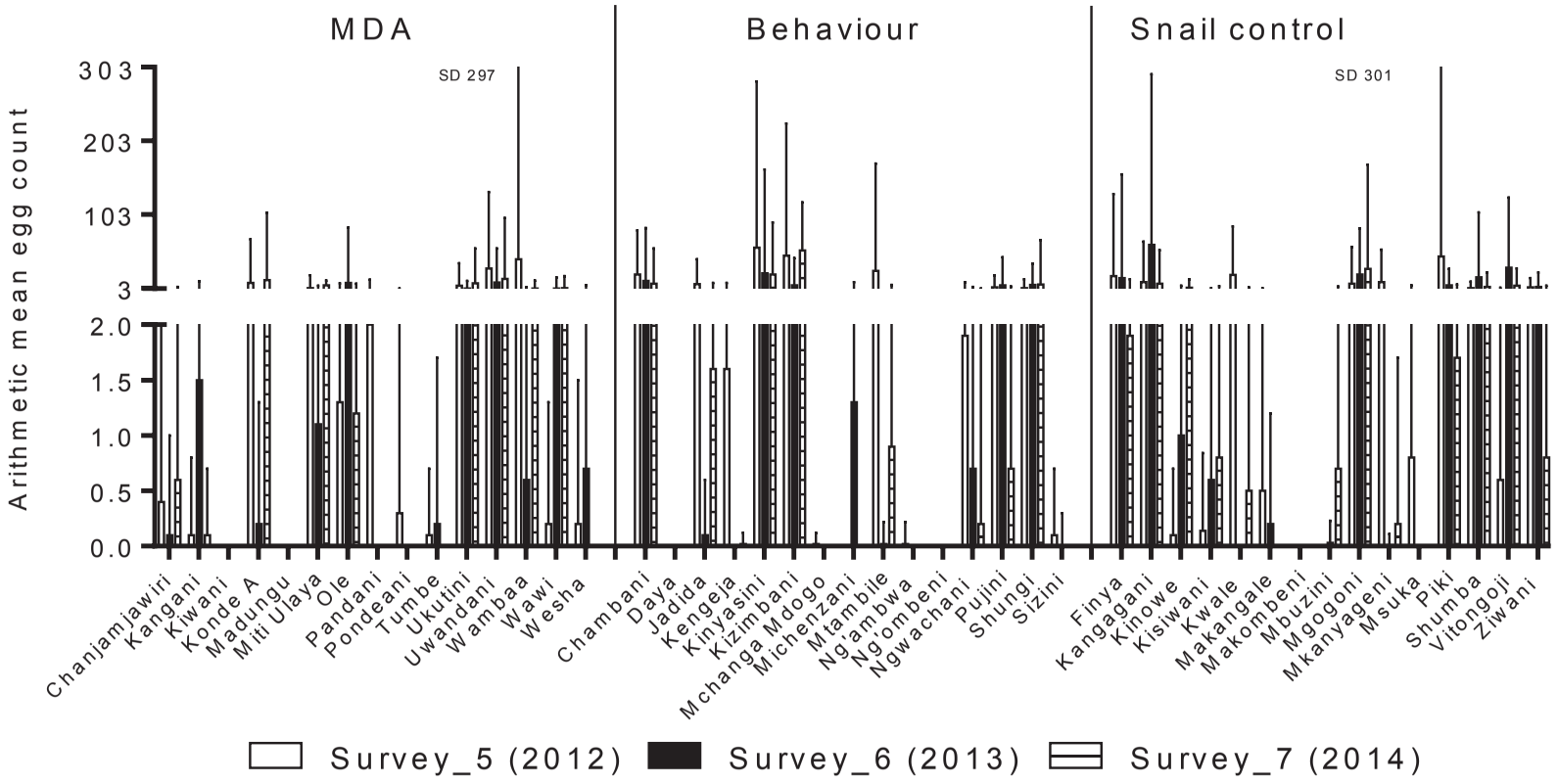


Figure 26: Mean (±SD) intensity of infection from the Std-4 children for the individual 45 schools



3.5.2.4 Impact of the individual SCORE interventions on mean intensity in SCORE Cohort 45.

The arithmetic mean values for all pupils by standard in each of the intervention arms are shown in Figure 27. It shows the mean intensities for the individual schools for Std - 3 and 4 children. Because of the high percentage of zero values in all of the data sets no statistical analysis is appropriate. Overall, at baseline, for Std-3 children, the mean intensity was almost two-fold higher (15.2 ± 115) in schools allocated to the Snail control arm but was comparable between MDA (7.7 ± 111) and Behavioural change (7.1 ± 58) intervention arms.

For the MDA arm both Std-3 and St-4 showed an overall drop in intensity between 2012 and 2013 and remained reduced at the 2014 survey. For the Behavioural change arm a very similar pattern of lower intensity after baseline was also seen. For the Snail control arm intensity also dropped over the three years despite an inconsistency in Std-4 initially showing an increase in 2013. Thus in general the mean intensities for children allocated to each of the intervention groups was progressively reduced as compared to baseline over a period of 2yrs and there was no evidence of major differences in the declines in the different interventions.

Figure 27: Intensity by standard and by SCORE intervention in Score Cohort 45.

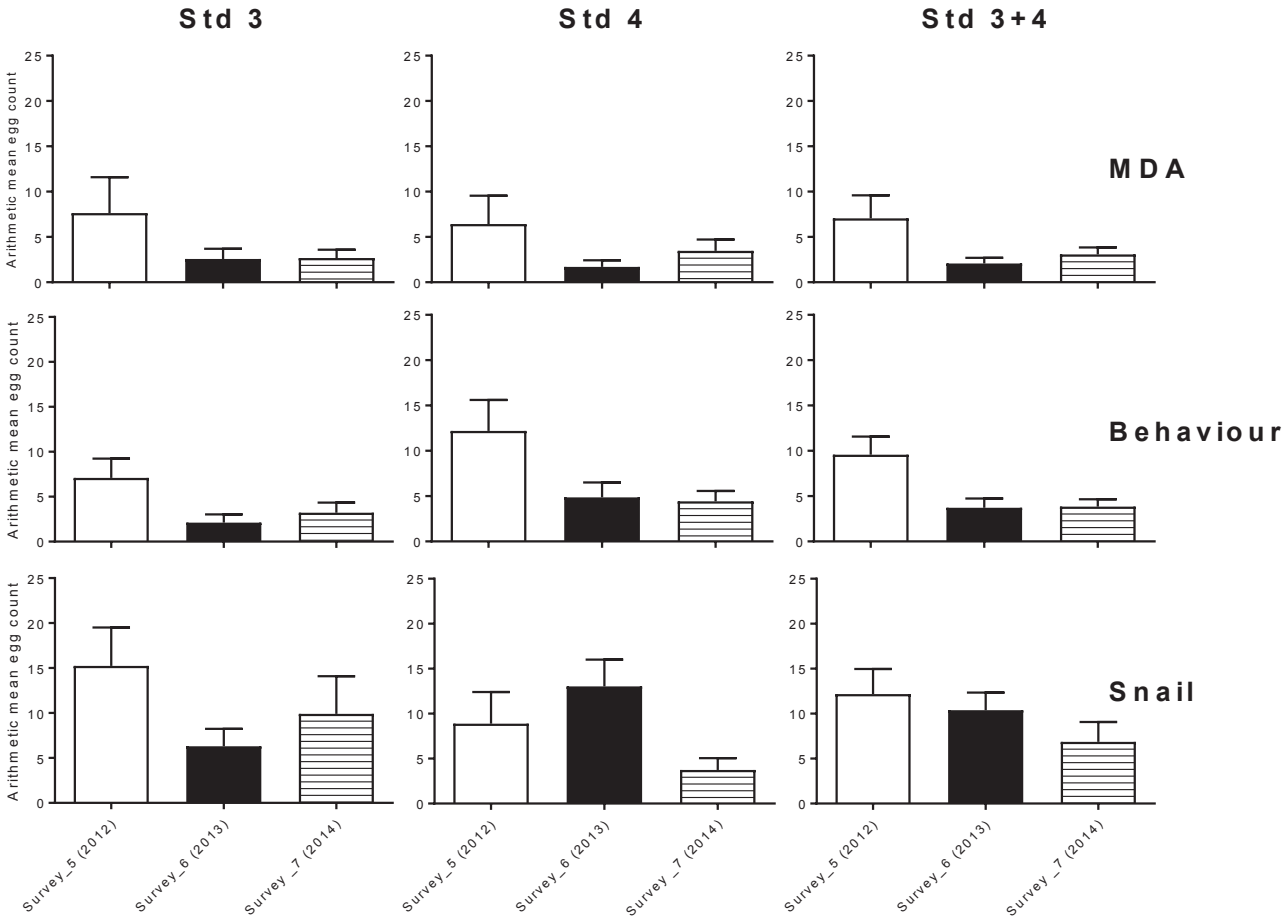


Figure 27 Mean (±SE) number of eggs/10ml of urine from individual children

3.5.2.5 Intensity by intervention strategy according to WHO classification. Considering intensity characterised as light (egg count <50/10ml of urine) or heavy (egg count ≥50/10ml of urine) in those infected, a higher percentage of light infections was consistently found (Table 3) with no markedly different trends between the different intervention arms.

Table 3: Percentages of children with light/heavy infections in the different interventions over 2012-14

Year	Intervention					
	Behaviour		MDA		Snail control	
	Light	Heavy	Light	Heavy	Light	Heavy
2012	(61.5)	(38.5)	(67.1)	(32.9)	(72.9)	(27.1)
2013	(83.7)	(16.3)	(82.9)	(17.1)	(72.1)	(27.9)
2014	(61.8)	(38.2)	(72.4)	(27.6)	(70.3)	(29.7)

Numbers in parenthesis are percentages

3.5.3 Assessment of the impact of MDA/SCORE using data from "Cohort 24" (surveys 1, 2 and 4):- PREVALENCE

Standard-1 children in the original 24 school cohort were tested at baseline in 2010, after one year of SBT with PZQ and again in 2013 after the two rounds of MDA in 2012. They provide a different data set from the above Score Cohort 45 (Stds 3 and 4) to look for any effects of the change in intervention policy.

3.5.3.1 Mean prevalence "Cohort 24".

The mean prevalence, before and after the introduction of MDA/SCORE, is shown in Figure 28. For comparison purposes data for the full 24 school cohort is shown in the top graph and its sub-set of 12 schools which only received chemotherapy (i.e. those schools which were allocated to MDA in the SCORE intervention) in the bottom graph. For Cohort 24 schools, at baseline, the prevalence of infection was (9.45% [95%CI = 7.78-11.11]) which increased significantly (z = -6.6; p = 0.0001); ($MH\text{-}_{OR}$ = 2.34; [95%CI = 1.81-3.04]; p = 0.00001) in survey_2 (18.85% [95%CI = 16.6-21.06]). However, the prevalence was significantly below baseline in survey_4 (2013) (7.2% [95% CI = 6.12-8.27] (z = 2.30; p = 0.02) following administration of two rounds each of SBT and MDA. Indeed, the score test for trend revealed significantly lower odds ratio ($MH\text{-}_{OR}$ = 0.87 [95% CI = 0.82-0.93]; p = 0.00001) over the period of 3yrs

Similarly, for the sub-set of 12 schools, the baseline prevalence was 6.67% (95%CI = 4.63-8.69) which was significantly increased to 15.97% (95%CI = 14.95-18.91); (z = -5.04; p = 0.00001) in survey_2. This prevalence (15.97% in 2011) was significantly (z = 6.85; p = 0.00001) reduced to 5.77% (95%CI = 4.37-7.16) in subsequent survey_4, although this prevalence was similar to that demonstrated at baseline. Furthermore, for the cohort of /12 schools, the score test for trend revealed significantly lower odds ratio ($MH\text{-}_{OR}$ = 0.86 [95% CI = 0.77-0.973]; p = 0.00001) over a period of 3yrs. So, generally, in both cohort 24 schools and its sub-set of 12 schools, the baseline prevalences were significantly increased in survey_2 but significantly reduced subsequently, though in some occasion, the reduction was somewhat similar with baseline.

Figure 28: Mean prevalence across the 24 school cohort

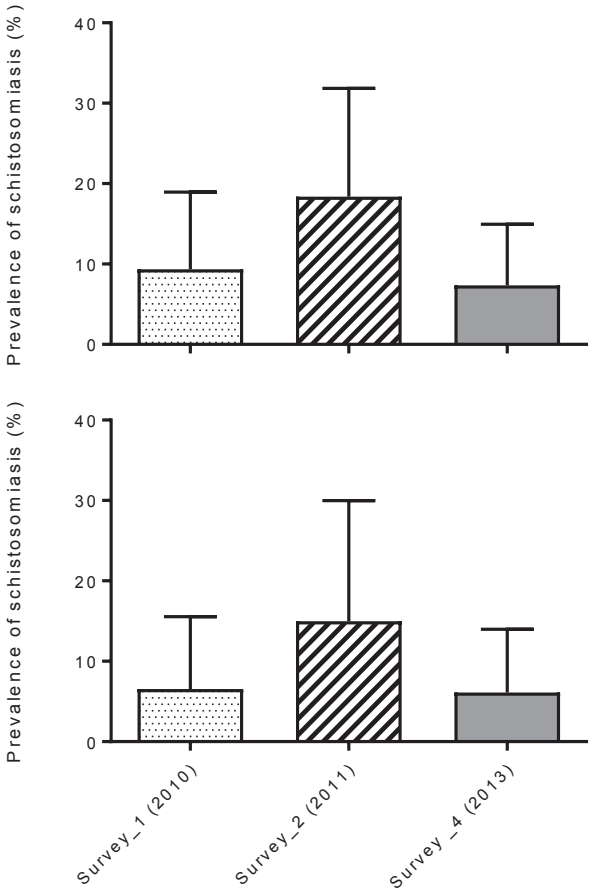


Figure 28 Mean prevalence of all schools in (i) the 24 school cohort (top graph) and (ii) the 12 schools which were only subject to the two different chemotherapy regimens i.e. the annual school based interventions in 2010 and 2011 and the bi-yearly MDA in 2012.

3.5.3.2 Mean prevalence “Cohort 24” by area

As shown in figure 29, at baseline (survey_1 2010), the mean prevalence of infection was highest in Mkoani district (14.67% [95% CI = 10.6-18.7]) and lowest in Micheweni (1.67% [95% = 0.2-3.1]). In the subsequent survey carried out a year later (survey_2 2011) following administration of one round of SBT the prevalence was quite markedly increased in all districts although the pattern of prevalences remained essentially similar to the baseline. Notably, in Micheweni district the infection increased to almost seven-fold (from 1.67% to 11.46%) ($z = -4.87$; $p = 0.00001$) as compared to the year before. The reason for the particular increase of infection was not apparent. The area is flat and sandy with relatively few transmission sites (water bodies) and there was no reported unexpectedly high rainfall which could result in formation of temporary water bodies or other ecological change that could facilitate transmission of the infection. In survey_4 2013 carried one year later after introduction of twice/yearly MDA, the mean prevalences in all districts were noticeably reduced and were more comparable between districts than at baseline.

Figure 29: Prevalence of schistosomiasis pre and post MDA/SCORE by area

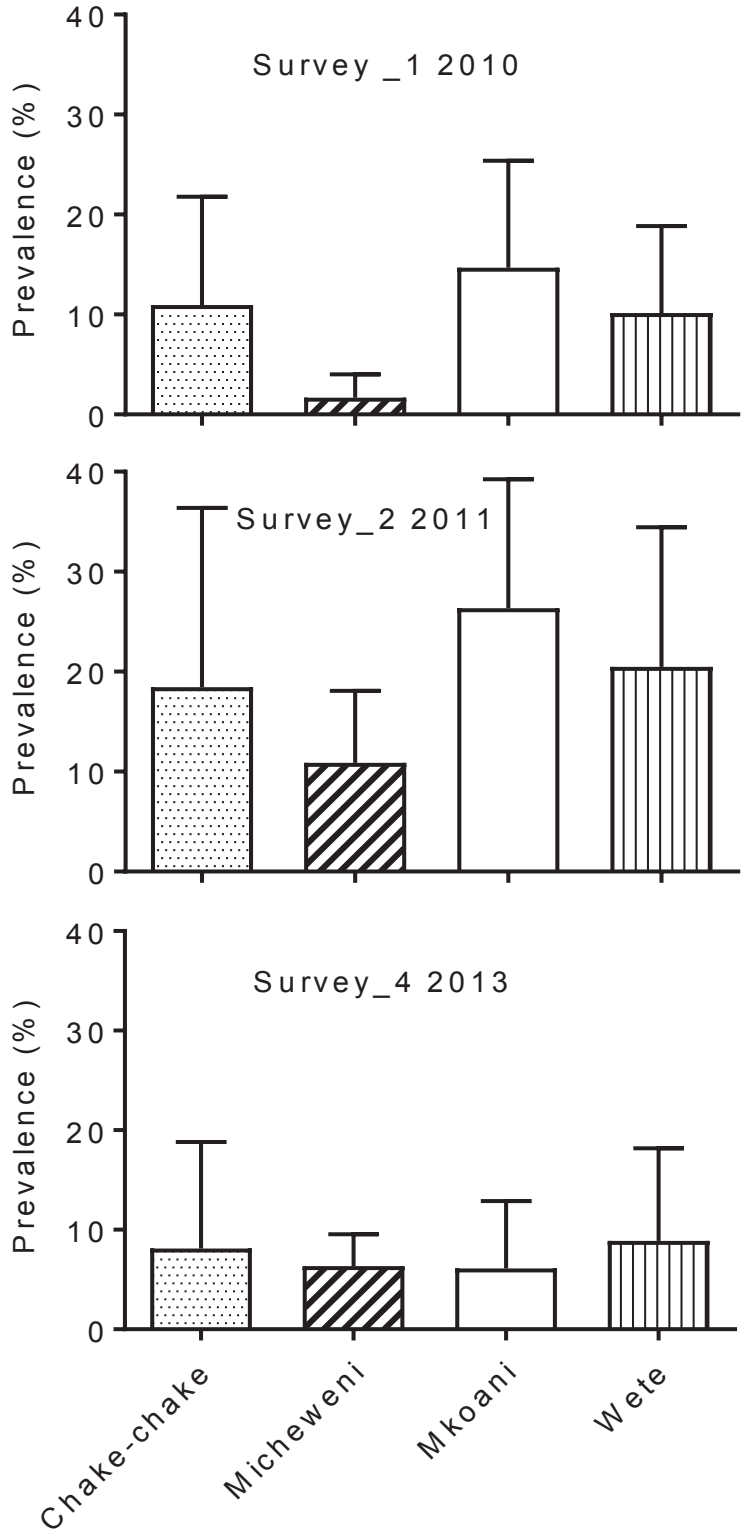
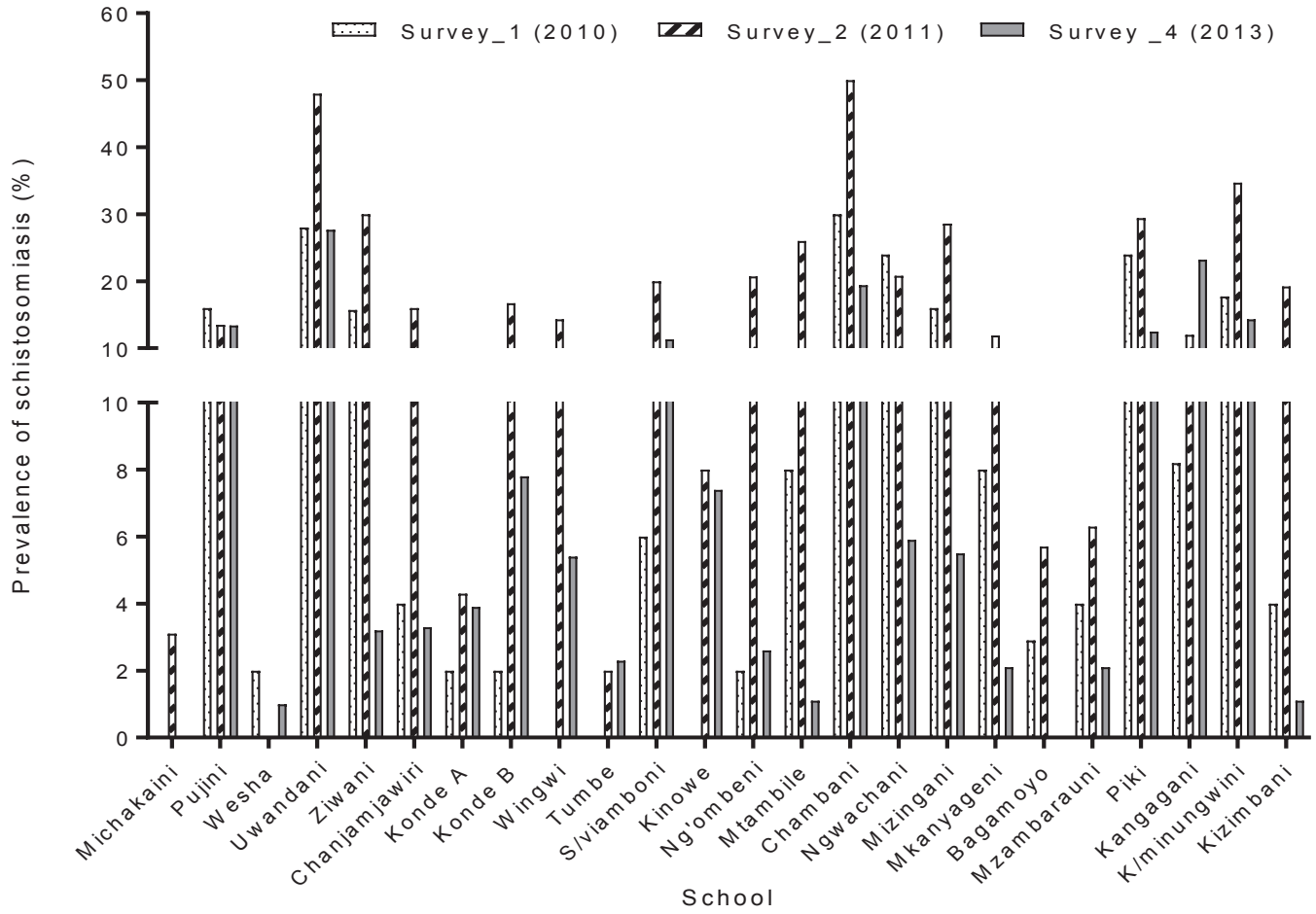


Figure 29 Mean prevalence (±SD) of Standard 1 children in "Cohort 24" by area.

3.5.3.3 Individual school prevalence

Figure 30 shows the prevalence data for each of the 24 schools over this period. As shown in Figure 8 and Appendix 3.1 some of these 24 schools were included in the different arms of SCORE but all would have been given chemotherapy. It can be seen that, overall, prevalence varied between and within the schools over time, some of the schools at baseline showing low and even zero prevalence while others had prevalences close to 30%. Although some schools showed consistently high prevalence over the study period e.g. Pujini, Chambani, Piki, Uwandani, S/viamboni and K/minungwini others showed consistently low prevalences e.g. Michakaini and Tumbe. In almost all of the schools, prevalence recorded at baseline was somewhat increased in survey_2 and then decreased in survey_4 and so, applying Mantel-Haenszel analysis to assess the trend of odds of infection over the years when controlled by schools, there was a significant odds ratio ($OR\text{-}_{MH}$ = 0.83 (95%CI = 0.77-0.89; p = 0.00001) (Appendix 3.2)

Figure 30: Individual school prevalences for Std-1 children in the 24 schools (2010-2014)



3.5.4 Assessment of the impact of MDA/SCORE using data from "Cohort 24" (surveys 1, 2 and 4):- INTENSITY

3.5.4.1. Intensities for the individual schools 2010-2013

The data on intensities for the individual schools is shown in Figure 31. No consistent trend is seen across the schools. In the majority (16/24) of schools mean intensity was reduced somewhat between 2011-13 although in most of the schools showing the highest intensities there was little change across the study period e.g. Pujini, Uwadani, Chambani, Mizingani.

3.5.4.2. Intensity by intervention strategy according to WHO classification.

As mentioned previously some of the Cohort 24 schools were included in the 45 schools involved in the ZEST/SCORE project and so they were allocated into different interventions strategies but also some of the schools were not included. Thus since MDA was administered across all schools despite whether they were involved or not in the ZEST/SCCORE project, for the purpose of this analysis, the schools not involved in the ZEST/SCORE project were allocated in the MDA intervention data.

Considering intensities as light (egg count <50/10ml of urine) or heavy (egg count ≥50/10ml of urine) (Table 4) clearly showed that overall many of the infected children had light intensity across the different interventions over the years but there was some variation with time in different intervention groups. For the Behavioural change group the proportion of heavy intensity infections was progressively increased while for the MDA alone heavy infections declined

Table 4: Proportion of intensities in Std-1 children in cohort 24 schools from 2010-2013 relative to different intervention strategies

Year	Intervention strategy					
	Behaviour		MDA		Snail control	
	Light	Heavy	Light	Heavy	Light	Heavy
2010	(66.7)	(33.3)	(61.5)	(38.5)	(80.6)	(19.4)
2011	(61.1)	(38.9)	(64.6)	(35.4)	(81.0)	(19.0)
2013	(48.8)	(51.2)	(74.2)	(25.8)	(82.1)	(17.9)

Numbers in parenthesis are percentages

Figure 31: Intensity for each of the “Cohort 24” schools 2010-2013

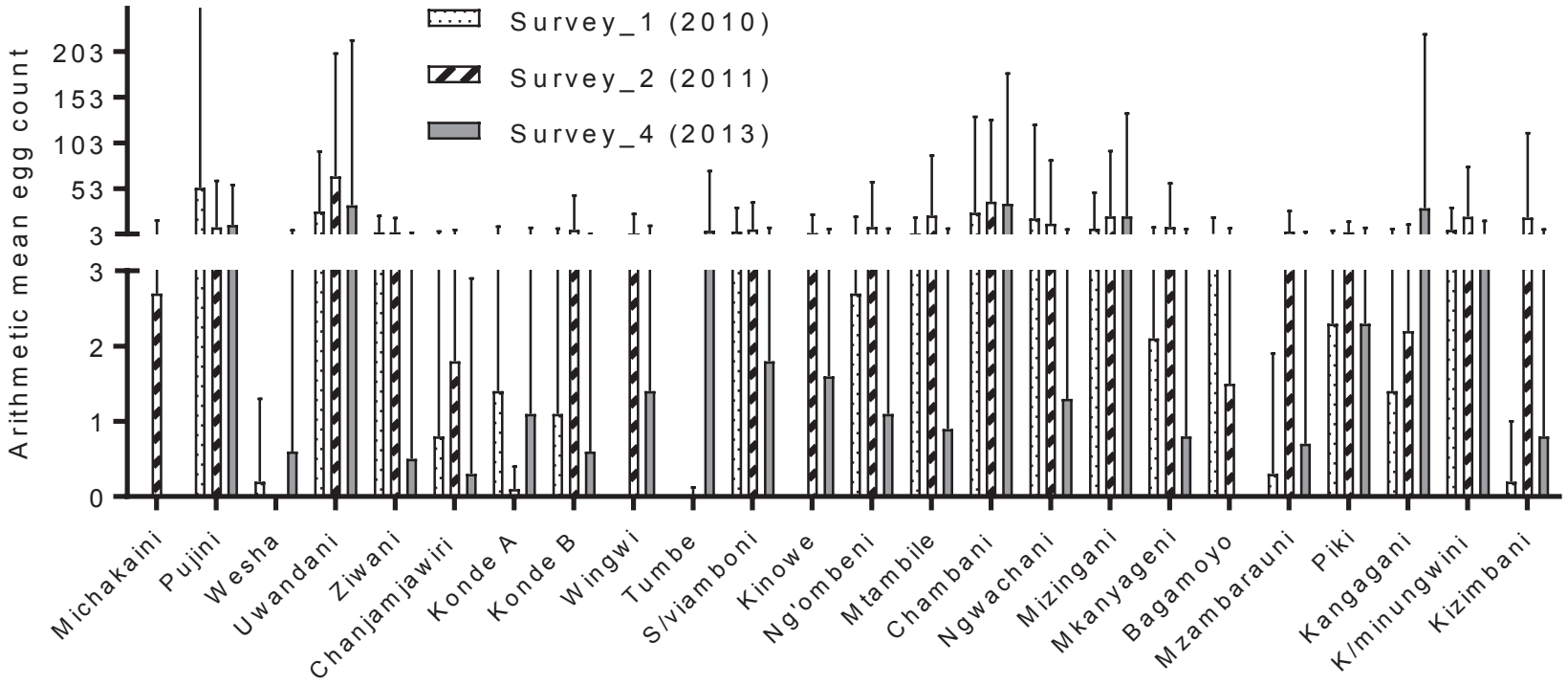


Figure 31 Arithmetic mean egg counts (eggs/10ml urine) from Std_1 children in each of the 24 schools following SBT in 2010 and 2011 and MDA (±other SCORE interventions) in 2012.

3.5.5 Impact of the introduction of the MDA compared with SBT on prevalence and intensity in "SCORE Cohort 5" surveys 1, 2, 5, 6 and 7)

Within "Cohort 45" there were 5 schools which were included in the original "Cohort 24" schools sampled during the SB treatment period (2010-2012) (surveys 1, 2 and 5) and which were also included in the MDA only arm of MDA/SCORE and so subject to the more intensive MDA after MDA/SCORE introduction. These give the opportunity to compare the infection levels following the successive SBT and MDA interventions. Standard-1 children were sampled in surveys_1 (baseline), 2, 5 (after successive annual SBT) and 4 (post one round of MDA) and Standard 3 children were sampled in surveys 2 (baseline), 5 (after one round of SBT), 6 and 7 (after one and two annual rounds of MDA, respectively).

3.5.5.1 Assessment of prevalence

The prevalence data for the 5 individual schools from 2010-2014 is shown in Figure 32. No consistent changes in prevalence over the period of the interventions are obvious for the individual schools with the exception of the data for the Std-3 children from Chanjamjawiri which suggested a persistently reduced prevalence following baseline. However, this was not reflected in the Std-1 data for the same school. Although the prevalence values vary over time for all of the schools, there is no consistent trend for values to be lower following the initial SBT interventions or following the subsequent MDA. Across the period as a whole, Mantel-Haenszel analysis to assess the trend of odds of infection over the years did not show a significant odds ratio ($MH\text{-}_{OR}$ 0.92 [95% CI = 0.77-1.11; p = 0.38]) (see appendix 3.3A). However, for Std-3 children, the Mantel-Haenszel test for trend of odds of infection over the years did produce a significantly lower odds ratio ($MH\text{-}_{OR}$ 0.79 [95% CI= 0.64-0.97]; p = 0.02) (see appendix 3.3B).

Overall there was no evidence from these 5 schools that the switch from SBT to MDA led to a reduction in prevalence over the period of study.

Figure 32: Prevalence of schistosomiasis during SBT and then MDA in each of the “Cohort 5" schools.

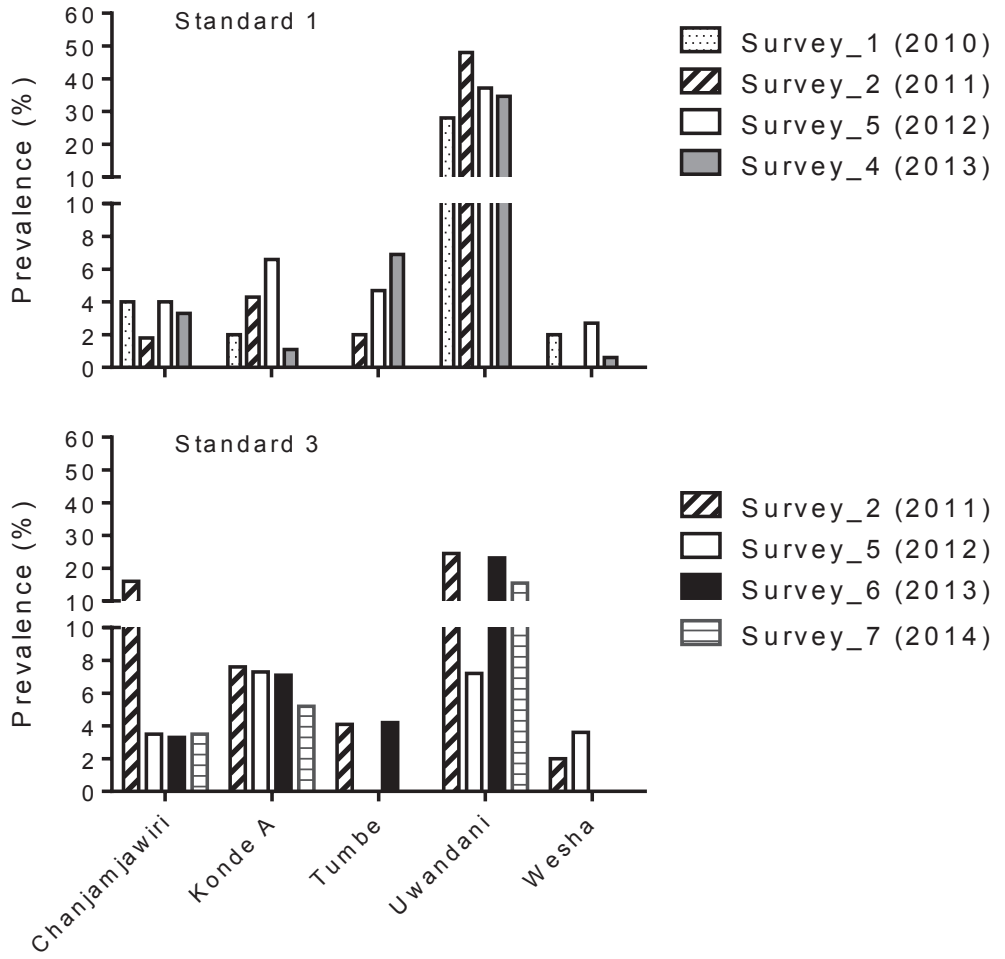


Figure 32 Prevalence in each of the “Cohort 5” schools.

3.5.5.2. Assessment of Intensity

Figure 33.shows the mean school intensities for the Cohort 5 schools. As with the prevalence there was an indication of slightly lower intensity following baseline for the Std-3 from Chanjamjawiri but as with prevalence this was not seen with the Std-1 children from this school. Overall there was no consistent pattern of change across the schools and, in general egg counts were broadly similar at the end of the intervention periods as at baseline e.g. for both Std-1 and Std-3 in Uwandani. Overall, as with the prevalence data, there was little evidence from these 5 schools that the implementation

of SBT or the switch from SBT to MDA led to reductions in intensity over the period of study.

Figure 33: Comparison of Intensity for Cohort 5 during SBT and MDA

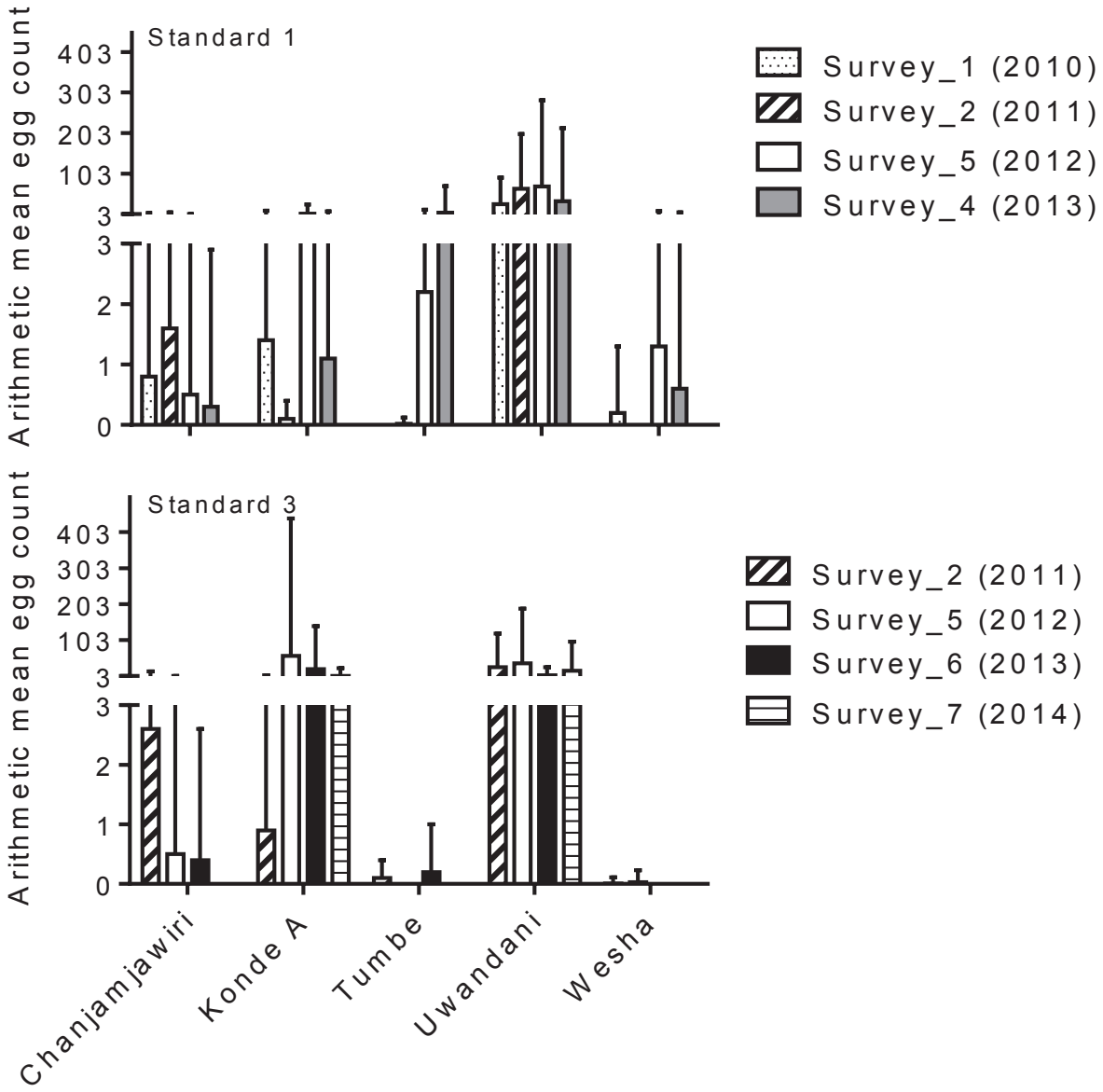


Figure 33 Mean ± SD egg counts for the SCORE Cohort 5 schools for Standard 1 and Standard 3 children

3.6 Discussion

Chemotherapy based control for schistosomiasis using PZQ is the fundamental approach advocated by the WHO and is adopted by most countries where these infections are prevalent. Generally, the chemotherapy is either targeted to school-children or community based MDA. Such interventions are often regarded as aiming to control morbidity rather than significantly reducing transmission. Both approaches have been used in Pemba but the relative impact of these on transmission has not been formally compared and this was one of the aims at the start of this study in 2010 since SBT was to be reintroduced then after a period of three years without routine treatments and also there was a proposal to implement a new National Control Programme in 2012 involving more intensive treatment i.e. MDA twice yearly. In fact the baseline survey in 2010 reported here showed a prevalence of *S. haematobium* of only 9.5% and so Zanzibar was considered and selected for inclusion in the newly proposed SCORE programme aimed at evaluating the use of other control methods (snail control and education) in addition to chemotherapy to progressively reduce transmission with a view to elimination in selected countries with schistosome prevalences of ≤10%. So the original study was expanded to evaluation of the different arms of this intervention. The study was envisaged to include various aspects of the monitoring of the implementation and impact of these control measures including human infection and disease, snail populations/infection levels, coverage and compliance, and drug efficacy.

3.6.1 Impact of chemotherapy

Schistosomiasis is a major public health problem across tropical and sub-tropical countries including Zanzibar. Current figures show that over 207 million people worldwide have schistosomiasis and many more are at risk of infection (Webster et al., 2009). In endemic countries, children aged between 5-15yrs are the most infected population because of their tendency of frequent contact with fresh water that in turns increase exposure to the infection. Furthermore, there is evidence of development of resistance to reinfection from mid-teenage years (Pinot de Moira et al., 2010, De Moira et al., 2010). For this reason, most countries engaged in the control of this devastating disease focus on MDA targeted at school-aged children with provision of praziquantel (40 mg/kg). Furthermore, school-children are easily accessible (Brooker et al., 2004).

The frequency of drug provision whether annual or biannual remains the choice of individual countries although the WHO proposed guidelines for the frequency of drug distribution and measures to be taken depending on the prevalence of the infection and methodology used (WHO, 2006). Such chemotherapy-based control has been reported to be successful in reducing disease morbidity (Savioli et al., 2004a), prevalence (Ming-Gang, 2005) and reversing pathology (Kabatereine et al., 2007, Brooker et al., 2004). Using mathematical modelling, it has been postulated that focusing control of schistosomiasis through school-aged children may interrupt and possibly eliminate transmission (Muchiri et al., 1996). The present study started with evaluating the impact of single annual treatment for the control of schistosomiasis in school children in Pemba based on parasitological data. To our knowledge this is the first formal study of this approach to be carried out in this setting.

3.6.2 Baseline characteristics and the effects of single annual SBT

The initial baseline monitoring in 2010 involved sampling of the Std-1 children who had not been mass treated previously and who therefore gave an indication of transmission in the community. By follow up in 2011 any impact of the single annual SBT on this transmission could be evaluated. In addition in 2011, by comparing the Std-1 and Std-3 children (who had had a single school based treatment one year previously) the impact of this on infection a year afterwards could be evaluated and compared.

The prevalence observed at baseline was 9.5%, the majority (67.9%) having light infections. This was surprising since, although the intensive (annual, community based) MDA, involving triple therapy with provision of ALB, ivermectin and PZQ in 2004-2006 had reduced the prevalence from 63% to 18% by 2007 (Guidi et al., 2010), there had not been any systematic drug treatment between 2006-2010 and it might have been expected that prevalence would have rebounded from the 2007 level. A similar trend of remarkable reduction (76.3%) [54.1% in 1986 to 12.8% in 1988] in haematuria was demonstrated in the earlier attempts to control the disease by selective chemotherapy in the late 1980s (Savioli and Mott, 1989). The observation of 9.5% prevalence was encouraging but the real cause for this apparently persistent low level infection since 2006 in the absence of efficient intervention such as snail control, intensive chemotherapy or effective behavioural change is unclear. Multiple possible factors

might have contributed to this reduction: increasing availability of safe water supplies in some villages where the investigated children come from; relatively reliable power supplies; increasing awareness of schistosomiasis and accessibility of PZQ leading to change of health seeking behaviour; or realization of the health impact of the disease. Also artesunate derivatives for the treatment of uncomplicated malaria were introduced and intensively used since late 2003 (Froberg et al., 2012) and have been shown to have a potent killing effect on schistosomes albeit at higher dose levels than usually used for malaria (Utzinger et al., 2001)..

However, despite the low prevalence in 2011, the prevalence determined at follow-up in the same Std-1 age group the following year after a single annual SBT showed the prevalence of schistosomiasis to be 18.9%. Similarly the number of the children with either light or heavy schistosomiasis was higher than that of the 2010 baseline even after stratification by class. For example, 73 (73%) of the Std-1 children had severe infection in 2011 as compared to 36 at baseline (32.1%). The reasons for this apparent rebound is unclear as there had been no change in health systems or climate e.g. increased rainfall that could have resulted in increased transmission sites. The fact that the 2011 estimate of 18.9% was similar to the previous most recent estimate of 18.9% in 2007 (Guidi et al., 2010) might be considered to raise questions about the reliability of the 9.5% value obtained in 2010. However, the sample sizes were large in both studies (n= 1531 in 2007; n= 2372 in 2011) and the same personnel and techniques were involved in the surveys headed by Shaali. Furthermore, the baseline for the SCORE study (see below) in 2012 showed comparable prevalences to that seen in the 2010 baseline for Std-1 children.

Despite the anomaly of the prevalence actually being higher in 2011 than in 2010 it is clear that the SBT did not result in lower prevalences in the Std-1 children attending school for the first time in 2011. It should be emphasised however, that the only potential impact the SBT in 2010 could have had would be to reduce environmental contamination of the 2011 Std-1 cohort in the year before they started school. It is perhaps unsurprising that this did not reduce worm burdens which would have accumulated in the 2011 Std-1 intake over their preschool years. There was however, some evidence that the SBT had a prolonged effect on the 2010-treated children themselves in that the prevalence in the Std-3 children in 2011 who would have also been treated in 2010 were significantly lower than that in the previously untreated Std1

children (11.4% cf 18.5%). However, the percentage difference was markedly lower than had been seen in earlier longitudinal studies whether monitoring haematuria or prevalence e.g. Savioli, Dixon et al. 1989 working in the same epidemiological setting (Savioli et al., 1989b) observed a remarkable reduction in both micro- and macro-haematuria, indicators of urinary schistosomiasis, following one year of selective chemotherapeutic (praziquantel) intervention (15.8→0.9% for macro-haematuria and 54.1→12.8% for micro-haematuria). It is also evident that as in recent years there has been some improvement in terms of availability of piped water supply, electricity, secio-economic aspects of individuals and school attendance which in some ways have contributed to the reduction of the prevalence of schistosomiasis compared to 1988 when the earlier studies were carried out. However, a similar observation was also reported in Kenya (Magnussen et al., 1997). Higher reductions were also reported by others (Toure et al., 2008, Koukounari et al., 2007, Tohon et al., 2008) who demonstrated 89.6% (from 59.6% to 6.2%), 89.2% (from 53.9% to 5.8%) and 49.6% (75.4% to 38%) decreases in prevalence after one year of praziquantel administration, respectively.

Infection intensity is the principal indicator in evaluating the effectiveness of schistosomiasis control programs based on chemotherapy (Brooker et al., 2004) since intensity is a measure of morbidity and a more sensitive indicator of worm reductions. In our study we observed progressive increases in overall mean intensity for the Std-1 children over the years (2010-2012) and also for the Std-3 during the period of 2011-2012, although, in both occasions, the majority of the infected children had light intensities. This was in contrast to what was observed in the above published longitudinal studies which showed significant decrease in the intensity of infection after few years of the program implementation (Koukounari et al., 2007, Toure et al., 2008). The Std-3 (older) children had lower overall mean intensity in each time period (2011-2012) which may be due to related to the fact that the Std-3 children had been previously treated at least twice in 2010 and 2011 during the SBT. Studies have shown that older age is associated with lower intensity (Etard et al., 1995) which could be explained by acquired immunity.

So overall the two years of SBT had no apparent beneficial effects on prevalence or intensity. However, the apparently modest impact of PZQ seen in the Std-3 children was not due to poor PZQ efficacy in Pemba as discussed below.

3.6.3. The effect of integrated control measures on prevalence of infection

From 2012 onwards (at least until this present study was completed), the NCP changed its approach and extended it to evaluate the possibility of elimination of schistosomiasis through implementation of integrated control measures. This involved introducing MDA twice a year, alone or in combination with Behavioural changes intervention or Snail control in defined areas.

In the Behavioural modification intervention, the "community co-designed process, based upon human-centred design" approach was implemented (Person et al., 2016). In this approach, community members identified their own problems and participated in designing related interventions which included school-based education and training, safe play activities, installation of community-designed washing platforms and installation of community-designed urinals (for both boys and girls). However, the urinals were found to be culturally unacceptable, complicated by various local factors and hence were not utilized so this strategy was abandoned by the community members. In all of the above-mentioned strategies, the main focus was directed to school-aged children.

The Snail control activities consisted of identification of fresh water bodies (in the intervention areas) and intensive snail searching followed by application of molluscicide, niclosamide (70% wettable powder (WP)). It was initially planned to apply niclosamide twice/year to coincide with PZQ distribution in communities (Knopp et al., 2012) but for some reasons that was impossible. It was realized that the Snail control team could spend little time in the field such that it could be difficult to achieve the desired outcomes and so it was decided to increase niclosamide application period to a total of ~8 months/year- which started from August-March each year.

For operational purposes, searching for snails as well as application of niclosamide were halted during the heavy rain seasons between April-July and resumed from August to March each year. Moreover, the application of niclosamide was focused i.e. was only applied in contact areas (areas with human activities) within the water bodies (Knopp et al., 2012). The focal application of niclosamide usually happen in large water bodies and this strategy has been found as one of the major challenge in snail control (Fenwick et al., 2006a).

The overall new baseline prevalence for this expanded study was based on evaluation of Std-1, 3 and 4 children and found to be 10.1%, the Std-1 children having a somewhat higher prevalence (12.4%) than Std-3 (7.9%) or Std-4 (8.8%) alone or the combined data between Std-3 and 4 (8.3%) which is consistent with the older children having had previous SBT. During the subsequent follow-up a year later, the Std-1 children were not sampled but the overall prevalence for the Std-3 and 4 was almost similar (8.2% [95%CI = 7.5-9.0]) with that of baseline. This disappointing finding led to changing the PZQ treatment strategies - (i) SBT to all school-aged children at school (ii) community-based MDA to pre-school-aged children and also adults (iii) provision of the SBT and MDA twice/year. This treatment approach in combination with other intervention strategies yielded a more marked reduction in the prevalence in subsequent years as detailed further below.

In the follow-up survey carried two years later after the baseline and a year after the move to twice-yearly SBT and MDA, the overall prevalence in Std-3&4 across all arms was significantly reduced to 5.31% (95%CI = 3.92-6.70) following implementation of integrated control measures for schistosomiasis (MH_{OR} = 0.8 [95%CI = 0.74-0.87]; (p = 0.0001]). However, the three different interventions showed quite marked differences in impact. The MDA alone only reduced the prevalence by 16% compared to baseline and this difference was not statistically significant.

Unlike the MDA alone, the combination of MDA with behavioural change and snail control appeared to enhance prevalence reduction (Fig 3.3.5). In the case of the behavioural modification arm, prevalence was reduced following the first year of intervention and further reduced after the second year giving an overall reduction or 48% over the two years. In contrast, for the snail control cohort, prevalence actually increased between baseline and the first year of intervention before subsequently declining in the following year. Overall prevalence for snail control was 32% lower over the two years. Whether these patterns reflect real differences in the speed of impact of the different approaches is unknown. The snail control and behavioural change interventions were initially implemented from Mid-August, 2012 just a few months before undertaking the first follow-up survey in 2013 giving little time for either to have a significant impact on prevalence. However, the results over the two years indicate the greater effectiveness of the integrated approach for schistosomiasis control. The validity of this conclusion will be tested following the ongoing control and evaluation as part of

SCORE. This further work will also determine if the decline is maintained and establish the rate of decline which is crucial in establishing whether such interventions could lead to elimination in such a setting as Pemba.

Although the results to date in this comparison of control interventions indicate that MDA alone was less effective than when combined with Behavioural modification or Snail control, it must be noted that the randomization of schools to the three interventions resulted in many of the shehia/schools allocated to MDA having lower baseline prevalence of infection compared to schools allocated in Snail control or Behavioural change intervention arms.

Despite the lack of impact of MDA alone in the present study it has been proposed that MDA could lead to interruption of transmission (Basanez et al., 2012, Wang et al., 2012b, Fenwick and Jourdan, 2016). However, for such MDA based control to produce better outcomes a longer implementation time may well be required, perhaps at least 6yrs (Wang et al., 2012b),

As part of the current study we were able to analyse the effect of more prolonged repeated chemotherapy in a small subset of 5 schools (Cohort 5) (Section 3.3.5) that were only subjected to chemotherapy, initially to SBT and then MDA from 2010-14 (8 rounds cumulatively: SBT [2] and [6] MDA). This data also failed to show any consistent trend in prevalence reduction. This failure in effectively interrupting schistosomiasis transmission by chemotherapy-based control raises concerns over its impact (Ross et al., 2015b). Concerns over possible lack of drug efficacy/emergence of resistance seem unlikely since we have assessed the efficacy of PZQ in two different time points (2011 and 2013) and all surveys have revealed high cure rates (CR [94.4%]) and egg reduction rates (ERR [76.1%]. In addition, an earlier study in the same setting (Guidi et al., 2010) did not indicate reduced PZQ efficacy. Other potential factors could be resistance of people in changing their behaviour or attitudes towards engaging in more risky activities or host determinant factors of the local people there and deterioration of public health services. Studies have shown that differences in the rate of transmission, pre-treatment infection intensity, rate of exposure and other host factors such as immunological (IgG level, especially IgG 1, 2 and 4; IgA, IgE and IgM) and demographic are significantly associated with schistosomiasis reinfection in a

population despite provision of regular treatment (Mbanefo et al., 2014, McManus and Bartley, 2004, Figueiredo et al., 2012, Jiz et al., 2009, Acosta et al., 2002).

The effectiveness of the combination of molluscicide and treatment with PZQ in terms of controlling schistosomiasis has been long appreciated (Sturrock, 1995, McCullough et al., 1980) and reported to be more effective than using them individually (Mangal et al., 2008). In this present study, snail control plus MDA resulted in a drop in prevalence in the Std-3 plus Std-4 cohort of 31.7% (from 10.88.2%-7.43%) in two years from 2012-2014. By comparison, in China it was shown that schistosomiasis integrated control measures using molluscicide with niclosamide, chemotherapy (PZQ), education and environmental modification substantially reduced infection from 1.7% to 0.4% in 8yrs (2005-12) (Chen et al., 2014). Similarly, in Morocco, the disease was eliminated through implementation of these strategies (Barkia et al., 2014, Laamrani et al., 2000a).

The current results suggest that the behavioural modification plus MDA had the best outcome. Despite this apparent success there are no direct explanations in relation to that achievement especially considering the timeframe to which the intervention was implemented. As indicated earlier, the behavioural modification strategy was effectively initiated in late 2012 giving just a short period of implementation of the strategy. Moreover some of the components of the strategy for example the installation of washing platform were progressively introduced as found feasible but also were inconsistently carried out. Notwithstanding this, behavioural modification is an integral component of the schistosomiasis control and if widely disseminated in communities could eventual lead to interruption of disease transmission (Fenwick et al., 2006a, Aagaard-Hansen et al., 2009, Useh and Ejezie, 1999). Indeed, several the countries (China, Morocco) which have reported successful elimination of schistosomiasis in certain areas have implemented behavioural change strategies (Xianyi et al., 2005b, Laamrani et al., 2000a).

With regards to intensity of infection as an indicator of the success of intervention measures, the initial phase of single annual SBT (2010-2012) was characterised by an increase in intensity as measured simply by arithmetic mean intensity or by the frequency of high intensity infections in Std-1 children. So this reflected the increase in prevalence also recorded during this time.

Regarding the more intensive interventions of the SCORE period, the simple arithmetic mean scores did not mirror the prevalence trends so well with the intensity for the MDA and Behaviour arms showing greater percentage declines than seen for prevalence. The trend for the Snail control arm was more similar for both. However, these trends in arithmetic mean values were not reflected in the percentage of "heavy" infections (egg count ≥50/10ml of urine) which were essentially the same at baseline in 2012 and in 2014 for all three of the intervention arms (Table 4). Similarly, for the original "Cohort 24" schools, 12 of which continued with MDA alone during the SCORE period, there was no consistent trend in reduction of the proportion of "heavy" infections (Table 3). As has been mentioned with regard to the trends in prevalence reductions the significance of these variations should become clearer with analysis of the subsequent monitoring of the SCORE intervention period.

To our understanding this present study is the first to attempt to evaluate comprehensively the impact on both prevalence and intensity of integrated interventions for schistosomiasis control in this setting. Many of the earlier studies that attempted implementing integrated schistosomiasis control focused only on prevalence (Chen et al., 2014, Katz, 1998, Amarir et al., 2011) and in some cases, the control measures were not implemented concurrently as carried out in Saint Lucia (Jordan, 1985). In fact in Saint Lucia, the implementation of schistosomiasis control measures were undertaken stepwise, initially involved snail control followed by increased distribution of safe water supply and selective chemotherapy. Nonetheless we could hypothesise that the concurrent implementation of integrated, synergistic, control measures for schistosomiasis in Pemba could lead to decreases of both prevalence as well as intensity as proposed by (Coura, 1995).

3.6.4. The impact of schistosomiasis control measures on anaemia

Infections with schistosomes has adverse effect on the health of children; anaemia being one of the major problems associated with this infection (Abd Ellah et al., 2015, Casmo et al., 2014). There are several hypotheses concerning the mechanisms that might be linked to the development of anaemia in children infected with schistosomiasis including inflammation, iron deficiency (due to direct ingestion of blood or regular blood loss resulting from rupturing small blood vessels), splenic iron retention and

autohaemolysis (Butler et al., 2012, Friedman et al., 2005b, Tolentino and Friedman, 2007, Friedman et al., 2005a, Mahmoud and Woodruff, 1972). Demonstration of improvement in terms of increasing haemoglobin concentration in children living in areas where this infection is prevalent is considered as a positive impact or a success indicator of control programs. The present study assessed the level of haemoglobin concentration in school-children as an impact of schistosomiasis control following 4 years of chemotherapy-based intervention. Unfortunately, most of the earlier studies conducted in this setting have focused on the relationship of anaemia and STH infection but not with schistosomiasis (Kung'u et al., 2009) and this report would serve as baseline data for the upcoming control initiatives. Measurement of anaemia was carried out in 2010 in the St-1 children in the Cohort 24 schools and then again in 2013. During this time control in the corresponding communities would have involved the initial SBT followed by one or other of the MDA, Behavioural modification or Snail control measures.

The data showed that at baseline, the mean haemoglobin concentration in Std-1 children was 11.3 g/dl with overall prevalence of anaemia being 56.6%; and only a small proportion (0.9%) had severe anaemia (Hb $\leq$ 7.0 g/dl). Following implementation of schistosomiasis control measures, the mean Hb concentration of the children in the population significantly increased to 11.9g/dl. Similarly, the proportions of children with anaemia (38.2%) or severe anaemia (0.6%) were significantly reduced. Our baseline mean haemoglobin concentration observed in the present study is similar to that obtained by (Koukounari et al., 2007) and (Tohon et al., 2008) one year after initial treatment implementation. The apparent improvement in Hb status mentioned above does not show an obvious relationship with the trends in prevalence (Figure 26) and intensity (Figure 30) over the same period although it a significant association of anaemia development with schistosomiasis intensity has been reported by others (King et al., 2005). This may indicate the complexity of development of anaemia with many factors necessary for its progression. Our data shows that most the anaemic children were from Mkoani district where the prevalence of schistosomiasis was higher than other districts. Unfortunately, resource constraints meant that it was not possible to monitor Hb levels in the other surveys reported in this project.

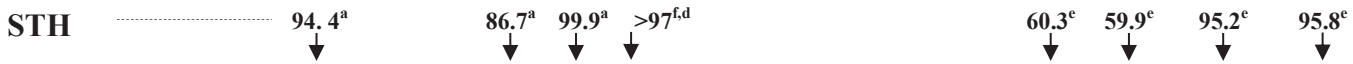
Chapter 4 Impact of Control Interventions on Soil Transmitted Helminth Infections

4.1 Introduction

The STH programme started in 1994 based on preventive chemotherapy. Since the beginning of the STH control programme, the benzimidazole derivates, ABZ and MBZ, had been regularly used to control the morbidity of STH in Pemba (Figure 34). However, the interventions were carried out using different approaches ranging from selective chemotherapy to community-based MDA. Furthermore, high prevalences (90%+) were demonstrated before interventions started (1986-1988) and although use of chemotherapy had been sporadic after that the prevalence remained comparably high throughout the 1990s. However, the use of MDA with albendazole as part of the GPELF and SCI programmes in 2000- 2006 led to lower prevalences (~60%) being recorded which, nevertheless, rebounded as soon as the MDAs stopped and school-based interventions were re-instated.

In parallel with the surveys for schistosomiasis described in Chapter 3 (for cohort 24) faecal samples were collected from schoolchildren from 2010 to 2013 for analysis of the presence of STH in order to assess the impact of the PC-based control in the school-based and MDA control initiatives.

Figure 34: Review of STH control in Pemba



	1986 - 1988	1989 - 1993	1994-1998	1999--2002	2003	2004	2005	2006	2007	2008	2009-2010	
Praziquantel (PZQ)	1	2	3	4		6		7			9	10
Mebendazole (MBZ)			3						8			
ALB + Ivermectin (IVM)					5			7				
Albendazole (ALB)						6					9	10

[a] Renganathan et al (1995)

[d] Albonico et al (1997)

[e] Haji –unpublished SCI data

[f] Stoltzfus et al (1997)

All based on school-children

1	Selective chemotherapy to children and adults - test and treat campaign, twice a year
2	School targeted treatment, twice a year
3	ZHCP: Initiated PZQ for school targeted treatment, only in high prevalence schools MBZ for school targeted treatment, twice a year
4	Irregular distribution
5	GPELF: Community MDA (> 5 years of age), once a year
6	ZHCP ,> 5 years of age, once a year 2004 - 2005, Community MDA in Pemba (supported by SCI - Schistosomiasis Control Initiative) 2006, School targeted treatment in both Islands
7	Integrated Triple Drug Administration, Community MDA, only once
8	Preschool targeted treatment (12 - 59 months), twice a year, supported by UNICEF
9	School targeted treatment, once a year: (The treatment was partially implemented)
10	School targeted treatment, once a year (during the National Baseline Survey)

4.2. Prevalence and intensity of STH infections in Std-1 schoolchildren at baseline (2010)

4.2.1 Prevalence in the different schools and districts

At baseline (2010) the overall prevalence of any STH was 93.9% (95%CI = 92.6-95.3). Prevalences of the three common species of STHs were 87.4% (95%CI = 85.5-89.3), 50.5% (95%CI = 47.7-53.3) and 46.7% (95%CI = 43.9-49.5), for *T. trichiura,* hookworms and *A. lumbricoide*, respectively. In all three types of infections, there was no statistical difference by gender (Table 5). Regarding the intensities of infection the overall arithmetic mean egg counts were: *Ascaris* 1457 ± 3679 eggs per gram (epg); *T. trichiura* 756 ± 1500) and hookworms 302 ± 1106 epg.

Table 5: Prevalence of the major soil transmitted helminths, *A. lumbricoides, T. trichiura* and hookworms, among school children in relation to sex in 2010

Type of infection	No. infected	Sex	
		Boys (%)	Girls (%)
A. lumbricoides	553	(47.0)	(53.0)
T. trichiura	1036	(49.1)	(50.9)
Hookworms	598	(49.8)	(50.2)

The prevalence of STHs by school is shown in Figure 35. *T. trichiura* was the most prevalent species in all schools reaching 100% in two schools. However, the prevalence of *A. lumbricoides* and hookworm varied between schools. Mkoani district had higher prevalences of all three species of helminth infection (Figure 37).

Figure 35 : Prevalence of STH in Std-1 schoolchildren among schools in Pemba in 2010

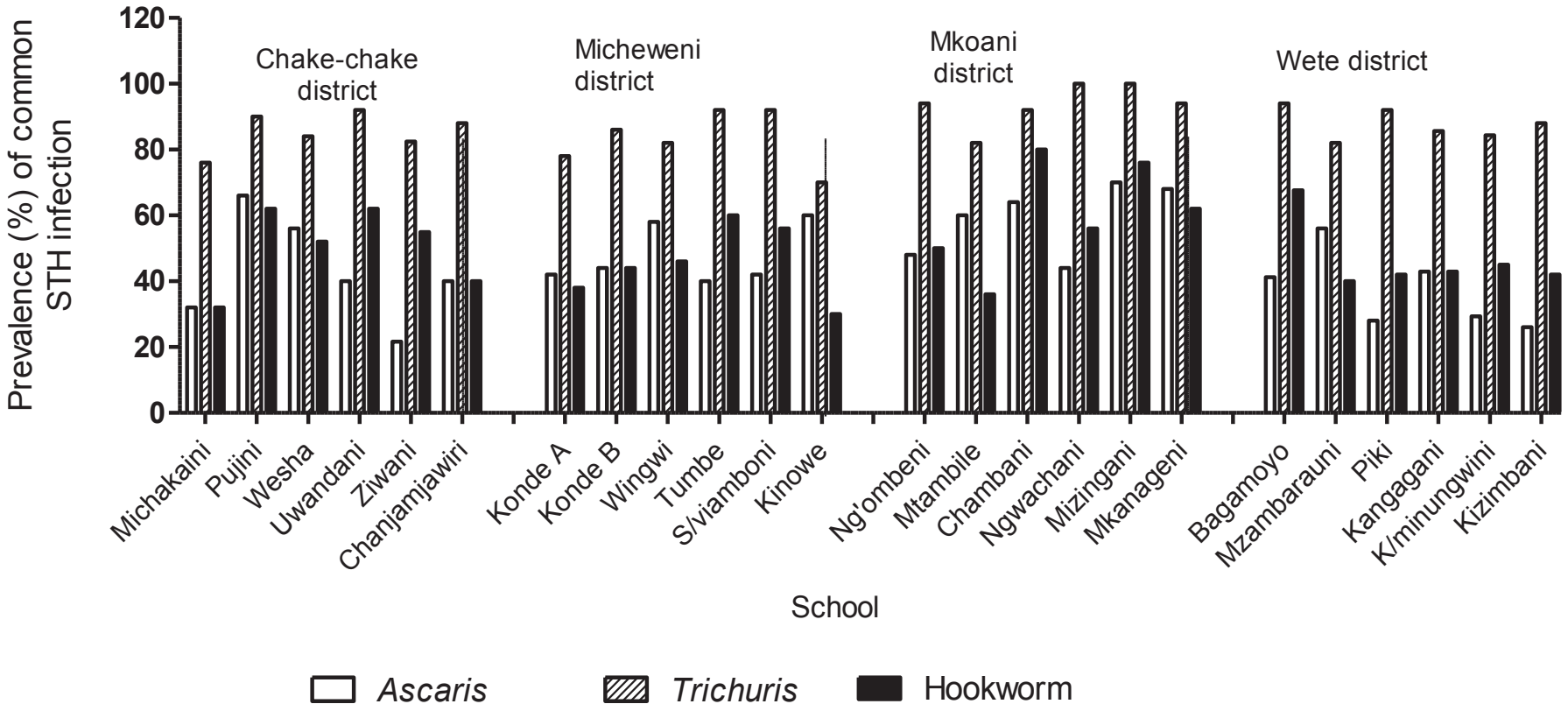
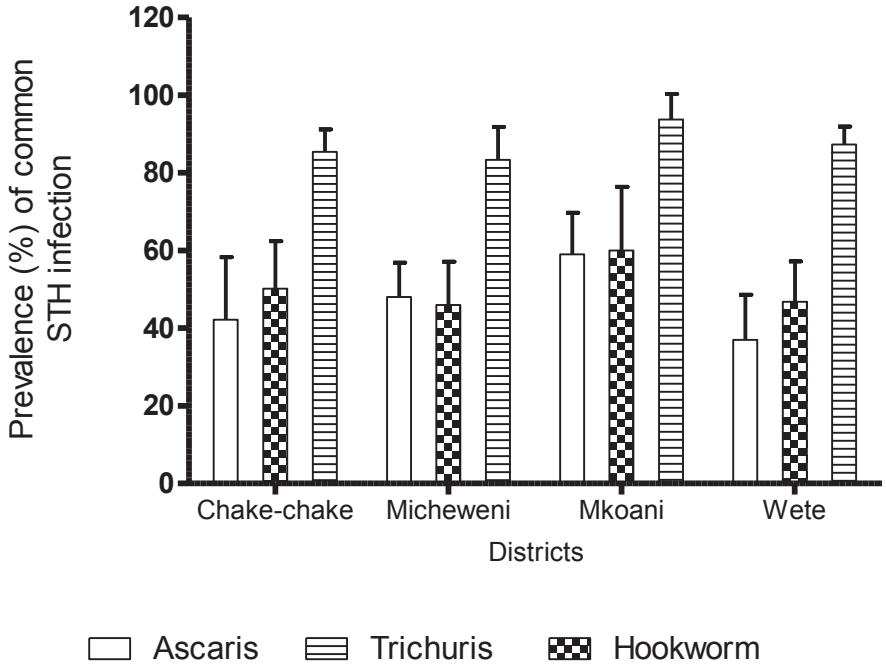


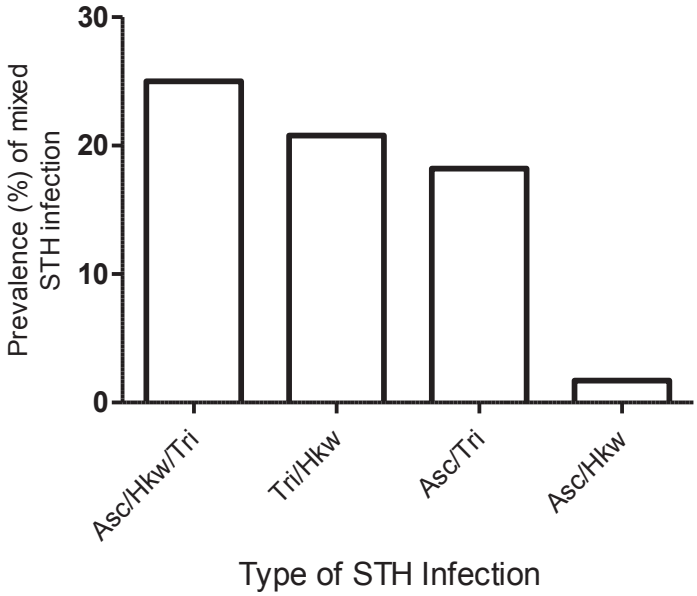
Figure 36: Prevalence (+SD) of the STHs among districts in Pemba in 2010



4.2.2 Multiple infections with STH

Many of the 1185 children had multiple STH infections as shown in Figure 37:- 296 (25%) of the children had triple infection with *A. lumbricoides*, *T. trichiura* and hookworms; 246 (20.8%) were co-infected with *T. trichiura* and hookworms; 216 (18.2%) were co-infected with *A. lumbricoides* and *T. trichiura;* 20 (1.7%) were co-infected with *A. lumbricoides* and hookworm. Thus, as shown previously, only 72 (6.1%) were not infected with any of the three worms. The remainder of the children, 36 (3%) were infected with hookworm alone, 278 (23.5%) were infected with *T. trichiura* alone and 21 (1.8%) were infected with *A. lumbricoides* alone.

Figure 37: Proportion of the children with mixed STH infection



4.2.3 Intensity of STH infection

Analysis of the intensity of helminth infections revealed that among the *A. lumbricoides* infected children, 80.1%, 11.9% and 8.0% had light (mean epg 119 ± 1101), moderate (mean epg 6712 ± 1467) and heavy (mean epg 17,208 ± 5368) infections respectively. Among the *T. trichiura* infected children, 79% had light infection (mean epg 364 ± SD 234), 20% had moderate infection (mean epg 2423 ± 1837) and 0.7% had heavy infection (mean epg 13,203 ± 1837). Among hookworm infected children 97% had light infection (mean epg 409 ± 403), 1.3% moderate infection (mean epg 3183 ± 447) and 1.7% had heavy infection (mean epg 9494.4 ± 6503.5).

4.3 Prevalence and intensity of STH infections in schoolchildren at follow-up in March 2011

In January 2010 following the above baseline survey, all primary school children on the Island aged from 7-13yrs were treated with albendazole (400 mg) for STH. The follow-up reported below was to determine the effects on helminth infection rates in the new Std-1 cohort a year later. Parallel sampling of infection in the Std-3 children also

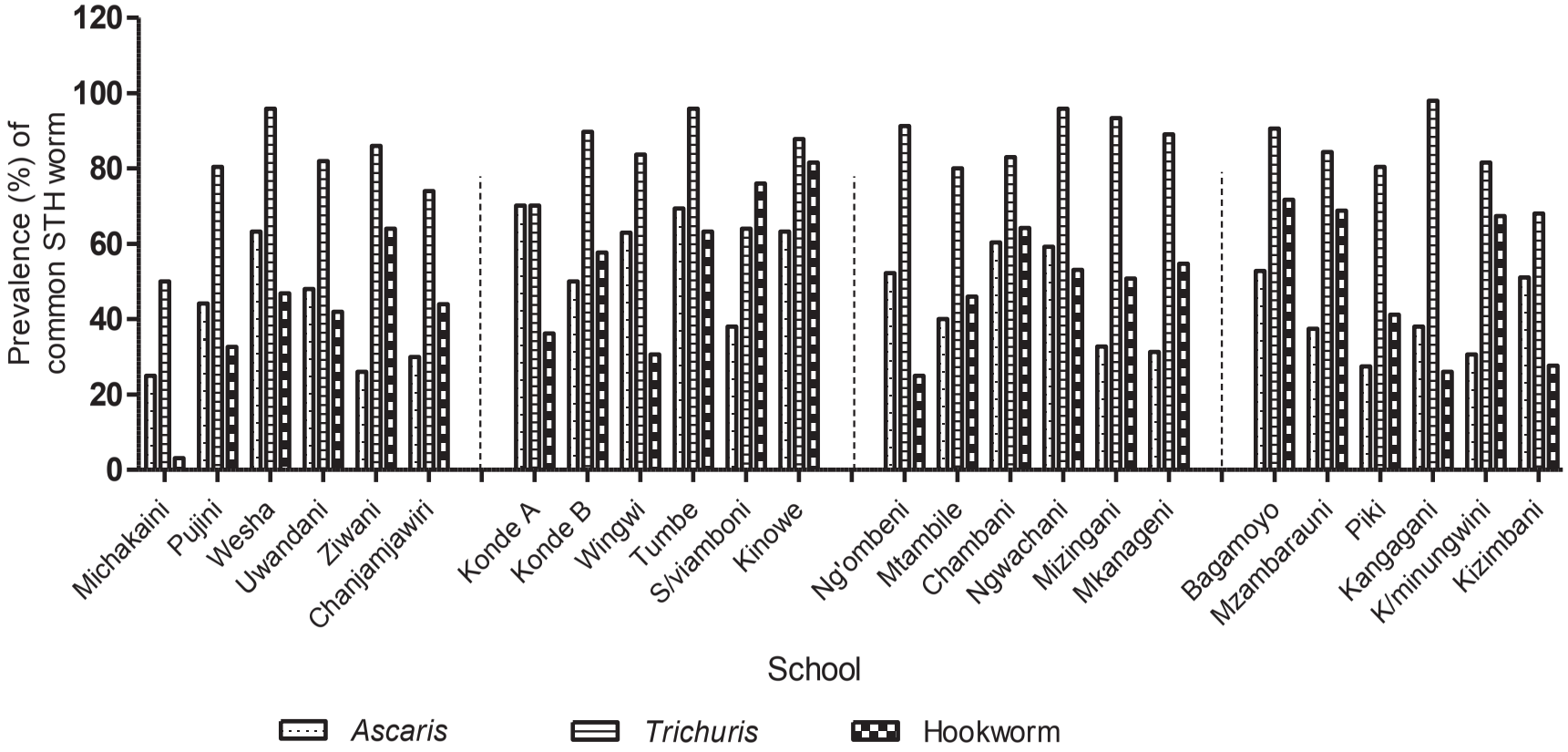
allowed some assessment of the impact of the 2010 SBT on worm burdens. In order to allow comparison of the data, Std-1 and Std-3 are considered separately.

Among the school children enrolled, 2355 (98.9%) produced stool samples although hookworm data was not available for 3 children. Of those with complete data, 1180 (50.1%) were in Std-1 and 1175 (49.9%) were in Std-3. The combined overall prevalence for any worm was 89.8% (95%CI = 88.6-91.0) and worm specific prevalences were 47.4% (955CI = 45.4-49.4) for *A. lumbricoides*, 83.3% (95%CI = 81.8-84.8) for *T. trichiura* and 46.3% (95%CI = 44.2-48.3) for hookworms.

4.3.1.1 Prevalence in Std-1 children in 2011

The overall prevalence for the Std-1 children was 90.7% (95%CI = 89.0-92.4) for any STH and worm specific prevalences were 83.9% (95%CI = 81.8-86.0) for *T. trichiura*, 50.3% (95%CI = 47.5-53.2) for hookworm and 46% (95%CI = 43.2-48.8) for *A. lumbricoides*. The data from the individual schools (for Std-1 children only) are shown in Figure 38. It is clear that *T. trichiura* infection was the predominant infection, although *A. lumbricoides* and hookworm infections differed remarkably across the schools. Michakaini School had the lowest prevalences for all three STH.

Figure 38: Prevalence of STH in Std-1 children among schools in Pemba in 2011



4.3.1.2 Intensity of infection for Std-1children

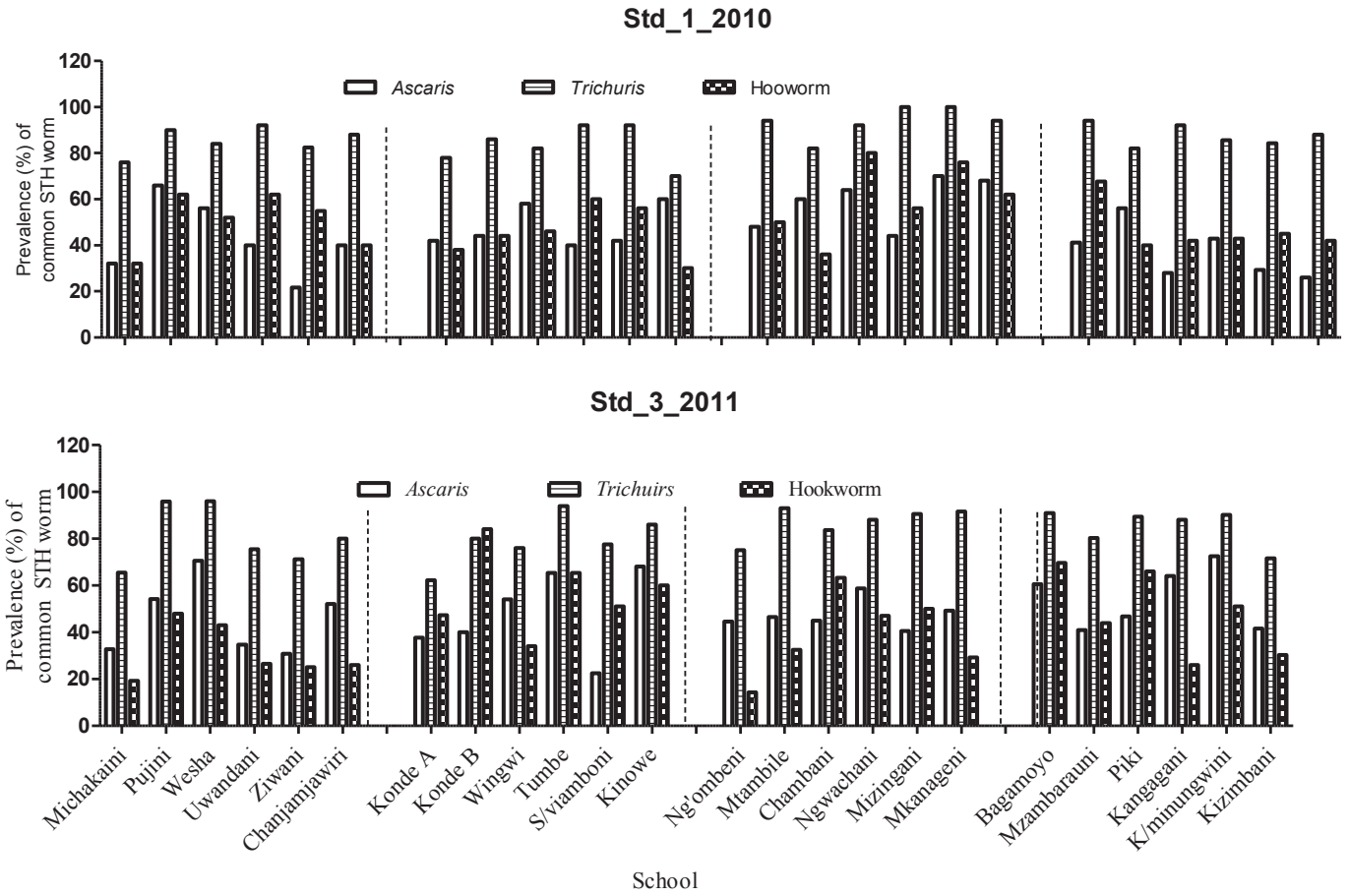
Regarding intensity of infection, 70.9%, 16.1% and 13% had light, moderate and heavy infection with *A. lumbricoides* respectively. The overall mean intensity for *A. lumbricoides* was 4794 (± 7417) egp. The children with light *A. lumbricoides* infection had an arithmetic mean egg count of 1491(± 1387) epg whilst those with moderate and severe *A. lumbricoides* infection had arithmetic means of 7190 (± 1447) and 19831 ± 10873) epg, respectively. Among the children infected with *T. trichiura*, 739 (74.2%) had light infection, 251 (25.2%) had moderate and 6 (0.6%) had severe infection and the overall intensity was 891 (± 1496) epg. Those with light, moderate and heavy infection had, respectively, arithmetic mean egg counts of 354 (± 253), 2176 (± 1521) and 13248 (± 3435) epg. Out of the hookworm infected children, 576 (96.64%), 15 (2.52%) and 5(0.84%) had respectively light, moderate and severe infections and the overall mean epg was 543 (± 706). These values for light, moderate and heavy infections were very similar to those in 2010 (*A. lumbricoides*: 80.1%, 11.9% and 8.0%; *T. trichiura:* 79.2%, 20.1% and 0.7 %; hookworm: 97%, 1.3% and 1.7%). There were no significant differences between the mean intensities of any of the STH infection between 2010 and 2011.

4.3.2. Effect of treatment on worm burden – comparison of Std-1 and Std-3

4.3.2.1 Prevalence

As indicated above, the parallel sampling of Std-3 children allowed assessment of worm burden after the Std-3 cohort had received the single round of SBT. Comparison of the data on prevalence of STH for individual schools for Std-3 and Std-1 children sampled at baseline does not show any remarkable difference (Figure 39). However, the overall prevalence for any worm was significantly different between the two classes ($z = -4.18$; $p = 0.0001$). The prevalences were 93.9% (95%CI = 92.5-95.3) for Std-1and 88.86% (95%CI: 87.1-90.7) for Std-3.

Figure 39: Comparison of the prevalence of STH infection among Std-1 in 2010 and Std-3 in 2011



Indeed, in Std-1 children, who were not treated previously, the overall prevalences were 90.7% (95%CI = 89.1-92.4) for any STH, 83.9% for *T. trichiura*, 50.3% for hookworm and 46% for *A. lumbricoides* compared with 88.9% (95%CI = 87.1-90.7) for any STH, 82.6% for *T. trichiura*, 42.2% for hookworm and 48.9% for *A. lumbricoides* for the Std-3 children. Only the hookworm prevalence was significantly higher in Std-1 children ($z = 3.9$, $p = 0.0001$).

4.3.2.2 Intensity of STH infection in Std-1 and Std-3 children

Table 6 compares the intensity of STHs infection between Std-1 and Std-3. Generally, the proportion of children with any intensity category between different STHs infection was comparable between the two classes. The only differences noted were for hookworm and *T. trichiura* but the actual number of children with severe infection was too small (5 for Std-1 and 1 for Std-3 for hookworm infection and 6 for Std-1 and 1 for Std-3 for *T. trichiura* infection) for a meaningful comparison. Nevertheless, the overall (arithmetic mean) hookworm epg for the two classes was significantly different ($t= 2.5$; $p = 0.01$) with mean epg of 273.4 for Std-1 and 220.1 for Std-3. However, the overall mean epg for both *A. lumbricoides* ($p = 0.5$) and *T. trichiura* ($p = 0.2$) were similar between the classes (Table 6).

Table 6: Comparison of the intensity STHs infection among Std-1 and Std-3 children.

	Intensity					
	Light		Moderate		Heavy	
Infection type	No.	(%)	No.	(%)	No.	(%)
A. lumbricoides						
Std-1	389	(71.4)	84	(15.4)	72	(13.2)
Std-3	404	(70.6)	86	(15.0)	82	(14.3)
Hookworm						
Std-1	576	(96.8)	14	(2.4)	5	(0.8)
Std-3	475	(96.4)	17	(3.5)	1	(0.2)
T. trichiura						
Std-1	735	(74.3)	248	(25.1)	6	(0.6)
Std-3	721	(74.2)	250	(25.7)	1	(0.1)

4.3.3 General features of the STH infections in 2011

4.3.3.1 Comparison of districts

As seen in Figure 41 taking the Std-1 and Std-3 children together, the prevalence of the different STH in the different districts was very similar to that seen for the Std-1 children in 2010 (Figure 37.)

4.3.3.2 Multiple Infections[10] with STH in 2011

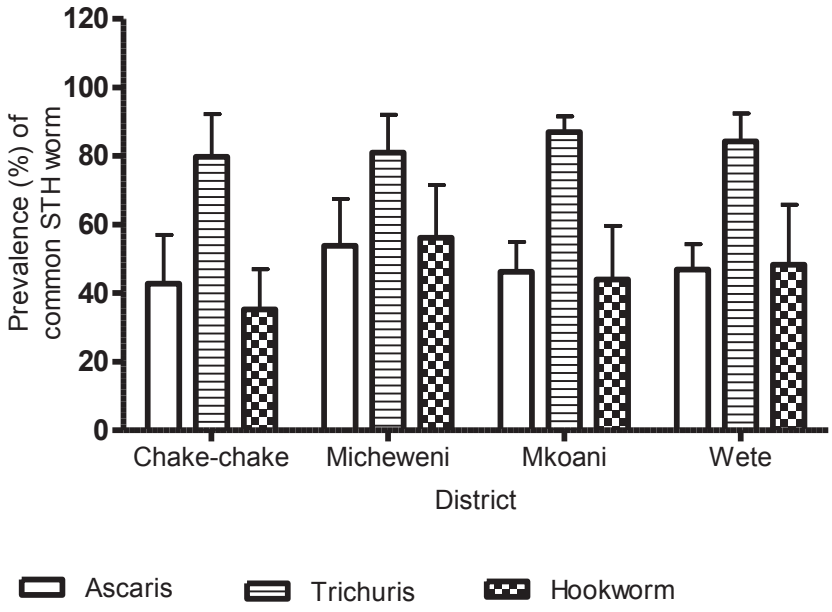
As in 2010 many of the children had multiple infections. Taking the Std-1 and Std-3 together 556 (23.6%) had triple infection, 444 (18.9%) were co-infected by *T. trichiura* and hookworms, 462 (19.6%) were co-infected by *T. trichiura* and *A. lumbricoides,* 33 (1.4%) were co-infected by *A. lumbricoides* and hookworms.

4.3.3.3 Impact of sex on STH infection levels

As in 2010 there were no strong associations of sex with prevalence of any of the STHs (see Table 4). The percentages in boys/girls were: *A. lumbricoides*, 47/ 53%; (χ^2 =0.9, p =0.33); hookworms: 49.8/50.2% (χ^2 =0.8, p = 0.36); *T. trichiura* 49.1/50.9% (χ^2 =1.2, p =0.3).

Figure 40: Prevalence of STH infections among the districts in Pemba in 2011.

Error bar indicates the standard deviation (SD) of the mean



[10] The denominator for the multiple infection was 2352. This is based on the number of children who had all records on STH

4.4 Prevalence and intensity of STH infections in Std-1 schoolchildren at follow-up in March 2012

4.4.1 Prevalence of STH in 2012

The present survey was carried out in March 2012, after two years of SBT. This survey allowed assessment of any trend of infection over the years. A total of 1074 Std-1 children were enrolled in the study. The children had a mean age of 7.9 yrs ± 0.8 (95% CI = 7.8-7.9). Of these 564 (52.5%) were girls and 510 (47.5%) were boys. Of the enrolled children, 1050 (97.8%) produced stool sample. The overall prevalence of any STH worm was 91.7% (95%CI = 90.0-93.4) and worm specific prevalence was: 52% (95%CI = 48.9-55.0) for *A. lumbricoides*, 87.8% (95%CI = 85.8-89.8) for *T. trichiura* and 51.2% (95%CI = 48.2-54.3) for hookworm. The analysis of STH infection in relation to sex revealed that in all worm species the prevalences were similar in boys and girls.

4.4.2 Prevalence of STH in schools and districts (2012)

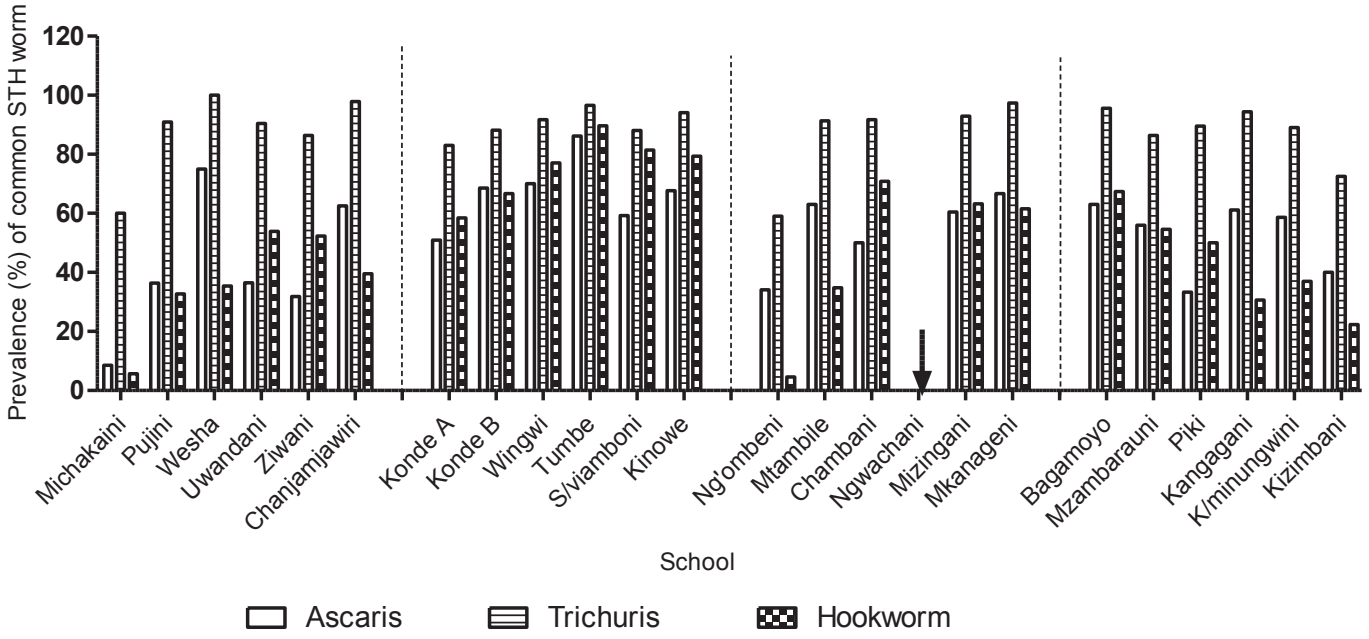
The prevalence of STH in schools and district is shown in Figure 41. In general, the prevalence of *T. trichiura* was comparable between schools and hence the districts, although, in some schools like Wesha the prevalence reached 100%. Surprisingly Michakaini School had very low prevalences of *A. lumbricoides* and hookworm as compared to other schools. There was no data of STH infection for Ngwachani School due to the fact that all primary school-children in this school were treated with albendazole few days prior undertaking the survey. *A. lumbricoides* and hookworm infections were predominantly found in Micheweni district as compared to other districts.

4.4.3 Intensity of STH infection (2012)

Table 7 shows the intensity of STH infections among the children. Generally, most of the children infected had light infections with any of the worms. A substantial number of children infected with *A. lumbricoides* had severe infection. The children with light *A. lumbricoides* infection had an arithmetic mean egg count of 1712 ± 1300 epg; those with moderate infection had a count of 7055 ± 1481 epg, whilst the children with severe

infection had a count of 22908 ± 13970 epg. The children with light, moderate and severe *T. trichiura* infections had, respectively, arithmetic mean egg counts of 394 ± 255 epg, 2212 ± 1592 epg and 15620 ± 5986 epg.

Figure 41: Prevalence of STH infections in schools and districts in 2012



Arrow head indicates area where stool samples were not collected

Table 7: Intensity of STH infections

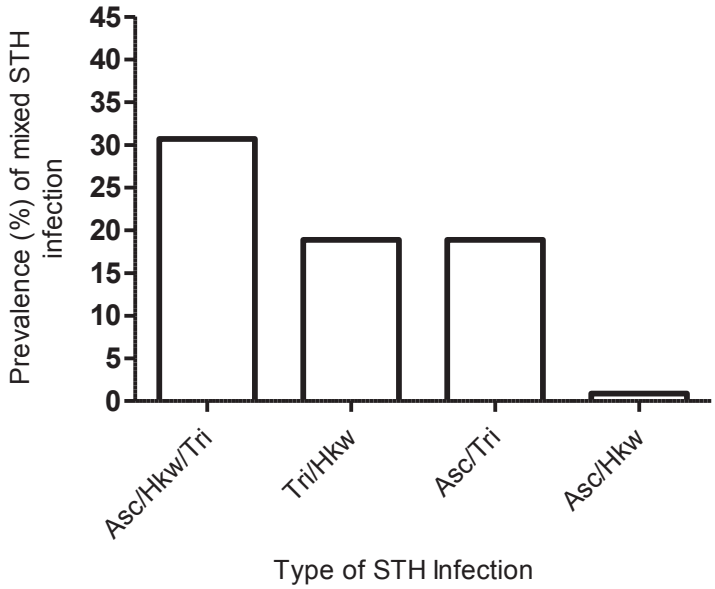
Type of infection	Intensity					
	Light		Moderate		Heavy	
	No.	(%)	No.	(%)	No.	(%)
A. lumbricoides	351	(64.3)	75	(13.7)	120	(22)
T. trichiura	651	(70.6)	267	(28.6)	7	(0.8)
Hookworm	515	(95.7)	17	(3.2)	6	(1.1)

The children with light hookworm infection had an arithmetic mean egg count of 388 ± SD 383 epg; those with moderate infection had a count of 2715 ± SD 584 epg while the children with severe infection had a count of 8044 ± SD 7035 epg. Regarding the intensity of STH relative to sex, comparable intensities were seen between boys and girls.

4.4.4 Multiple infections (2012)

Out of 1049 children who produced stool samples, 322 (30.7%) had triple infection with *A. lumbricoides*, *T. trichiura* and hookworm. 195 (18.6%) children were co-infected with *T. trichiura* and hookworm or *T. trichiura* and *A. lumbricoides*. Only 9 children (0.9%) were co-infected with *A. lumbricoides* and hookworm (figure 43).

Figure 42: Prevalence of multiple STH infections in 2012



4.5 Comparison of STH infection in 2010 - 2012

Following reintroduction of SBT for control of STH infections, single annual treatment with ALB (400mg) was administered to school-children aged between 7-13yrs. Later, in 2012, during the course of implementation of the PC strategy focussed on control and possibly elimination of schistosomiasis, the drug administration approach was changed from single annual SBT to twice/yearly community wide treatment or MDA. Thus the STH surveys reported above were aimed at assessing the impact of single annual SBT in terms of reduction of prevalence and intensities. For this comparison only Std-1 data collected from 2010-12 were considered.

Figures 44 and 46 show comparisons of the prevalences and intensities of STH infection in school-children from 2010 to 2012. Clearly the data shows that the overall prevalence of STH (any worm) has been minimally reduced as a result of provision of two rounds of SBT. For the three common STH worms, there were inconsistent reduction and increase of the prevalences over the years especially for *T. trichiura* and hookworm infections. For *A. lumbricoides*, the situation was somewhat different by observing

steady increase of the prevalences over the years. However, the increase was non-significant between the successive years.

The intensities of infection were generally maintained at a low level (light intensity), although the percentage of light infections in fact decreased slightly over the time for *A. lumbricoides* and *T. trichiura*. For example, a higher proportion (80.1) of children with light *A. lumbricoides* infection was demonstrated in 2010 compared to 64.3% in 2012.

Figure 43: Comparison of the prevalence of any STH infections from baseline (2010-2012) and after two rounds of administration of ALB

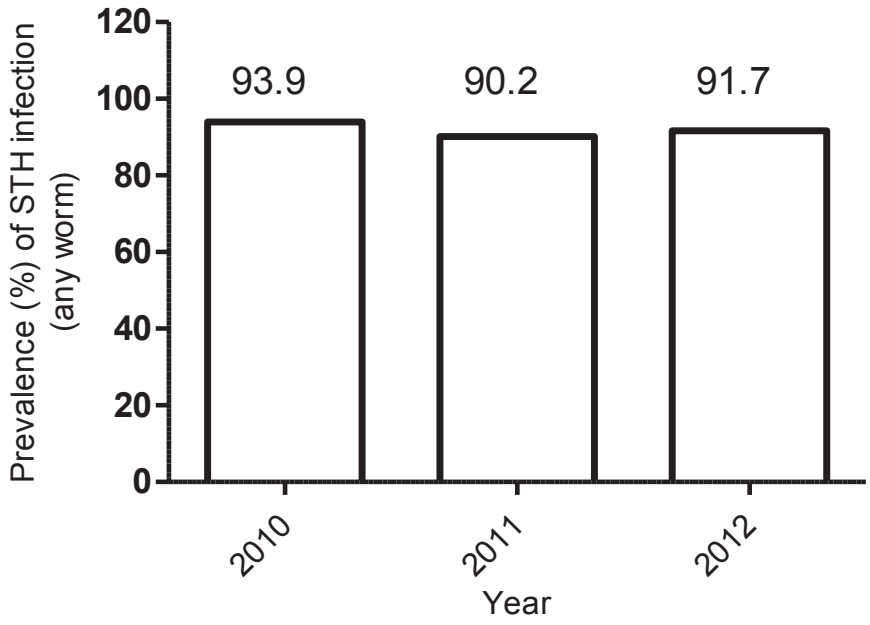
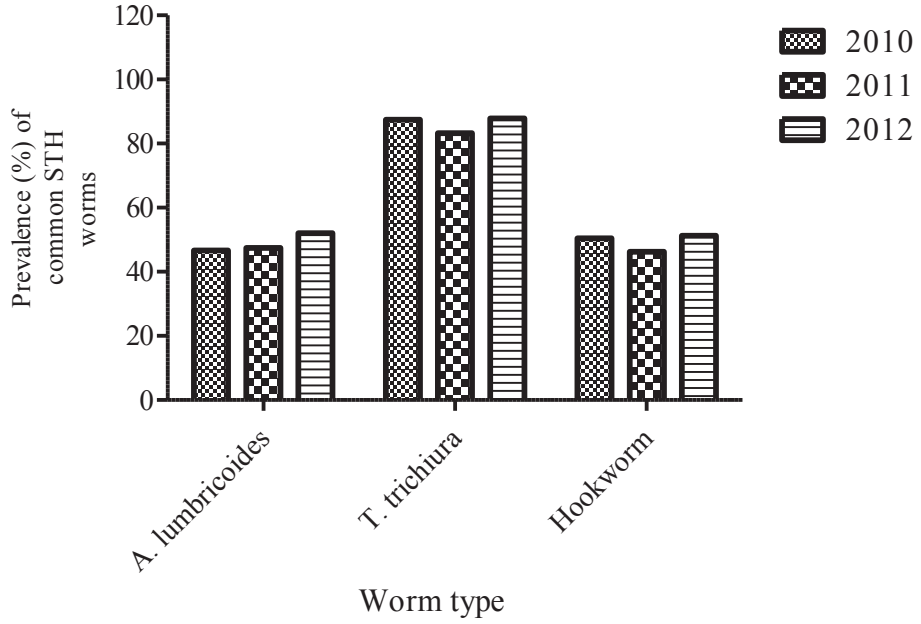
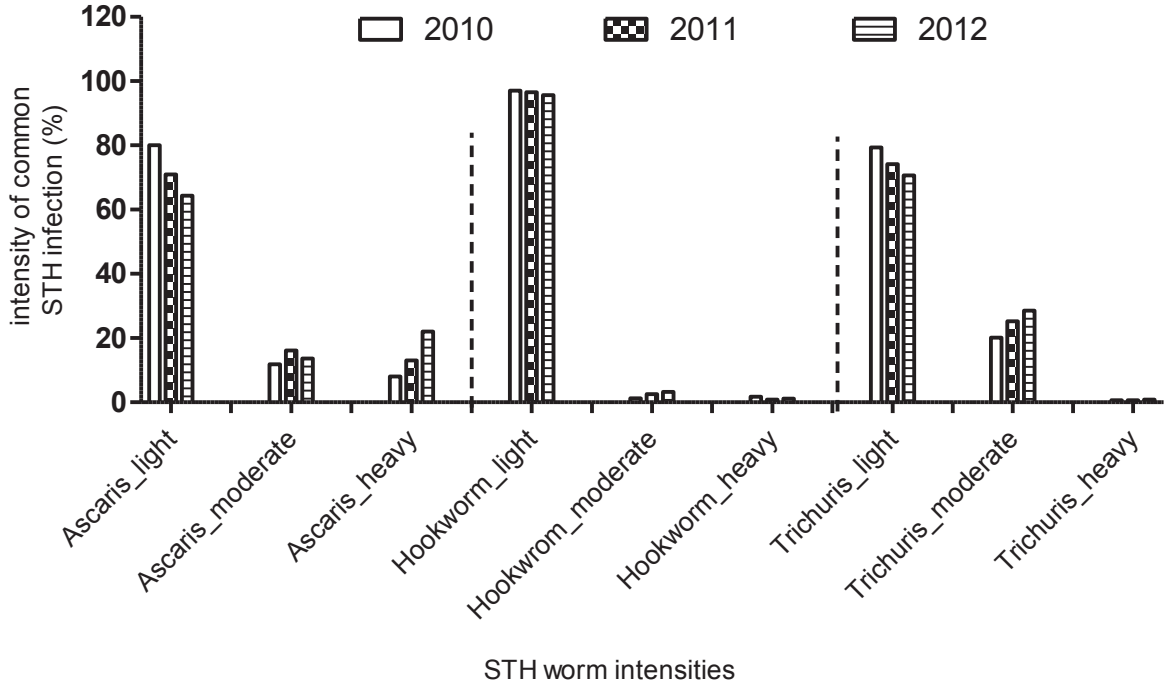


Figure 44: Comparison of the prevalence of the three common STH worms from 2010-2012 after two rounds of administration of ALB



Conversely the proportion of children with severe *A. lumbricoides* infection was steadily increased over time reaching to 22% in 2012, almost two-fold higher than at baseline (2010). Similarly, a higher percentage of children (79%) with light *T. trichiura* infection was observed in 2010 as compared to 2012 paralleled by an increase in moderate infections. Interestingly hookworm intensities were comparable between the years.

Figure 45: Comparison of the intensities of STH infections in Std-1 children in 2010 (baseline) to 2012 (after administration of two rounds of ALB)

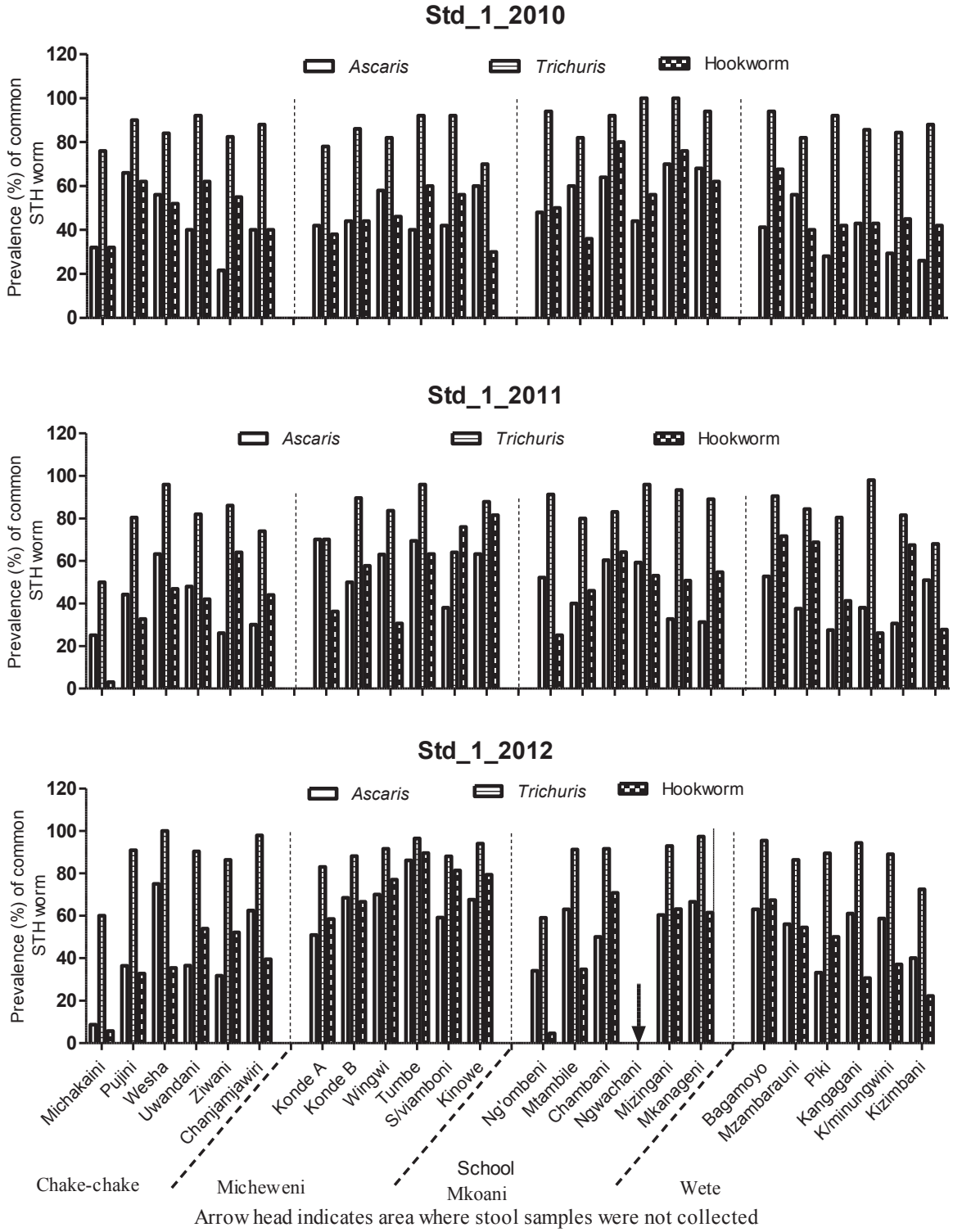


4.6 STH infection in individual schools in 2010 - 2012

As shown in Figure 42 above, the overall prevalence of any STH worm was comparable from 2010-2012. However, STH worms (*A. lumbricoides*, *T. trichiura* or hookworm) specific prevalences (Figure 43) varied between the years and the schools. As in 2010, *T. trichiura* infection was most predominant reaching 100% in some schools (Wesha) but decreasing in other schools e.g. Mizingani. Unfortunately, there were no data in Ngwachani School for 2012. This was due to provision of ALB to all primary school-children just prior to undertaking the survey. Applying the Mantel- Haenszel analysis for the score test for the trend of odds of *T. trichiura* infection showed that there was slightly increased odds of *T. trichiura* infection across the schools (Odd ratio [OR] = 1.02; 95% CI =0.90-1.15; p = 0.7) The detailed table for this analysis is shown in Appendix 4C. A similar trend of increase or decrease of hookworm infection was also demonstrated in some schools. But the overall trend of odds of infection increase was

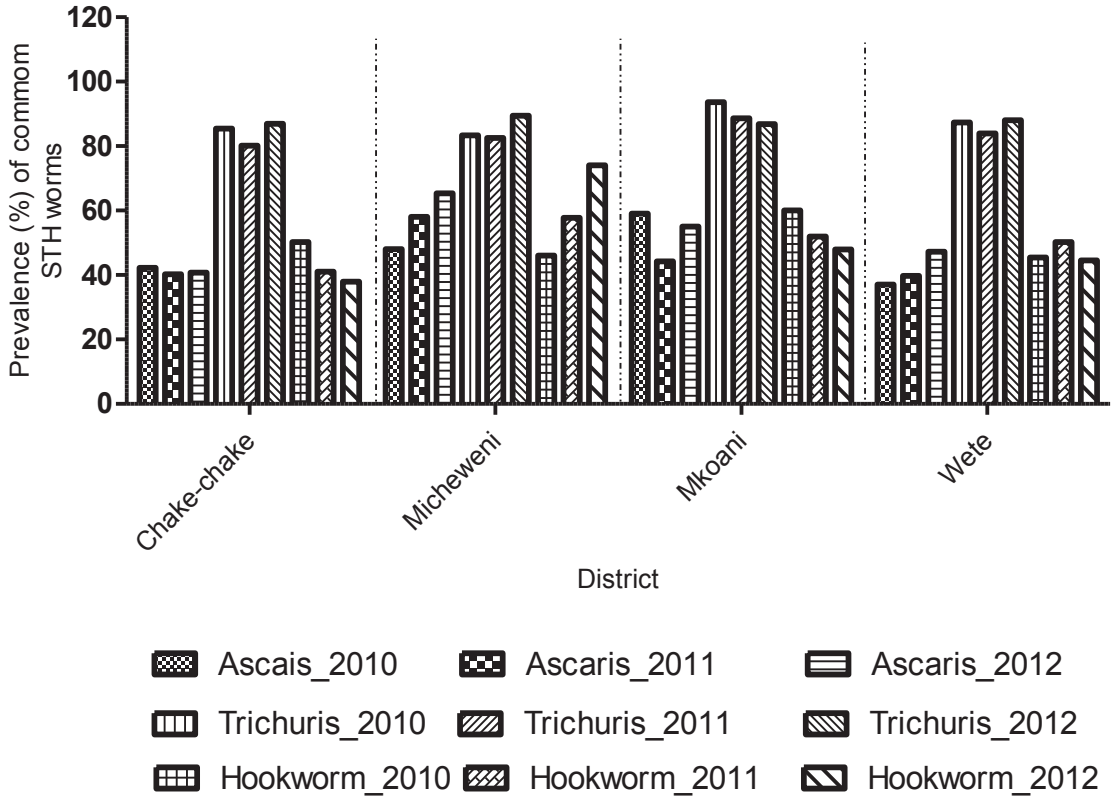
not significant (OR = 1.0; 95% CI=0.92-1.1; p = 0.9), (see appendix 4B). *A. lumbricoides* infection also showed a decrease and some schools and increases in others. In Tumbe the infection rate increased by over 100% in 2012. Indeed, the score test for trend of odds revealed there was a slightly increased odds of *A. lumbricoides* infection across the schools (OR = 1.12; 95% CI=1.02-1.21) and this was significant (p = 0.01) (see Appendix 4A).

Figure 46: Comparison of STH infections between schools in 2010-2012



Arrow head indicates area where stool samples were not collected

Figure 47: Comparison of the prevalences of STH infections in districts from 2010 - 2012



In comparing the prevalences of STH infections in relation to districts between baseline (2010) and 2012, after two rounds of ALB administration (Figure 47), the prevalence of *T. trichiura* was broadly similar across the districts over time with a slightly increased trend for odds of infection (OR= 1.02, 95% CI = 0.90-1.15; p = 0.80). The situation was very similar for hookworm infection, where the overall trend of odds of infection was OR= 1.02, (95% CI = 0.93-1.11; p = 0.67) across the districts. Nevertheless, a marked increase of the hookworm infection was detected in some of the districts. For example, in Micheweni district the infection steadily increased over time reaching 74.1 % in 2012, a 61.1% increase. Compared to *T. trichiura* and hookworms, there was slightly higher trend of odds of *A. lumbricoides* infection across the districts (OR= 1.12, 95% CI =1.03-1.21; p = 0.01). Appendix 1d-f illustrates the detailed tables for the above analysis.

4.7 Prevalence and intensity of STH infections in Std-1 schoolchildren at follow-up in March 2013

4.7.1 General prevalence of STH infection in 2013

Between January-March 2013, a survey to assess the impact of one year of biannual MDA for the control of STH infection was conducted in Pemba. This survey was designed to provide data for comparing two strategies or approaches on the frequency of drug delivery: (1) single annual treatment in 2010-2012; (2) biannual treatment (April 2012 and Nov 2012) in terms of reduction of the prevalence and intensity of STH.

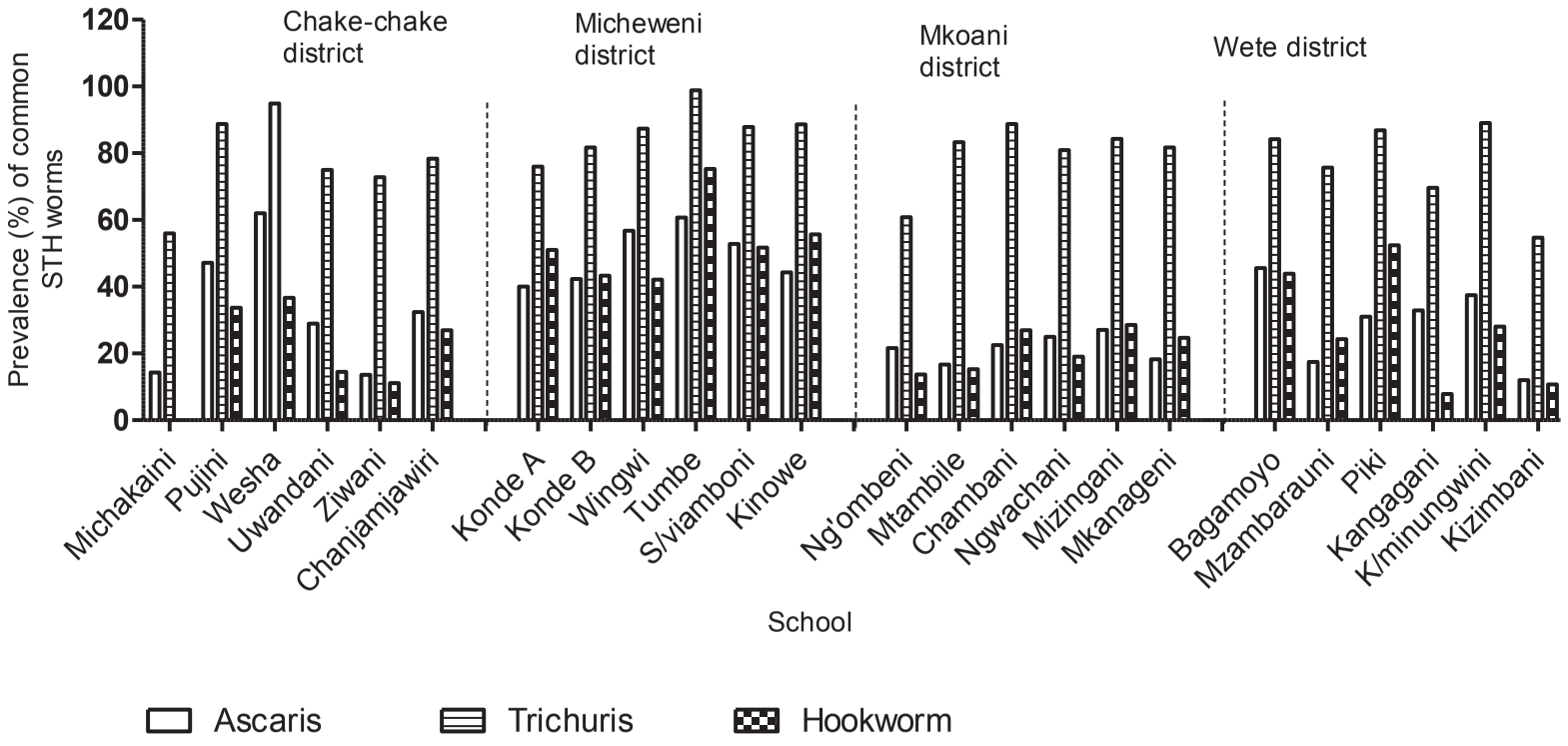
During this survey a total of 2309 Std-1 school children were enrolled. Of these 1201 (52%) were girls and 1108 (48%) were boys. The children had mean age of 7.3yrs (95% CI= 7.25- 7.31) with a range of 6-13yrs.

Of the enrolled children only 1971 (85.4%) produced stool samples. Overall the prevalence of any worm was 85.4% (95%CI = 83.8-86.9) and the worm specific prevalences were 34.1% (671) (95%CI = 32.0-36.2) for *A. lumbricoides*, 32.1% (632) (95%CI = 30.0-34.1) for hookworm and 80.8% (1592) (95%CI = 79.0-82.5) for *T. trichiura*. More boys were infected with *A. lumbricoides* (52.9%) than girls (47.1%) and the infection was significantly associated with sex (p = 0.001*)* but this was not the case for hookworm and *T. trichiura* infection.

4.7.2 Prevalence of STH in schools and districts in 2013

Figure 49 illustrates the prevalence of STH in schools and districts. This figure shows that Tumbe School had higher prevalences of all common STH as compared to other schools while Michakaini and Kizimbani schools had the lowest prevalences for any of the worm infections. Generally, most of the schools had <40% for *A. lumbricoides* and hookworm infections (Michakaini had 0% of hookworm infection). Micheweni district had the highest prevalence for *A. lumbricoides*, hookworm and *T. trichiura* infections.

Figure 48: Prevalence of STH infection across Pemban schools and districts in 2013



4.7.3 Intensity of STH infection in 2013

Among the *A. lumbricoides* infected children 482 (71.8%), 55 (8.2%) and 134 (20%) had light, moderate and heavy intensity, respectively. The children with light *A. lumbricoides* infection had an arithmetic mean epg of 1321 ± 1000, those with moderate infection epg of 6962 ± 1328and those with severe infection epg of 26501 ± 14451. Most (95%) of the hookworm infected children had light infections with mean epgof 416 ± 396; 4% had moderate infections with mean epg of 2540 ± 462 epg; and1% had heavy infections with mean epg of 5564 ± 1786. Among those children infected with *T. trichiura*, 64.8% had light infection (mean epg 436 ± 263), 34.4% had moderate infection (mean epg 1997 ± 1307) and 0.8% had heavy infection (mean epg of 16735 ± 6700).

4.7.4 Intensity in relation to sex

The intensity of the three common STH worms in relation to sex is shown in Table 8. This table clearly shows that the intensities of *A. lumbricoides* and *T. trichiura* infections were comparable between sexes. The trend for hookworm infection was somewhat different as the proportion of boys with moderate or severe hookworm infection was slightly higher in boys than girls. However, the actual number of children with severe hookworm infection was remarkably low.

Table 8: Intensity of STH worms in relation to sex in 2013.

Infection type/intensity	Sex			
	Female		Male	
	No.	(%)	No.	(%)
A. lumbricoides				
Light	235	(74.4)	247	(69.6)
Moderate	21	(6.7)	34	(9.6)
Heavy	60	(19.9)	74	(20.9)
Hookworm:				
Light	303	(96.5)	298	(93.7)
Moderate	9	(2.9)	16	(5.0)
Heavy	2	(0.6)	4	(1.3)
T. trichiura :				
Light	523	(64.1)	509	(65.6)
Moderate	283	(34.7)	264	(34.0)
Heavy	10	(1.2)	3	(0.4)

4.7.5 Multiple infections (2013)

Among the children who produced stool samples, only 288 (14.6%) were not infected with any of the common STH whilst 681 (34.6%) of the children were infected with *T. trichiura* alone, 42 (2.1%) had *A. lumbricoides* alone, 34 (1.7%) had hookworm alone. Fifteen (0.7%), 328 (16.6%), 297 (15.1%) of the children were respectively co-infected with *A. lumbricoides* and hookworm, *A. lumbricoides* and *T. trichiura,* and hookworm

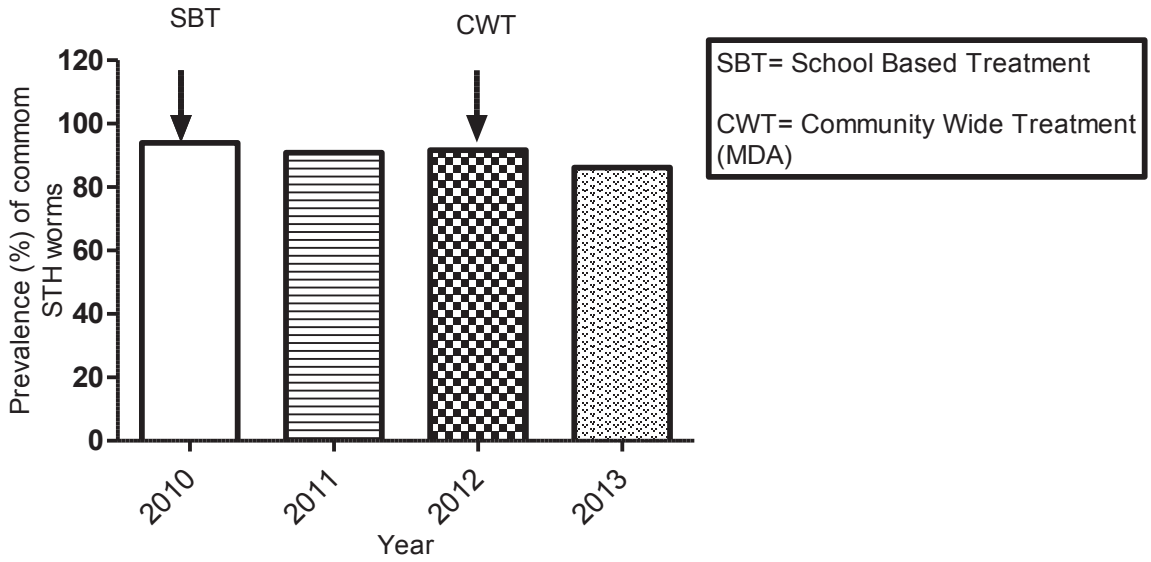
and *T. trichiura*. Only 286 (14.5%) of the children had triple infection. The observation of triple infection with STH worms among the children would not be a surprise thing in this setting as it is more common in Zanzibar, though with varying levels (Knopp et al., 2009).

4.7.6 Comparison of STH prevalence between 2010 and 2013

Due to the fact that, wherever schistosomiasis is endemic, STH infections usually also prevail, WHO has urged schistosomiasis endemic countries to take advantage of any planned PC for schistosomiasis and concurrently control STH infections (Montresor et al., 2002). In view of this, following implementation of the elimination of schistosomiasis project in Zanzibar there was a shift of strategy from annual school based treatment with PZQ and ALB to twice yearly provision of both drugs through MDA, and therefore it was important to assess the impact of each of these strategies in terms of reduction of infection prevalence and intensity.

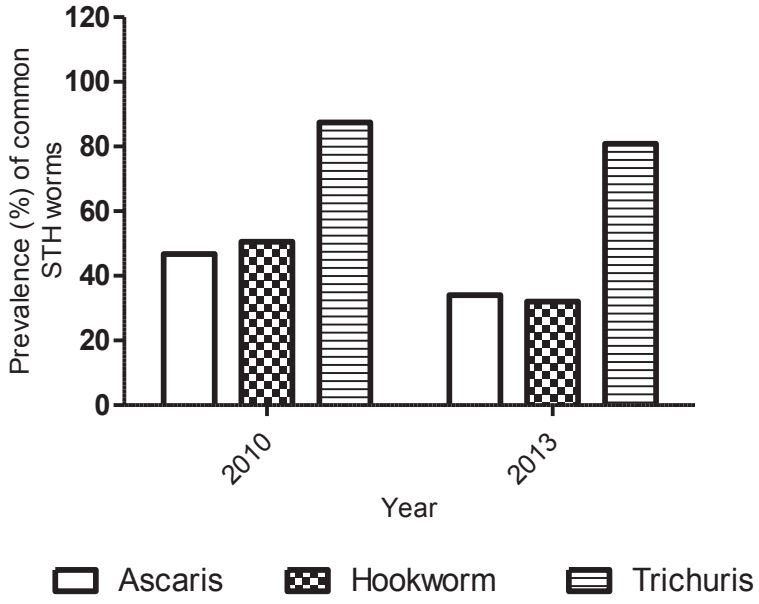
The baseline (2010) prevalence of any worm of 93.9% (95%CI = 92.6-95.3) was modestly but significantly reduced to 86.2% (8.2% reduction) (z = 6.7; p = 0.0001) in 2013 after two rounds each of school-based treatment and MDA (Figure 49). Equally, worm specific prevalences for any of the common STH infection were considerably reduced. For instance, hookworm was reduced from 50.5% to 32.1% (z = 10.3 p = 0.0001), *A. lumbricoides* was reduced from 46.8% to 34.0% (z =7.1, p = 0.0001) and *T. trichiura* infection was reduced from 87.4% to 80.8% (z = 4.5, p = 0.0001) (Figure 50) following provision of twice-yearly treatment with ALB in the communities as MDA. The trend of decrease of STH infections was also apparent across the individual schools. As at baseline, *T. trichiura* infection was predominant across the schools but only one school (Tumbe), during the follow-up in 2013, recorded a comparable prevalence (98.9%) noticed in some schools (Mizingani and Ngwachani 100%) in 2010. Nevertheless, when applying the Mantel- Haenszel analysis revealed that there was no increased odds of *T. trichiura* infection across the schools (Odds ratio [OR] = 0.6; 95% CI =0.49-0.71).

Figure 49: Comparison of the prevalence of STH infection between 2010-2013 following administration of ALB as SBT and MDA.



Similarly, overall there was remarkable variation of the prevalence of hookworm infection across the schools. Indeed when applying the Mantel- Haenszel analysis indicated that there was no increased odds of hookworm infection across the schools (OR = 0.44; 95% CI =0.38-0.51). Likewise, there was increase or decrease of *A. lumbricoides* infection across the schools but when applying the Mantel-Haenszel analysis shows that there were no odds of infection increase (OR = 0.56; 95% CI =0.49-0.66).

Figure 50: Comparison of worm specific prevalence for the common STH infection between 2010 and 2013



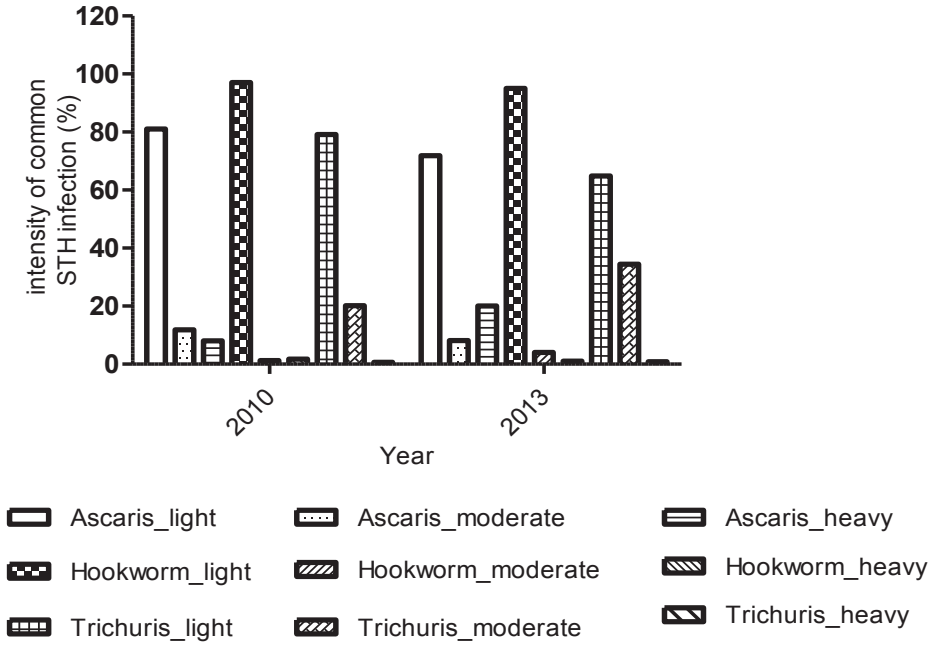
With regards to STH infections in the districts, the prevalence of *A. lumbricoides* between the districts varied over the years. However, when applying Mantel-Haenszel analysis it revealed that there was no significant odds of the infection increasing across the districts (OR = 0.57; 95% CI = 0.49-0.67). The similar trend of variation in the prevalence of the infections in the districts between the years was also demonstrated for *T. trichiura* and hookworm and overall, for any of these infections, there were no increasing odds. The OR for *T. trichiura* infection was 0.60 (95% CI= 0.49-0.74; p = 0.0001) and the OR for hookworm was 0.45 (95% CI = 0.39-0.52; p = 0.0001).

4.7.7 Comparison of STH infection intensity between 2010 and 2013

The intensities of infection for any of the common STH worm remained at low level (light intensity) (Figure 51) although there was variation between the years. For instance, the percentage of children with moderate intensity for *T. trichiura* infection increased in 2013 (34.4%) as compared to 2010 (20%) and the proportion of children

with severe intensity infections with *A. lumbricoides* increased by almost two-fold (from 8-20%). For hookworm infection, the proportions of children with light, moderate or severe intensity were comparable between the years. The increased proportion of children with severe *A. lumbricoides* or moderate *T. trichiura* infections may raise concern over the possible emergency of drug resistance.

Figure 51: Comparison of the STH infection intensity between 2010 and 2013



4.8 Assessment of anaemia in relation to STH Infection

4.8.1 Baseline

The children that produced stool samples were also assessed for their haemoglobin levels. The mean haemoglobin concentration was 11.3 g/dl ± 1.7 (95%CI = 11.21 - 11.41). Overall the prevalence of anaemia was 56.6%. Out of the anemic children only 1.3% had severe anaemia (Hb ≤ 7.0 g/dl) and so 98.7% had moderate anaemia. The proportion of girls (56.7%) and boys (56.5%) with anaemia was comparable.

The analysis of anaemia in relation to common STH infections is illustrated in Table 9. It was clearly shown that a high proportion of children regardless their STH infection status, were anaemic. However, *A. lumbricoides* infection was significantly associated with anaemia according to Chi-square analysis. Furthermore, univariate analysis using

logistic regression also showed a significantly increased odds of anaemia in children infected with *A. lumbricoides* (OR = 1.28; 95%CI = 1.02-1.61).

Table 9: Proportions of children with and without anaemia in relation to STH infections in 2010

Type of STH infection	Anaemic		Not Anaemic		*p*-value
	No.	(%)	N	(%)	
Any STH					
Infected	630	(56.6)	483	(43.4)	
Not infected	41	(56.94)	31	(43.06)	0.95
A. lumbricoides:					
Infected	331	(59.9)	222	(40.1)	
Not infected	340	(53.8)	292	(46.2)	0.04
Hookworm:					
Infected	341	(57.0)	257	(43.0)	
Not infected	330	(56.2)	257	(43.8)	0.8
T. trichiura:					
Infected	589	(56.8)	447	(43.2)	
Not Infected	82	(55.0)	67	(45.0)	0.7

4.8.2 Follow-up assessment of anaemia in 2013

A high proportion (98.8%) of the 2283 enrolled children was assessed for haemoglobin (Hb) level. Of the children assessed for the presence of STH infections, 1966 (99.7%) also had blood test for Hb level. The children had a mean Hb level of 11.9 g/dl (± 1.4) (95% CI =11.8-11.9). Of the children assessed for Hb, 871 (38.2%) were anaemic (Hb<11.5g/dl). The proportion of girls with anaemia (49.7%) was comparable to that of boys (50.3%) and anaemia was not associated with sex ($\chi^2 = 3.04$; $p = 0.08$).

Moreover, among those with anaemia, only 5 (0.6%) had severe anaemia and 866 (99.4%) had moderate anaemia.

The proportion of children with anaemia relative to STH infection is shown in Table 10. With this data, unlike that for *A. lumbricoides* in Table5 there was no evidence of a statistically significant correlation between status with any of the worm species and anaemia. Due to the relatively small number of children with severe anaemia, further analysis in relation to STH infections was not possible.

Table 10: Proportions of children with and without anaemia in relation to STH infections in 2013

Type of STH infection	Anaemic		Not Anaemic		*p*-value
	No.	(%)	N	(%)	
Any STH					
Infected	636	(37.92)	1041	(62.08)	
Not infected	104	(36.11)	184	(63.89)	0.55
A. lumbricoides:					
Infected	262	(39.1)	408	(60.9)	
Not infected	478	(36.7)	818	(63.1)	0.3
Hookworm:					
Infected	246	(39.1)	384	(60.9)	
Not infected	494	(37.0)	841	(63.0)	0.3
T. trichiura:					
Infected	601	(37.9)	986	(62.1)	
Not Infected	139	(36.7)	240	(63.3)	0.6

4.8.3 Comparison of anaemia between 2010 and 2013

The overall prevalence of anaemia was significantly reduced from baseline (56.6%) in 2010 to 38.2% in 2013 (z =10.3; p = 0.0001) following administration of single annual

treatment or twice yearly in the community with albendazole and praziquantel in schools. Equally, the mean Hb concentration significantly increased from 11.3g/dl to 11.9g/dl (t= -11.1; p = 0.0001). Nevertheless, the absolute number of children with severe anaemia (Hb $\leq$ 7.0g/dl) was too low at both baseline and follow-up for a meaningful comparative analysis. However, as shown in tables 9 and 10 the proportion of children with anaemia who were infected or not infected with any STH was comparable.

4.9 Discussion

Control of STH based on preventive chemotherapy (PC) using benzimidazole drugs is the fundamental approach advocated by the WHO and is adopted by most countries where these infections are prevalent. Generally the PC is either targeted to school-children or to the community as MDA (WHO, 2006). Such interventions are often regarded as aiming to control morbidity rather than reducing transmission. Both approaches have been used in Pemba but their relative impact on transmission has not been formally compared. This was one of the aims at the start of this study in 2010 since school-based treatment was reintroduced after a period of three years without routine treatments and also because of changes in treatment strategies following implementation of new National Control Programme in 2012 involved more intensive treatment i.e. community MDA twice yearly. Despite that ALB has been reported to have low cure rate for the treatment of STH worms especially *T. trichiura* and *A. lumbricoide* in Zanzibar (Stothard et al., 2009) which is similar setting to the study area where this project was carried out.

The baseline study in 2010 followed repeated PC interventions with MBZ or ALB since 2000 directed either to school-children or preschool children with occasional MDAs (see Figure 34). As seen from Figure 34 there has been limited systematic effort to monitor effects of the STH treatments. Evidence showed that the MDAs for filariasis and schistosomiasis in Pemba caused temporary reduction in STH prevalence (from ~95% to ~60%) but with a rapid rebound of infection when MDA stopped. Likewise, in Unguja where the epidemiological setting is similar, an overall reduction of 39.6% prevalence of STH infection with species- specific prevalence of 46.6%, 21.6% and 16.9% for *T. trichiura*, hookworm and *A. lumbricoides* was demonstrated after several years (from 1994 to 2007) of PC intervention (Knopp et al., 2009). The present study assessed the current prevalence of STH namely: *A. lumbricoides*, hookworms and *T. trichiura* infection after several years of implementation of MDA and SBT in Pemba. The Std-1 children examined at baseline in Feb 2010 should have been included in the ongoing UNICEF community based MBZ provision to pre-school children in 2009. Nevertheless, a very high prevalence of STH infection was seen (93.9%) including *T. trichiura* [87.4%] and hookworms [50.5%]), exceeding the WHO threshold (≥ 50%) set for continuing PC intervention as one of the main control measures.

4.9.1 Effect of single SBT in 2010

The present study was not longitudinal as the impact of treatment in the children was not assessed in a cohort but, rather, by studying the Std-1 groups (children who have not been treated previously), we looked for any impact of school-based treatment on transmission during the previous year. There was no evidence of this overall, (94% cf 91% overall prevalence of STH) or within the individual schools. Furthermore, when comparing St1 and Std3 children (the latter having been treated in 2010) there were no significant differences in prevalence for STH or for any individual worm infections except for hookworm (50% in Std1 cf 42% in Std-3). The lack of difference likely reflects the fact that the BZ are only moderately effective against the STH and also that there is rapid reinfection. The children may be re-infected as early as six months post treatment, as has been demonstrated early in the control programme (Albonico et al, 1995), and so the re-infection rate looks not to have changed despite multiple rounds of PC. Furthermore the subsequent follow-up of the Std-1 population following two rounds of SBT revealed marginal reduction of the overall prevalence comparing baseline in 2010 (93.9%with that a year later (2011) (90.2%and then remaining comparable in 2012) (91.7%) (Figure 43). Similarly worm specific prevalences were comparable between the years, with *T. trichiura* being more prominent. Indeed there was marked variation of the prevalences of STH infection across the schools and districts over the years that may reflect changes in transmission dynamics. At baseline Mkoani district had high prevalences for any worm as compared to other districts. However subsequent analysis revealed high prevalence of STH in Micheweni district. This finding is not surprising as the Micheweni district has a high illiteracy rate and is also considered the poorest district, two factors that are linked with high transmission of STH. Furthermore, a relatively high proportion of children had multiple infections with STH even after implementation of two rounds of SBT. This suggests that single annual SBT for STH did not significantly reduce transmission, contrary to what other workers believe (Truscott et al., 2014b). However, other studies have reiterated that for the MDA treatment to interrupt transmission a high drug coverage has to be ensured in a wide age groups in the population (Anderson et al., 2014). In this setting, the drug coverage was as high as 80%. Despite this relatively good coverage, the very high STH transmission appears to sustain rapid reinfection (Truscott et al., 2014a). Nevertheless, overall, many of the children remained with light intensity for any of the STH worm over the entire study period. An earlier study conducted by Stoltzfus and colleagues

(Stoltzfus et al., 1997) found minimal reduction in prevalence of hookworms (from 94.5- 89%) and *T. trichiura* (from 96.7-92%) even after treating children twice/year with mebendazole (500mg) in a one year period. The consistent finding of high prevalence of these worms in this population clearly indicates an intense transmission and rapid reinfection though the likelihood of emergence of drug resistance cannot be excluded. A recent study has shown that treatment of children with albendazole alone resulted in significantly lower CR against *T. trichiura* infection compared with oxantel pamoate or other drug combinations (Speich et al., 2014). Indeed, it has been confirmed in meta-analysis study that ALB has limited efficacy against *T. trichiura* infection (Keiser and Utzinger, 2008). Likewise previous studies carried out in in the same epidemiological setting observed low CR of benzimidazoles for the treatment of STH but there was no evidence of occurrence of mutation of the beta (β) tubulin gene at codon 200 (Albonico et al., 2004b) that is responsible for BZ resistance in animals.

The overall objective of the PC strategy is to reduce morbidity with the ultimate goal of preventing debilitating disease sequelae of chronic STH infection. Thus, in this context, maintaining light intensity of STH might be perceived as a positive impact as the STH morbidity is directly correlated to intensity.

4.9.2 Impact of community-based MDA

Implementation of the schistosomiasis elimination project was concurrently undertaken with morbidity control for STH infection through PC. So changes of approach in drug delivery in terms of frequency would have an impact on the integrated control of both infections directly. As such, in 2012, there was a change of drug delivery approach from single annual SBT to biannual community wide MDA. The follow-up study presented here was carried out after two rounds of community MDA. The results suggested that the MDA led to a moderate reduction in the overall prevalence (to 86.2%) for any worm as well as of worm-specific prevalences for *A. lumbricoides* (34.0%) and hookworm (32.1%). *T.trichiura* infection, however, remained still as high (80.8%) as demonstrated before initiation of regular PC treatment (2010). Generally, a high proportion of the children had light intensities for any worm but an increase in the proportion of children with moderate and heavy intensities for *T. trichiura* and *A. lumbricoides* respectively was recorded, as compared to baseline. In addition, a substantial proportion of children had multiple infections even after two rounds of MDA. The finding of a significant decline of the overall prevalences and sustaining a high proportion of light intensities

during the course of project implementation is encouraging although the reductions were not as high as that reported for other settings (Supali et al., 2013, Oqueka et al., 2005). However, Supali and colleagues (Supali et al., 2013) assessed the impact after several (six) rounds of implementation of MDA whilst the present survey was after just two rounds of MDA. Nevertheless, at the time of our survey, considering cumulative treatment rounds offered in our setting, the overall rounds of treatment were comparable between the two studies. Despite the overall declining of the prevalence of infection over the years, still a reasonable proportion of children had double or triple infections. The observation of triple infection with STH worms would not be a surprise thing in our setting as it more common in Zanzibar, though with varying levels (Knopp et al., 2009).

There is overwhelming evidence on the impact of the SBT or MDA in terms of reduction of prevalence and intensity of STH but it is unlikely that these treatment approaches alone could effectively interrupt transmission in schools or communities. Nevertheless, continuation of the provision of antihelminthic drugs in school-aged children or adults would still be essential to maintain infection at light intensities and so to prevent STH negatively affecting the health of the population. Regarding the efficacy of BZ drugs, recent multi-centre trials have shown that both ALB (Vercuysse et al, 2011) and MBZ (Levecke et al., 2015) efficacies are sustained globally. However, the very low efficacy of BZs in Pemba, especially against *T. trichiura*, may warrant more in-depth studies to explore the potential occurrence of drug resistance.

4.9.2.1 Anaemia

Infections with STH have adverse effect on the health of children; anaemia being one of the major problems associated with these infections especially hookworms (Stoltzfus et al., 2000, Kung'u et al., 2009). Several hypotheses or mechanisms are linked to the development of anaemia in children infected with these worms. For instance, STH are associated with mal-absorption, loss of appetite, chronic blood loss (Gilles et al., 1964) or haemorrhage which eventually lead to iron deficiency anaemia. The present study assessed the level of haemoglobin in school-children as an impact of helminth control following several years of PC. At baseline in 2010 the data showed that mean haemoglobin concentration in Std-1 children was 11.3 g/dl with overall prevalence of anaemia being 56.6%; and a small proportion (1.3%) had severe anaemia (Hb $\leq$ 7.0 g/dl). However, during the subsequent follow-up of the Std-1 children (not the same individuals) in 2013 after two rounds each of SBT and MDA, the mean Hb

concentration of the children has significantly (t = -11.1; p = 0.0001) increased to 11.9 g/dl and that, inversely, the proportion of children with anaemia was significantly reduced from 56.6 to 38.2% (z = 10. 3; p = 0.0001). Nevertheless our baseline data (on anaemia) was somewhat lower than that observed by (Mwandawiro et al., 2013) in nearby Kenya in a similar epidemiological setting, albeit based on a smaller sample size (the Kenyan study examined only 492 children). Nonetheless, the observation of the reduction in the prevalence of anaemia and the gain in Hb concentration is promising and may indicate a positive impact of the control measures. However, it would be difficult to conclude that this achievement was a direct impact of deworming alone and other factors may have contributed, as many diseases and conditions are implicated in causing anaemia (Brooker et al., 2008). To assess confounders of anaemia, in this present study (at least at baseline) it was determined that malaria parasite prevalence and intensity in the children was very low (data not shown) but other possible causes of anaemia were not explored in the study.

There is overwhelming evidence on the relationship of anaemia and STH infection (Brooker et al., 2006a). We present here anaemia data as a possible consequence of helminth infection and in relation to its control. In this study there was slightly higher percentage of children with anaemia who were infected with *A. lumbricoides* compared with those who were not and this difference was statistically significantly and also there was increasing odds of anaemia in *A. lumbricoides* infected children. However, no significant difference in prevalence of anaemia was seen for *T. trichiura* or hookworm infected children. Interestingly, the association of anaemia with *A. lumbricoides* infection was only apparent at baseline but not during the subsequent follow-up. At that period none of the STH worm infection was associated with anaemia. This may be due the fact that most infections were light and development of anaemia has been clearly shown to be strongly correlated with intensity of infection (Brooker et al., 2008, Stoltzfus et al., 2004). Development of anaemia is also crucially dependent on iron intake and so another possible explanation could be that the children are now better nourished. So, albeit the development of anaemia is a complex and multifactorial process the improvement in haemoglobin concentration in children living in areas where STH infections are prevalent could be considered a positive impact and, although not specific to STH, an indicator of success of the ZHCP.

Chapter 5 Assessment of the coverage, compliance and attitude to mass drug administration intervention for the control of STH and schistosomiasis

5.1 Introduction

Schistosomiasis and soil transmitted helminthiasis (STH) caused by *Ascaris lumbricoides*, *Trichuris trichiura* and hookworms pose a great disease burden in tropical and sub-tropical countries resulting in growth retardation during childhood, iron deficiency anaemia, malnutrition and poor school performance (Stephenson and Holland, 1987, Hotez et al., 2004). On the other hand these infections are associated with decreased economic productivity in the adult population (Taylor-Robinson et al., 2012).

Periodic administration of anthelminthic drugs to populations at risk can revert most of the morbidity and prevent the chronic debilitating consequences related to schistosomes and STHs infections if administered correctly and in a timely manner (Richter, 2003, Savioli et al., 2004b, Simeon et al., 1995).This strategy has been recently branded as "preventive chemotherapy" (PC), often referred to as deworming, and has been recommended as the cornerstone of control strategies (Who, 2002, Taylor-Robinson et al., 2012, WHO, 2006). So far two common approaches: 1) school-based treatment (SBT) 2) community wide treatment (CWT) or mass drug administration (MDA) are being employed for preventive chemotherapy (Massa et al., 2009a). Mathematical modelling has shown that treatment of school-aged children significantly reduces morbidity and may interrupt schistosomiasis transmission to the level of elimination (Carabin et al., 2000). Despite this potential, for these strategies to yield maximum impact in helminth control, attention must be paid to optimum coverage and community compliance (Massa et al., 2009b, Prichard et al., 2012, Mathieu et al., 2004, Worrell and Mathieu, 2012). This postulate comes from the fact that if a proportion of the individuals within the community remains untreated they act as reservoir of the infection (Nandha et al., 2013). The key question is what level of coverage is necessary for effective intervention. In an attempt to eliminate lymphatic filariasis (LF) during the era of the Global Program on Elimination of Lymphatic Filariasis (GPELF), an estimated 65->80% treatment coverage with 5-6 rounds of MDA was considered

sufficient to achieve interruption of transmission and eventually elimination (Mohammed et al., 2006, Cantey et al., 2010, Nandha et al., 2013). Since control of schistosomiasis and STH uses a similar strategy (preventive chemotherapy) to that of LF, at least 75% of treatment coverage for the population at risk (school-age children) is the target set by WHO for morbidity control (WHO, 2006).

The drugs used for preventive chemotherapy against STHs and schistosomiasis are administered as a single dose (Albendazole [ALB] - 400mg/ Mebendazole 500 mg and Praziquantel [PZQ] 40mg/kg) in order to sustain compliance (adherence) for a successful chemotherapy-based control strategy. However, there is limited data related to coverage and compliance in the use of ALB or PZQ for MDA campaigns against STH infection and schistosomiasis. Studies were, however, carried out to assess MDA compliance for the elimination of LF mentioned above. This demonstrated that clients not realising the importance of taking the drugs, unfavourable provider's attitude, unacceptability of drug distributors (Nujum et al., 2012), and uncertainty of side effects (Sunish et al., 2013) were among the factors associated with non-compliance. In another study that assessed factors associated with participation in an MDA campaign against lymphatic filariasis it was shown that knowledge on the mode of the disease transmission (Mathieu et al., 2004), age and sex (Babu and Kar, 2004) and social mobilisation strategy (Nandha et al., 2013) were significantly associated with MDA compliance. Coverage is a key performance indicator of PC control programmes and is the most important data that is required to demonstrate success of such a Programme (WHO, 2010). Mass drug administration has been the common approach utilized in chemotherapy-based control for schistosomiasis and STHs infection. This delivery mechanism is found to be most cost-effective (Lo et al., 2015, Turner et al., 2016, Leslie et al., 2011) and has shown a great impact in terms of reduction of disease morbidity as well as interruption of disease transmission (Truscott et al., 2014a). However, there remains a paucity of information related to coverage and compliance of PC drugs (PZQ and ALB) administered in mass campaigns against schistosomiasis and STH infection.

Zanzibar embarked on MDA treatment for helminth control since 2001 at the time of implementation of GPELF where co-administration of ivermectin (IVM- 200mg/kg) and ALB (400mg) was delivered to the eligible population excluding children <5yrs, pregnant women, lactating mothers at first week and ailing chronic patients, once a year, for a period of 5yrs (Mohammed et al., 2006). Parallel to that, in 2004, morbidity

control of schistosomiasis and STHs through the support of the Schistosomiasis Control Initiative (SCI) was launched. In the latter programme ALB (400mg) and PZQ (40mg/kg) were co-administered in the communities as MDA using the platform established for elimination of LF. In 2006 there was shift of approach from community-based MDA to school-based treatment with the provision of the same regimen to all primary school-children aged between 7-15yrs. Since then there had been sporadic implementation of diverse helminth control programmes up to 2010. Subsequently, with the current ambitious goal of elimination of schistosomiasis - twice yearly co-administration of ALB and PZQ was implemented and included even younger children (≥3yrs) (Knopp et al., 2012). Despite the importance of coverage[11] and compliance[12], these factors had not been assessed previously in PC for schistosomiasis and STH in Zanzibar. Therefore, the studies described below were undertaken to provide data on these variables during the new ZNCP/SCORE initiatives."

5.2 Results

Coverage and compliance was studied during the 2nd round of MDA in the ZNCP/SCORE undertaken in November 2012.

5.2.1. Assessment of coverage

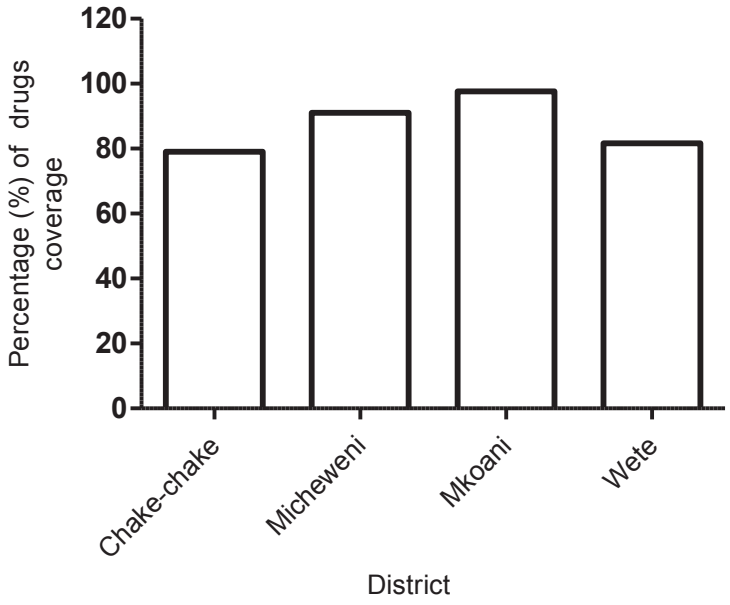
This was assessed from data collected across the whole of the Pemba. Overall, a total of 448891 individuals were registered during initial CDD house visits but only 362255 (80.7%) of them were treated. However the majority, 36490 (42.1%), of the untreated individuals were children who, during the allocation of drugs by visiting CDDs, were deemed to be ineligible for treatment because they were found to be aged <3yrs or below the with shorter height for determining drug dose according to the estimated height indicated on the dose pole The remainder of the untreated individuals had other reasons including moving (19716 [22.8%]) from their actual residence (19716 [22.8%]), being pregnant (4697 [5.4%]), breastfeeding (3898 [4.5%]) or being chronically ill

[11] Coverage is defined as the percentage of individuals in a given population that received drug or drug combination in an intervention area.

[12] Compliance (sometimes referred to as adherence) is a term used to denote a degree to which a client correctly follows advice. In this study it indicates how correctly the individuals followed instructions from drug distributors.

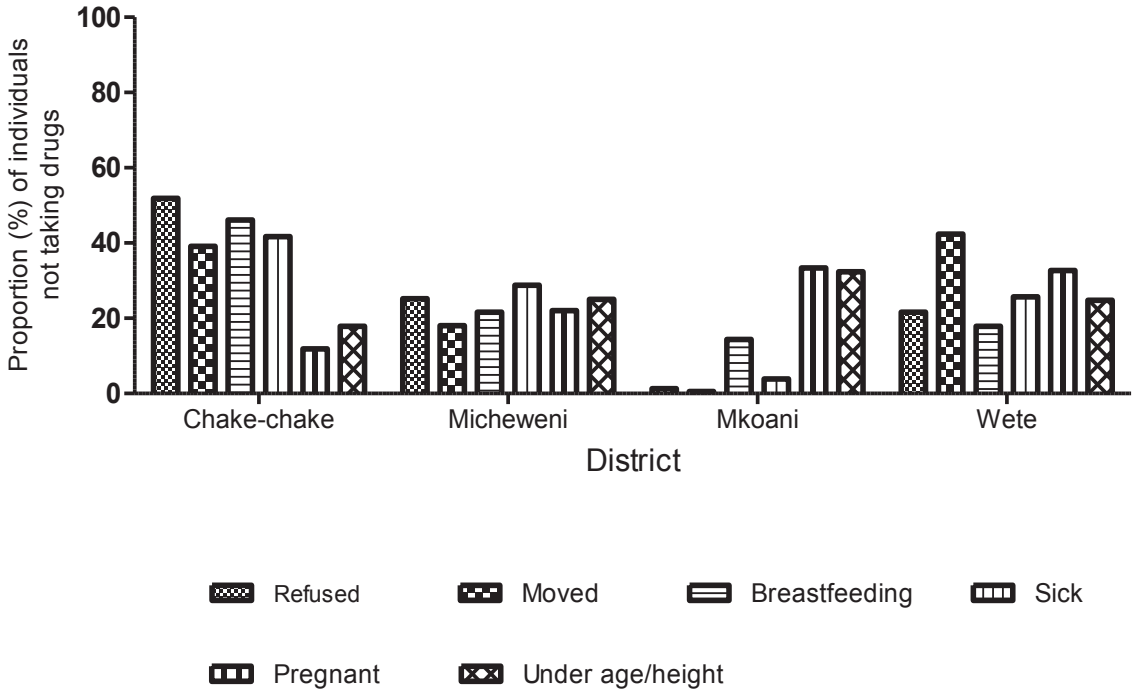
(2519 [2.9%]) at the time of drug delivery. Others, (22.3%), refused to accept the drugs from CDDs. 5.4.2 Geographical coverage. Figure 52 illustrates drug distribution coverage across the various Pemban districts. This shows that Chake-chake district had the lowest drug coverage as compared to other districts. Similarly, Chake-chake had high proportions (69.4%), (39.1%), (46.1%), (41.7%) of those individuals denied the drugs from CDDs, moved from their locations, breastfeeding and those that had chronic illnesses, respectively (Figure 53). The reasons for the overall lower coverage and increased proportion of individuals who did not take the drugs for various reasons at Chake-chake district are unclear but increased population movement due to the presence of many government offices and businesses along with temporary mobile residences may have roles in this.

Figure 52: Level of drug coverage across different districts in Pemba achieved during MDA in 2012



It is also notable that there was a very low drug refusal rate. The reasons for this were not obvious. Drug coverage data for individual shehias is not presented here but will be analysed further.

Figure 53: Distribution of individuals not taking the drugs provided during MDA in Pemba for various reasons in 2012



5.3.1 Assessment of Compliance

Following drug distribution, a post mass drug administration (MDA) survey was undertaken in 15 shehias in Pemba to assess possible factors involved in compliance/non-compliance and potential adverse events (AE). A total of 1464 individuals were enrolled in the study. Among these 679 (46.4%) were male and 785 (53.6%) were female. The individuals had a mean age of 30.4yrs (95%CI: 29.6-31.2) with a range of 10-91yrs. Table 11 illustrates the shehias participating in the survey.

Table 11: List of shehias involved in the post MDA survey

S/No	Shehia name	No .participants
1	Chokocho	100
2	Kangagani	100
3	Kizimbani	101
4	Kojani	93
5	Konde	99
6	Makangale	100
7	Mgelema	99
8	Michenzani	99
9	Mjini Wingwi	100
10	Mtangani	78
11	Muambe	97
12	Shengejuu	102
13	Shumba mjini	99
14	Wawi	99
15	Wesha	98

5.3.2 Drug compliance

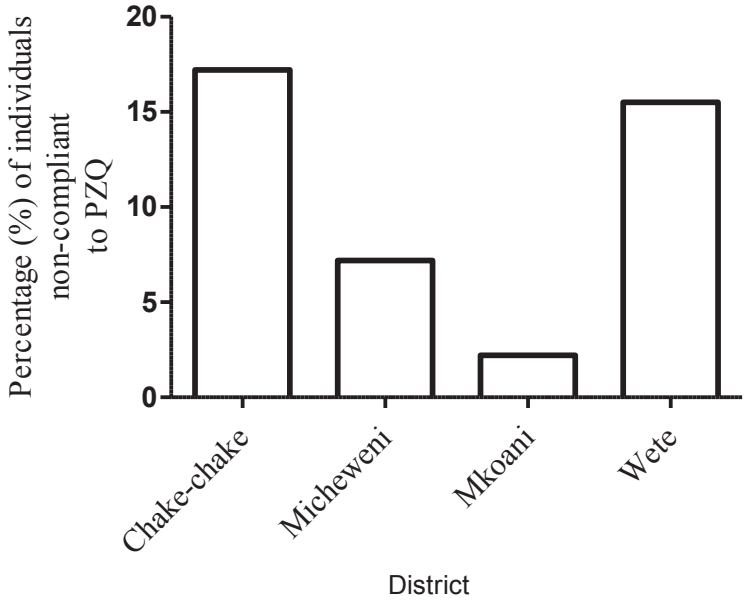
5.3.3.1 Praziquantel compliance

For this analysis only 1438 (98.2%) of the enrolled individuals are included as there was an inconsistency between the number of tablets given and does reported as that taken. Of these, 146 (10.2%) were non-compliant to PZQ. These individuals were either did not take the drug at all or did not take the prescribed amount of tablets provided to them or did not take the drugs according to the WHO guideline. Overall there were 58 (8.8%) males and 88 (11.3%) males and females who respectively that were non-compliant to PZQ, the difference not being significant. Although, further analysis revealed that there was no association of PZQ non-compliance between sex ($\chi^2 = 2.6$ df 1; $p = 0.1$).

5.3.3.2 Praziquantel compliance in relation to districts

Figure 54 indicates the proportions of individuals non-compliant to PZQ in relation to the districts. Quite marked differences in compliance for the different areas were seen, being high in Mkoani and lower in Chake-chake and Wete. The reasons for this were not apparent.

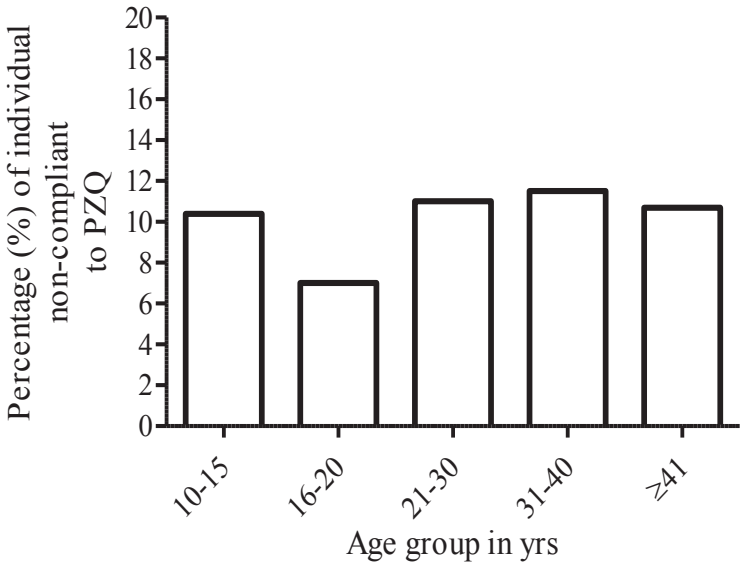
Figure 54: Proportion of individuals non-compliant to praziquantel drug



5.3.3.3 PZQ compliance in relation with age group

This analysis includes only 1431 (97.7%) of the enrolled individuals as unfortunately for some of them age was not recorded. Interestingly the percentage of individuals non-compliant to PQZ was generally similar between the different age group (Figure 55). Although, was slightly lower in the age group between 16-20yrs.

Figure 55: Proportion of individuals non-compliant to PZQ in different age group



5.3.3.4 Compliance with PZQ relative to knowledge on schistosomiasis and its risk behaviours.

Drug compliance was also assessed in relation to the treated individual's knowledge about schistosomiasis and its associated risk behaviours which was considered might influence drug uptake to individuals given the drugs in mass treatment campaigns. Of the 1462 respondents, 1067 (73%) were aware of schistosomiasis. However, this did not significantly alter compliance (110 [(10.5%]) compared to 35 [9%] non-compliant to PZQ. Indeed, it shows that there is no statistical association between schistosomiasis awareness and PZQ compliance ($\chi^2 = 0.74$, df 1; $p = 0.3$).

Further analysis of PZQ compliance in relation to awareness of previous treatment for schistosomiasis revealed that among 1437 responded regarding previous treatment for the disease, 881 (61.3%) reported had never having been treated, 540 (37.6%) reported having been treated and 16 (1.1%) were unaware i.e. they did not know if they had previously received treatment for schistosomiasis. Overall, 63 (7.3%), 80 (15.1%) and 2 (12.5%) of those reported to be previously untreated, treated and unaware (who did not know if treated for schistosomiasis) respectively, were non-compliant to PZQ and this was statistically significantly by Chi square ($\chi^2 = 22.1$, df 2; $p = 0.0001$), although, in univariate analysis using a logistic regression model, it revealed that there was no significant association between PZQ non-compliance and having being treated previously (odds ratio [OR] =1.0; 95%CI = 1.0-1.01; $p = 0.6$).

Regarding compliance in relation to previous haematuria, 1446 individuals responded on whether they had ever experienced haematuria during their life time. Among those 1211 (84.8%) reported never having had haematuria, 225 (15.6%) reported having had haematuria and 10 (0.7%) were unaware of ever having experienced haematuria. Of those who had not experienced haematuria, 113 (9.5%) were non-compliant to PZQ. Similarly, among those having experienced haematuria, 29 (13.2%) were non-compliant to PZQ. Interestingly, none of the individuals unaware of having haematuria were non-compliant to PZQ, although the actual number of respondents in this category was very low. There was no association between experiencing with haematuria and compliance to PZQ ($\chi^2 = 3.9$ df 2; $p = 0.1$).

With regards to health status of the individuals prior to MDA medication, of the 1433 respondents with a compliance record, 1317 (91.9%) had good health and 116 (8.1%) were unwell. Among those with good health, 130 (10%) were non-compliant to PZQ. Similarly, of those who were unwell, 14 (12.1%) were non-compliant to PZQ. Further analysis reveals no significant association between PZQ compliance and health status ($\chi^2= 0.6$, df 1; $p = 0.5$). Additionally, individuals were asked if they had had any of the NTD diseases controlled through MDA (STH, schistosomiasis or LF) during their lifetime. Of the respondents, 215 (16.6%), 1046 (81%) and 31 (2.4%) reported to have any of those diseases, not having the disease or did not know, respectively. This was significantly associated with non-compliance according to Chi-square analysis ($\chi^2 = 6.0$, df 2; $p = 0.05$). When looking specifically at schistosomiasis, the proportion of PZQ non-compliants were 9.9% for those without disease, 14.4% for those had disease and 19.4% for those did not know. Nevertheless, logistic regression analysis did not demonstrate a significant association between having the NTD disease and non-compliance to PZQ (OR =1, 95%CI for the OR =1.0-1.001; $p = 0.1$).

5.3.4.1 Albendazole compliance

For logistical reasons similar assessment of drug uptake in relation to knowledge of risk factors associated with transmission of STH, and of previous treatment history with albendazole was not studied. This analysis only focused with compliance with albendazole distributed during the MDA.

The same number of individuals (1438) included in the analysis of PQZ compliance is also used in the analysis of ALB compliance. Of those, only 36 (2.5%) were non-

compliant to ALB. The percentage of males and female who did not comply was comparable (3.0% [males] vs. 2.1% [females]). Non-compliance to ALB was not associated with sex (χ^2 =1.2 df 1; p = 0.2).

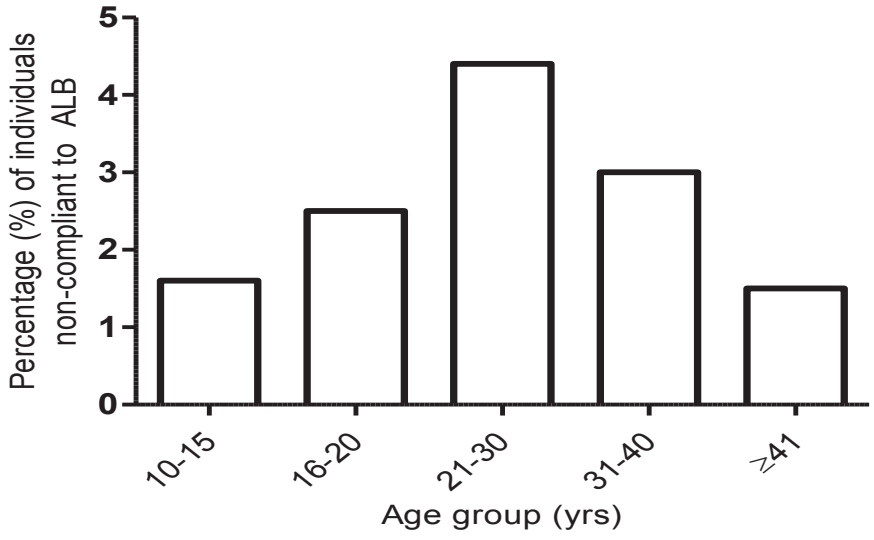
5.3.4.2 Albendazole compliance in relation to districts

With regards to the analysis of ALB compliance in relation to the districts, it was found that Wete district had the highest proportion (6.5%) of non-compliance as compared to other districts. The proportions of ALB non-compliance for other districts were 1.7% and 1.5% for Chake-chake and Micheweni respectively and 0% for Mkoani district. The reasons for this small difference in non-compliance rate across the districts are unclear and could be difficult to establish but the most plausible reasons could be greater awareness and self-motivation.

5.3.4.3 ALB compliance in relation to age group

Figure 56 shows the non-compliance to ALB. The figure indicates that the percentage of individuals non-compliant to ALB was broadly comparable between different age groups although it was slightly higher in the 21-30yr age group.

Figure 56: Proportion of individuals non-compliant to ALB in different age groups



5.4 Adverse Events

The analysis of adverse events[13] (AE) included 1449 (99.0%) out of 1464 individuals enrolled. Among those, 192 (13.3%) reported experiencing AE. The list of different AEs shown to participants is illustrated in Table 12.

Table 12: Number of individuals experiencing different adverse events (side effects) during MDA.

Signs/Symptoms	No. individuals with AE	(%)
Dizziness	123	(9.3)
Sleepy	35	(2.8)
Abdominal pain	42	(3.4)
Nausea	66	(5.3)
Diarrhoea	5	(0.4)
Sweating	5	(0.4)
Low back-pain	3	(0.2)
Headache	56	(4.5)
Tiredness	43	(3.4)
Cramp	6	(0.6)
Vomiting	11	(0.9)
Blood in faeces	1	(0.1)
Night fever	2	(0.2)

Numbers in parentheses are percentages.

From this table it is clearly observed that there were small proportions of individuals who experienced one or more adverse event as a result of consumption of the drugs. However, it was not established which among the two drugs was associated with those signs/symptoms. The majority (103 [62.0%]) of individuals experiencing any AEs reported symptoms happening during the first day of drug distribution and only a few

[13] Adverse Event is defined as medical incident that takes place following receiving of medical intervention (such as drug(s) or vaccination) that is suspected to be, but it is not necessarily - caused by the medicine used in the intervention".

individuals reported onset of AE between the second day and one week after drug distribution. All AEs resolved without requiring any medical attention. No perceived life-threatening AEs were observed during the campaign.

5.5 Discussion

5.5.1 Drug Coverage

This is the first study to be conducted in Zanzibar since 2006 that analyses in some detail the National coverage of an NTD Control Programme. Analysis of the National data set for coverage as reported by CDDs, showed high drug coverage overall (80.7%) but there were quite marked differences between the districts (69.3-88.2%). Notably, Chake-chake district had the lowest coverage rate with the lack of coverage due to: a high proportion (69.4%) of the overall individuals refusing the drugs from CDDs; having moved (39.1%) from their residence; high pregnancy rate (46.1%) and chronic illnesses (41.7%). In contrast, in Mkoani district<2% of individuals either denied the drugs or had moved from their residence. The reasons for the overall lower coverage and increased proportion of individuals who did not take the drugs for various reasons at Chake-chake district are unclear but increased population movement due to the presence of many government offices and businesses along with temporary residences may have a role in this. Other studies have reported that individuals living in the urban settings are most likely to resist treatment -resulting to lower coverage than in the rural areas (Ranganath and Reddy, 2012). Although the entire Pemba Island is considered as rural, it is certainly that case that Chake-chake is the most "urban" area of the island. The possible explanations for motivation or de-motivation of individuals to accept drugs during MDA have been considered by others. In some countries, political persuasions (Parker and Allen, 2013), misconceptions about the drugs delivered under MDA (Joseph et al., 2011), mistrust of international aid, relationships between CDDs and the drug recipients, and other social aspects have been linked with low coverage (Parker and Allen, 2013).

In spite of the regional variation in coverage, the overall finding of 80.7% coverage is encouraging as it is higher than that found is some other studies e.g. in the Philippines (48.3%) and Kenya (58.2%) (Tallo et al., 2008, Mwinzi et al., 2012). However, these latter studies were more restricted than the current one which involved 121 shehias with several villages whilst the Philippine and Kenyan studies covered only 50 and 25 villages, respectively. In fact, in this setting, most of the CDDs have been repeatedly involved in the distribution of PC based drugs, PZQ and ALB, during MDA campaigns; hence they have gained reasonable knowledge, experience and became more organised

and accepted in the communities. These reasons have, perhaps, contributed to higher coverage rate demonstrated in this study.

Despite the apparently high coverage achieved it should be noted that the drugs were delivered house-to-house but the actual swallowing of the drugs was not witnessed as direct observed therapy (DOT) was not incorporated in the programme. Therefore, it is unverified whether this programme coverage coincided with therapeutic coverage (i.e. the proportion of individuals who swallowed the drugs). Since drug taking was not witnessed it is possible that a considerable number of individuals were untreated and hence increase the potential of disease transmission. Furthermore, there is evidence that it is challenging to obtain the comprehensive understanding of the proportion of adult population who were taken the drugs delivered under MDA (Parker and Allen, 2013). The unexpected low drugs coverage experienced in community-based treatment during MDA campaigns has raised concerns over the effectiveness of this strategy particularly if schistosomiasis elimination is targeted (Tallo et al., 2008).

Since helminth control using MDAs is likely to continue in Pemba, consideration must be given to possibilities to improve coverage further. In particular consideration should be given to the reasons for the poor coverage in certain shehias. Certain causes, notably population mobility, as observed in Chake-chake, are more difficult to overcome but other aspects related to compliance could perhaps be improved. Further work could be done to establish possible reasons associated with low coverage in subsequent MDAs. It is relevant that studies have demonstrated that SBT usually attain high drug coverage as compared to community-based treatment (Kabatereine et al., 2006, Mwinzi et al., 2012). For example, in Uganda, coverage of 91.4% in schools compared with 64.7% in communities has been demonstrated (Kabatereine et al., 2006). Other studies have suggested that community sensitization i.e. meeting with community members to discuss about MDA program aiming at increasing their awareness regarding the target disease, impact of the intervention(s) or any expected side effects would improve coverage (Fleming et al., 2009). Unfortunately, in this setting, community sensitization directed towards MDA, in most cases, has not been undertaken comprehensively.

The studies on compliance discussed below were intended to give further insight into coverage issues.

5.5.2 Drug compliance - PZQ

There is overwhelming evidence that for chemotherapy based control strategies such as that implemented for schistosomiasis and STH infections, compliance (adherence) to treatment is fundamental for ensuring and sustaining good coverage (Joseph et al., 2011). In this study we have assessed compliance with single dose ALB and PZQ delivered through an MDA campaign complemented by other control measures in fighting against STH infection and schistosomiasis that are currently underway in Zanzibar. Overall, 10.2% of individuals were found to be non-compliant to PZQ but there was remarkable variation between the districts similar to what has been detected in coverage. The reason for the lower proportion of PZQ non-compliance observed in Mkoani district is unclear but could be related to the high prevalence of schistosomiasis infection there resulting in increased awareness of the consequences of the infection. As described previously, there is limited data regarding compliance to PZQ and ALB in the context of MDA but other studies have demonstrated similar figures i.e.11.1% and 14.7% for non-compliance to PZQ syrup and tablet formulation respectively (Navaratnam et al., 2012). Nevertheless, the latter study was carried in young children aged between 1-5yrs who are likely to be distracted and ready to cry resulting in non-compliance. Other factors have been related to poor compliance in the wider age range such as the characteristic nature (choking smell, size of the tablet and taste) of PZQ, fear of the occurrence of side-effects (Cao et al., 2011) mistrusting of dose pole (as measures height instead of weight) to define dosage (Fleming et al., 2009, Parker et al., 2008, Parker and Allen, 2011). The fear of development of side-effect of drugs, for instance PZQ, has been associated with low compliance (Woelfle et al., 2011, Parker et al., 2008, Fleming et al., 2009).

As mentioned it has been hypothesized that side-effects resulted from ingestion of PZQ can hamper compliance (Cao et al., 2002). In this current study investigation of side-effects related to consumption of PZQ revealed a wide range of symptoms. Dizziness, abdominal pain, nausea, headache and tiredness were the frequent symptoms experienced by most individuals. The less frequent treatment-attributed symptoms were bloody diarrhoea, sweating, abdominal cramp, vomiting and night fever. None of these symptoms persisted or threatened an individual's life or led to hospitalization. The side-effects reported here correspond to those described by other workers (Erko et al., 2012, Berhe et al., 1999, Garba et al., 2013) and are reported to be avoided if the drug is taken

along with food (Muhumuza et al., 2015). In addition, our study population has been subjected to several rounds of PZQ and ALB treatment and given the low intensity of helminth infection, side effects which are mainly due to the allergens of dying of parasites, were minimised compared to naïve endemic areas with high intensity of schistosomes and STH infections (WHO, 2011a).

It was postulated that knowledge of the disease (transmission or signs and symptoms) would increase drug compliance, however; this was not necessarily the case in the current study which showed overall 10.5% of those with knowledge of schistosomiasis were non-compliant to PZQ. Possibly such individuals did not see treatment as important for them for different reasons including apparent absence of the disease or not considering themselves as exposed to the infection in terms of engagement with risk behaviour for the disease. This might in fact be more or less true as the MDA is carried in the whole community regardless of infection status and to all individuals at risk of infection. The linkage of the absence of overt disease and non-compliance to drugs has been demonstrated by other workers (Christensen, 1978). The proportion of individuals who were unaware of schistosomiasis and were non-compliant to PZQ was comparable to those with awareness and there was no association of awareness of schistosomiasis and compliance. These findings are in agreement with those observed by (Nujum et al., 2012) and highlighted the need for increasing and consolidating efforts focusing on awareness campaigns and the importance of treatment among the community members.

MDA directed to the control of schistosomiasis focuses on a wide population at risk of infection, although, the infection is predominantly found in school-children aged between 5-15yrs. Awareness of this age/intensity relationship might increase sentiments and beliefs among the adult population that they are free of infection and so more likely to be non-compliant to treatment. Greatest compliance was found in the age-group 16-20yrs (93%) but lower again for the younger age-group (≤15yrs). It is possible that this younger age group were influenced by their elders who were not compliant as it is very likely that if parents resisted treatment they may also be unwilling for their children to be treated. Overall, these results are not in agreement with those observed by (Brieger et al., 2011) and (Abd Elaziz et al., 2013) who found high compliance rate among individuals' ≥24yrs of age. Further, wider, study would be needed to confirm if the age pattern reported above is true and to establish the reasons if so.

5.5.3 Drugs compliance - ALB

Schistosomiasis and STH infection usually co-exist in most schistosomiasis-endemic countries and for this reasons WHO has urged endemic countries to take advantage of integrated implementation to control more than one NTD with the PC strategy (WHO 2011). Unlike schistosomiasis where in some countries like in Zanzibar there is an attempt towards eliminaton, the objective for STH remains morbidity control through provision of BZ derivates. Todate, in many endemic countries, ALB is the most common antihelminthic drug used for STH morbidity control based on its efficacy against a variety of STH species. Currently, Zanzibar is focussed on controlling STH using MDA including administration of ALB, and therefore the assessment of drug compliance is of utmost importance. This is believed to be the first study carried out in this setting to assess compliance of individuals towards PC-based control for STH. Overall, a high ALB compliance rate (97.5%) was found across the island with some district (Mkoani) reaching 100%. The particularly high compliance rate for ALB relative to PZQ is believed to be related to: the relatively sweet and aromatic smelling characteristic nature of ALB, the fact that treatment involves taking a single tablet, less fear for the development of side-effects, and awareness of the higher prevalence of STH infection in the societies.

This present study found an overall very promising coverage in the MDAs running in Pemba. However, the lower than average coverage in some areas may hamper acheiving the expected program goal, interruption of disease transmission and ultimately elimination. Furthermore there was a significant proportion of individuals who did not take the drugs for various reasons highlighting the need to strengthen community mobilization strategies so as to increase coverage and adherence and hence eliminate residual untreated individuals. Additionally further studies are required to establish the demotivation factors associated with low coverage.

Chapter 6 Investigation of the transmission of *S. haematobium* infection in infants <6yr

6.1 Introduction

Studies have demonstrated that in endemic countries, school children aged between 5-15yrs are the most infected population (Hotez et al., 2006a) and harbour heavy disease burdens (Chan et al., 1999, Betson et al., 2010). Thus, control efforts to combat the disease are focused in this age group (Betson et al., 2010). However, a few studies have indicated the importance of schistosomiasis in young children before the age of school. Most of these have been concerned with *S. mansoni* infection e.g. in a study carried out in Uganda, it was shown that 27.2% of pre-school children (mean age 3yr, range 4 months to 6.5yr) had infection (Betson et al., 2010). However, a study on *S. haematobium* was conducted in Mali and found that 51.2% of the pre-school children (mean age 2.9yr, range 1-4 years) were infected (Dabo et al., 2011). For decades, Zanzibar has been identified as an endemic country for *S. haematobium* infection but only one small study has been carried out in preschool children there, on the Island of Unguja, and it showed a very low prevalence (3.9%). No such study has been carried out in Pemba and so the aim of this work was to assess the prevalence of *S. haematobium* infection in a relatively large cohort of pre-school children so as to inform decisions on the target population for the introduction of the ZNCP/SCORE MDA described above. In parallel to the parasitological testing, a further sub-study involved assessing the use of haemastix for diagnostic and morbidity indications in this infant population.

It was also of interest to get some insight into the likely circumstances associated with exposure to schistosome infection in the infants and so information was gathered from the mothers by means of a questionnaire.

6.2 Results

6.2.1 Assessment of the prevalence and intensity of S. haematobium *in infants*

A community survey was carried out in pre-school children living in seven *shehias* around the cohort 24 schools (|Chapter 3: survey_1) found to have high schistosomiasis transmission. The list of the *Shehia* with the number of children enrolled is shown in

Table 13. A total of 911 children were enrolled in the study. The children were aged between 24-71months (mean 3.8 yrs, median 4yrs) according to birth certificate/reported age by parents. Amongst the children 407 (44.7%) were girls and 504 (55.3%) were boys. 909 (99.8%) of the children produced urine samples.

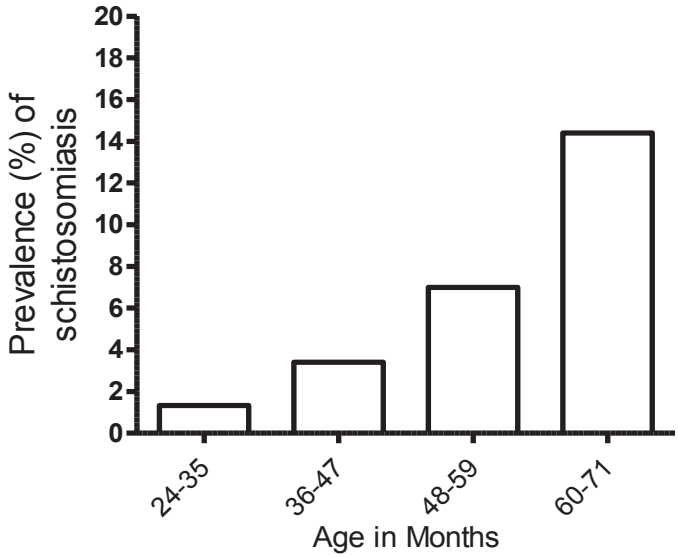
Table 13: List of Shehias surveyed for determination of schistosomiasis in pre-school children in Pemba

Shehia	N	(%)	School surrounded
Chambani	132	(14.5)	Chambani
Kiuyu Minungwini	131	(14.4)	Kiuyu Minungwini
Mgagadu	131	(14.4)	Mizingani
Mtambile	129	(14.2)	Mtambile
Piki	130	(14.3)	Piki
Uwandani	130	(14.3)	Uwandani
Ziwani	128	(14.1)	Ziwani

6.2.2 Prevalence of infection

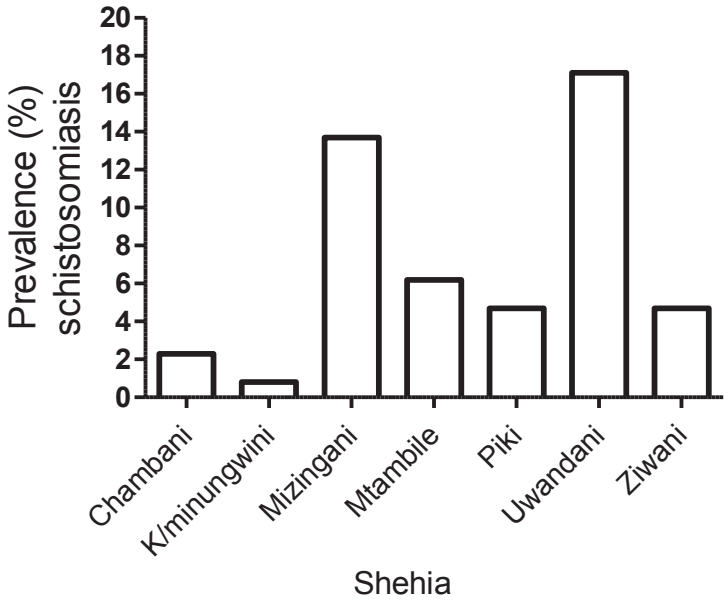
Schistosomiasis was found in 64 (7.04% [95%CI = 5.3-8.7]) of the 909 children (2/150 [1.3%] in the age range 24-35 months, 7/208 [3.4%] (aged 36-47 months), 18/258 [7%] (aged 48-59) and 37/256 [14.4%] (aged 60-71 months). Among the enrolled boys, 37 (7.3% [95%CI = 5.1-9.6]) had schistosomiasis compared to 27 (6.7% 95%CI = 4.2-9.1]) girls. The infection rate clearly increases with age as shown in Figure 57.

Figure 57: Prevalence of schistosomiasis in different age ranges



When comparing schistosomiasis between different villages, Uwandani had the highest prevalence as shown in Figure 58 whilst other areas showed very low prevalences. This pattern generally reflects the prevalences also seen in school-aged children (Chapter 3).

Figure 58: Prevalence of schistosomiasis in under 3-5yrs children in the seven shehias of Pemba



6.2.3 Intensity of schistosomiasis

Among the infected children, 14 (21.9%) and 50 (78.1%) had severe and light infections respectively. The children with light infection had a mean egg count of 12.1 ± SD 10.7 eggs/10ml of urine (range: 1-46 egg/10ml) while those with severe infection had a mean egg count of 185.3 ± SD 175.1 egg/10ml of urine (range: 50-700 egg/10ml of urine). Severity of infection was not statistically associated with sex ($\chi^2 = 0.30$ $p = 0.57$) (35.7 [female] vs. 64.3% [male]). The percentages of severe infections in each of the age bands were: 2yr – 0%; 3yr – 7.1%: 4yr – 42.9% 5 yr - 50%.

6.3 Performance of hemastix for detection of schistosomiasis and evaluation of morbidity (haematuria) in pre-school children

The presence of micro-haematuria was also analysed and its performance for diagnosing schistosomiasis in young children was compared against the gold standard method, microscopy (Table 13).

Table 14: Comparison of urine filtration and haemastix for schistosomiasis diagnosis in children

	Haemastix						
Microscopy	+ve	-ve	Total	Sensitivity	Specificity	PPV	NPV
+ve	50	14	64	78.1	96.4	62.5	98.3
-ve	30	814	847				
Total	80	828					

Of the samples examined, 815 were true negatives (detected negative by the two methods), 14 were false negatives (detected as negative by haemastix method alone), 30 samples were false positive (detected as positive by haemastix method alone) and 50 were true positive (detected as positive by the two methods). The sensitivity and specificity of the haemastix for the detection of S. *haematobium* infection in young children was 78.1% and 96.4% respectively. Additionally, the positive predictive value (PPV) for haemastix was 62.5% and negative predictive value (NPV) was 98.3%. The detection rate difference between haemastix and microscopy was -1.8% (95%CI= 0.2—

3.2). There was a statistically significant difference in the diagnosis of schistosomiasis between the two methods (McNamara's $\chi^2 = 5.8$ $p = 0.01$). The detection of haematuria as indicated by haemastix demonstrates a significant level of morbidity in these young children.

6.4 Investigation of risk factors for infant infection by questionnaire to mothers.

The mothers or guardians of the children enrolled in the survey were questioned about the risk behaviours leading to transmission of schistosomiasis to their children. The questionnaire used is shown in the Appendix 6.1. A total of 911 mothers/guardians were enrolled. The mothers/guardians had a mean age of 32.1yrs with a range of 15-70 yrs (median 30yrs). Among the mothers/guardian enrolled, 9 (1%) had higher education (finished college/university), 229 (25.2%) had secondary education, 293 (32.2%) had primary education, 339 (37.3%) had Koranic knowledge alone and 40 (4.4%) were illiterate. Interestingly none of the child whose mother/guardian had higher education was infected with schistosomiasis although this relates to just 9 mothers. Of the children whose mothers/guardians had Koranic knowledge alone, were illiterate, had primary or secondary education, respectively, 26 (7.7%), 3 (7.5%), 23 (7.9%) and 12 (5.2%) were infected with schistosomiasis. Based on this data there was no statistically significant association with educational experience ($\chi^2 = 2.4$ $p = 0.67$). Surprisingly, the majority of mothers/guardians of the infected infants (58 [90.6%]) had heard about the disease and indeed there was no significant difference in infection prevalence whether the mothers reported knowing how the disease is transmitted or not (Table 15). However, Table 15 also clearly demonstrates the heightened risk from water contact but the data did not identify any significantly greater risk associated with different types of water contact and did not establish a link with frequency of water contact.

Table 15: Risk behaviours of the mother/guardians associated with transmission of schistosomiasis

Risk factor	N	%	Children infected		Children not infected				
			N	%	N	%	OR	P	95%CI
Knowing how disease is transmitted									
Yes	376	41.5	28	7.45	348	92.55	1.1	0.69	0.7 - 1.9
No	532	58.5	36	6.8	496	93			
Use pond/stream water for washing utensil									
Yes	250	27.6	31	12.4	219	87.6	2.67	0.0001	1.6 - 4.5
No	656	72.4	33	5.03	623	94.97			
Use pond/stream water for washing clothes									
Yes	453	49.9	46	10.15	407	89.85	2.7	0.0003	1.6 - 4.8
No	455	50.1	18	4	437	96			
Go to pond/stream with their children									
Yes	358	39.4	47	13.1	311	86.9	4.7	0.00001	2.6 - 8.5
No	550	60.6	17	3.1	533	96.9			
Frequency of going to pond/stream with their children									
Every day	160	17.7	22	13.75	138	86.25	0.8	0.8	0.07 – 6.6
Once/week	9	1	1	11.1	8	88.9			
Twice/week	90	10	10	11.1	80	88.9			
Whenever possible	644	71.3	31	4.8	613	95.2			

6.5 Discussion

The current efforts toward controlling schistosomiasis are largely focused on school-aged children and national control programmes in endemic countries have not involved pre-school children in the treatment campaigns. This has been attributed to (i) insufficient convincing prevalence data as well as safety records on the use of praziquantel in this age population; (ii) practicality and (iii) speculation that younger children are infrequently in contact with freshwater bodies (Mutapi et al., 2011). With recent safety data together with modification of the dose pole used for the delivery of praziquantel (Sousa-Figueiredo et al., 2010b) there has been a growing interest to investigate the prevalence of schistosomiasis in pre-school children worldwide where the disease is endemic (Stothard et al., 2011). We report here the first study to investigate schistosomiasis in this age population in Pemba. Infection was detected in 7.04% of the pre-school children, the majority (78.1%) being light infections but demonstrable in just 2 yr olds. This prevalence is lower than that observed by some workers (Dabo et al., 2011, Garba et al., 2010, Chu et al., 2010) but higher than that demonstrated by others (Mutapi et al., 2011, Sousa-Figueiredo et al., 2008). The latter study was conducted in similar epidemiological setting in Unguja (the other Island that makes Zanzibar). A possible explanation for the observed difference between the present study and that of Sousa-Figueiredo could be related to differences in the number and diversity of the villages/shehia involved. Here seven *Shehias* were investigated which included at least three villages in each (data for village level were not shown) whilst in Unguja three villages in one *Shehia* were studied, albeit in an area historically associated with significant schistosomiasis endemicity (Rudge et al., 2008). There is overwhelming evidence that schistosomiasis is a focal disease and so the restricted sampling may not have provided a representative prevalence value for the wider distribution. Another possibility could be timing (season) on which the investigation has been conducted as schistosomiasis transmission varies with season (Chandiwana and Christensen, 1988). It has been shown that transmission of *S. haematobium* infection often occurs during the hot dry season (Chandiwana et al., 1987). In this instance the hot dry season is between September- November which is the similar time that the present study has been conducted. Nevertheless, this situation might be different in Zanzibar as the dry hot season is between December to early March. A further plausible explanation could be difference in sample size. Sousa-Figueiredo et al. (2008) and

Mutapi et al. (2011) (Sousa-Figueiredo et al., 2008, Mutapi et al., 2011) examined only 102 and 104, respectively, while in the present study 911 children were sampled.

The study showed a clear albeit expected increase in prevalence and intensity of schistosomiasis with age from 2-5 years with older children being more frequently and heavily infected.

6.5.1 Risk behaviour for transmission of schistosomiasis

Contact with freshwater bodies where the snail intermediate host resides has been long recognized as the main risk factor and essential pre-requisite for acquisition of schistosomiasis. In interviews with mothers/guardians of the children participating in the pre-school survey it was found that a substantial proportion of respondents use pond/stream water for various reasons including washing of utensils, clothes or bathing. The data shows a strong significant association of schistosomiasis with these risky behaviours which is consistent with other studies (Lima e Costa et al., 1987). In the present study we found that frequency of water contact for mothers/guardians who accompanied their children in water bodies was statistically associated with acquisition of schistosomiasis by the children. Children whose mothers reported visiting water bodies with children everyday were more likely to be infected (13.75%) than mothers who visit once/week (11.1% [the absolute number of mothers in this category was extremely low [1]), twice/week (11.1%) and whenever possible (4.8%). However, the Mantel-Haenszel analysis revealed low odds ratio (OR = 0.8, $p = 0.8$). Notwithstanding, visiting of mothers along with children to water bodies put children at risk of acquiring schistosomiasis as children are inclined to want to spend significant time in the water. However, in the present study exposure times (duration) and frequency of individual mother's water contact, which could be assumed as a proxy for their children, were not investigated. Nevertheless studies show that women spend much time in contact with water bodies (Sow et al., 2011, Scott et al., 2003) regardless of season (Sow et al., 2011). Similarly, an association was observed between schistosomiasis and low education or illiteracy; none of the child whose mother/guardian had completed university/college had schistosomiasis. This observation is in agreement with that of (Firmo et al., 1996).

6.5.2 Usefulness of hemastix for the diagnosis of schistosomiasis in pre-school children

The use of haemastix (chemical reagent impregnated on absorbent paper) to detect the presence of blood in urine has been widely applied as an alternative, indirect and rapid diagnostic aid for schistosomiasis in endemic areas and has been used in Pemba to direct treatment in individual cases and in treatment campaigns (Savioli and Mott, 1989). Despite its usefulness in certain settings e.g. where parasitological diagnosis is lacking there are drawbacks i.e. its performance is influenced by age and sex as well as intensity of the infection (Etard, 2004). Also, it has been reported that haemastix have high sensitivity (97.8%) but low specificity (58.8%) in children aged between 5-10yrs (Robinson et al., 2009) whilst Kotb and colleagues (1986) have reported sensitivity of 85.5% and specificity of 94.4% for children aged between 6-15yrs. (Kotb et al., 1996). In the present study a high sensitivity (96.4%) but somewhat lower specificity (78.1%) was seen in the pre-school children aged 2-5yrs. This sensitivity was lower than the 100% sensitivity found in the 7-11 year olds in Std-1 (data not shown). The somewhat lower sensitivity may be related to the younger children having less bladder damage due to the shorter period of infection and so lower egg-induced blood loss. Overall, the findings support the earlier observations, above, that haemastix is a relatively reliable and useful tool in younger children.

In view of our findings, it is clear that, even in Zanzibar setting, children become infected with schistosomiasis at a very young age. It is difficult to assess the impact of infections in this pre-school age population on maintaining transmission but they should be considered in the planning of PC MDA programmes. Given that the ZNCP/SCORE programme in Pemba was angled towards elimination of transmission, the above demonstration of significant prevalence in the pre-school children in Pemba led to inclusion of the 3-5 year old children in the MDA programme. Praziquantel is currently only available in pill form and a relatively large dose is required so its administration to small children is far from ideal. This combined with the increasing awareness of infection levels in young children has led to a widespread call for development of paediatric formulation, suitable for younger children <5yrs. The pharmaceutical company, Merck Serono, who currently manufactures and donates praziquantel for control programmes, is striving hard to develop a paediatric PZQ formulation https://www.merckgroup.com/en/company/responsibility/our-strategy/health/schistosomiasis.html)

Chapter 7 Transmission dynamics of *Schistosoma haematobium* infections, and characterization of *Bulinus* populations prior to the implementation of the SCORE project

7.1 Introduction

Schistosoma haematobium is a blood fluke that is responsible for urogenital schistosomiasis and is predominantly found in Africa and the Middle East. Current estimates show that approximately 150 million people are infected with this parasite (Abbasi et al., 2007). Despite how widespread this parasite is, its transmission is considered to be focal and is dependent both on the presence of a suitable (or susceptible) intermediate snail host and fresh water contact. Additionally, environmental, ecological and socio-economic factors play a large role in transmission (McManus et al., 2010). Climatic changes may also favour extended transmission of schistosomiasis (Mas-Coma et al., 2009). For some time, the world has been experiencing global warming which can lead to a disruption in temperature and rainfall patterns (Martens et al., 1995) and the aquatic environment (Mas-Coma et al., 2009). Studies have demonstrated that increased rainfall leading to temporary surface water bodies, such as ponds and streams which constitute snail habitats and breeding sites can increase the snail's range (Dennis et al., 1983, Mas-Coma et al., 2009, Cantrell, 1981). Increased temperature also has a direct impact on transmission as it accelerates cercarial development within the snail, and thus their release from the snails (Shiff et al., 1979, Lee and Lewis, 1977, Martens et al., 1995).

It is of importance for any schistosomiasis control programme to establish which snails are responsible for transmission, their geographic location and where transmission is happening. This is vital in the planning and implementation of control measures. It has long been recognized that various *Bulinus* species serve as the intermediate host for *S. haematobium* in different parts of the world where schistosomiasis is endemic (McCullough, 1959). However, *Bulinus* species display differing levels of susceptibility to infection by *S. haematobium* in differing endemic locations. For instance, in Zanzibar and the nearby island of Mafia, *B. nasutus* has been proved as refractory to infection (Stothard et al., 2013a, Stothard and Rollinson, 1997a) whereas in coastal Kenya *B.*

nasutus has been reported to play a role in transmission (Kariuki et al., 2004a). Another example of regional variation in schistosome/*Bulinus* compatibility comes from an experimental study which showed that *B. globosus* from Kinshasa, Zaire were only compatible with *S. intercalatum* from Zaire but not strains from Cameroon (Frandsen, 1979). Therefore, snail-schistosome "incompatibility" and specificity are factors which must be considered with regard to schistosomiasis transmission (Rollinson et al., 2001, Allan et al., 2009).

Traditionally, discrimination of snail species within the *Bulinus* genus was done by assessment of shell and reproductive organ morphology (Vidigal et al., 1998, Carvalho et al., 2001). Nevertheless, *B. globosus* and *B. nasutus* are morphologically very similar and are sympatric in some geographical areas (Barber et al., 2000). Additionally *B. nasutus* can also be infected by other schistosome species i.e. *S. bovis* (Barber et al., 2000) thus reliable, specific and sensitive techniques are required to discriminate these snails and to establish the 'real' intermediate host role. So far, molecular methods have been used for the detection of genetic variability and to elucidate phylogenetic relationships especially using cytochrome oxidase subunit-1 (Cox-1). Allele specific amplification of the ribosomal internal transcribed spacer (ITS) has also been developed to differentiate and characterize *Bulinus* species (Rollinson et al., 2001, Akinwale et al., 2011a, Jorgensen et al., 2013, Kane et al., 2008). Cox-1 is a highly conserved gene and has been utilized in assessing the relationship of parasites as well as their intermediate hosts and their differentiation (Kandil et al., 2010) and is thought to produce superior result to ITS-2 (Kane et al., 2008).

Evaluation of the level of infection in wild snail populations is critical in understanding the distribution of schistosomiasis. This is often carried out by assessing snail populations for patent infections (i.e. the shedding of cercariae). The rate of prepatent infection (snails with immature infections not shedding cercariae) is usually neglected probably because of the lack of suitable, simple detection methods (Hamburger J, 2004), although snails can be crushed between glass plates to observe developing parasites. Surveillance of snail populations is also helpful in the assessment of the effectiveness of the control strategies on parasite transmission (Hamburger et al., 2004, Rollinson et al., 2009). Therefore, specific and sensitive methods for identification of infection in the snails are required. The polymerase chain reaction (PCR) assay based on the detection of *Dra*1 (a tandemly repeated DNA sequence) in the genome of *S.*

haematobium, and loop-mediated isothermal amplification (LAMP) have shown promising results being able to detect a single cercaria (Hamburger et al., 2004, Hamburger et al., 2013). Both conventional PCR and LAMP have proved to be equally sensitive for the detection of schistosome parasite within the snail host (Kumagai et al., 2010).

The ZNCP/SCORE initiatives described in this thesis were aimed at schistosomiasis elimination through integration of snail control by molluscide (niclosamide), mass drug administration (MDA) and behavioural change. Given the importance of understanding which snail species are responsible for transmission, their geographic location and the foci of transmission, malacological surveys were carried out prior to embarking on the implementation of the control programmes in order to: (i) identify potential transmission sites (ii) assess transmission dynamics of *S. haematobium* infection (iii) characterize the *Bulinus* snail hosts involved in transmission.

7.2 Results

In a period of one year (October 2010- September 2011), six potential sites (water bodies) were visited once/month to assess transmission dynamics of *S. haematobium* in the snail intermediate hosts. The sites were arbitrary chosen based on high prevalence of schistosomiasis observed in our baseline survey described above in Chapter 3 (cohort 24 schools, survey_1), historical data and easy accessibility of the areas. Table 11 below illustrates the nature and characteristics of the water bodies in each individual site. Table 7.2 indicates the geographical coordinates of the water bodies visited over a 1yr period. A total of 1831 (mean 32.12/sampling, range of 0-236) *Bulinus* snails were collected from all six sites. The distribution of the snails collected is shown in Figure 60. There was marked variation in the numbers collected from the different sites with Mkungu yielding the highest number of snails as compared to other sites. The reasons for variation in number of the snails collected across different sites could be (i) ecological (with some water bodies had abundant vegetation), (ii) size (iii) nature (stream or ponds) of the water bodies and (iv) seasons- as some of the water bodies are temporary and tend to dry-up during dry seasons. Of the collected snails, only 18 (0.98%) shed cercariae. Of these 17 (94.4%) were from Kangagani and 1(5.6%) was from Mavungwa. Interestingly, most (16 [88.9%]) of the shedding snails were collected at Kangagani between the months of October and December. The only positive snail

from Mavungwa was collected in June. It should be noted that in this small study all shedding snails were recovered from large permanent ponds rather than streams. Table 16 also shows that Kangagani had somewhat different water characteristics than the other sites (lower salinity, TDS and conductivity) but the low number of sites studied means that no firm conclusions could be drawn from this regarding correlation with snail infection rates.

Table 16: Chemical characteristics of the water bodies surveyed for assessment of transmission of schistosomiasis over 1yr period (2010-11)

Sampling Site	Nature	Chemical characteristics					Other characteristics
		Salinity[a]	TDS[b]	Temp[c]	pH	conductivity[d]	
Kangagani	large pond	0.02	55.7	31.1	8.38	110.0	permanent
Kimbuni	stream	0.19	203.8	28.3	7.86	429.9	temporary and dries during dry seasons
Mavungwa	large pond	0.19	177.9	30.4	7.91	350.7	permanent
Mkungu	stream	0.2	209.4	27.7	7.96	433.2	temporary and dries during dry seasons
Piki	stream	0.25	250.6	29.2	7.98	516.8	permanent but large part dries during dry seasons
Vitongoji	large pond	0.12	177.0	31.0	8.01	237.7	temporary and dries during dry seasons

Concentration:

The numbers represent mean values for the various sampling times and the units are: a (%), b (mg/l), c (centigrade) and d (μ/s). TDS = (Total Dissolved Salts)

Figure 59: Potential schistosomiasis transmission areas sampled for snail host characterization

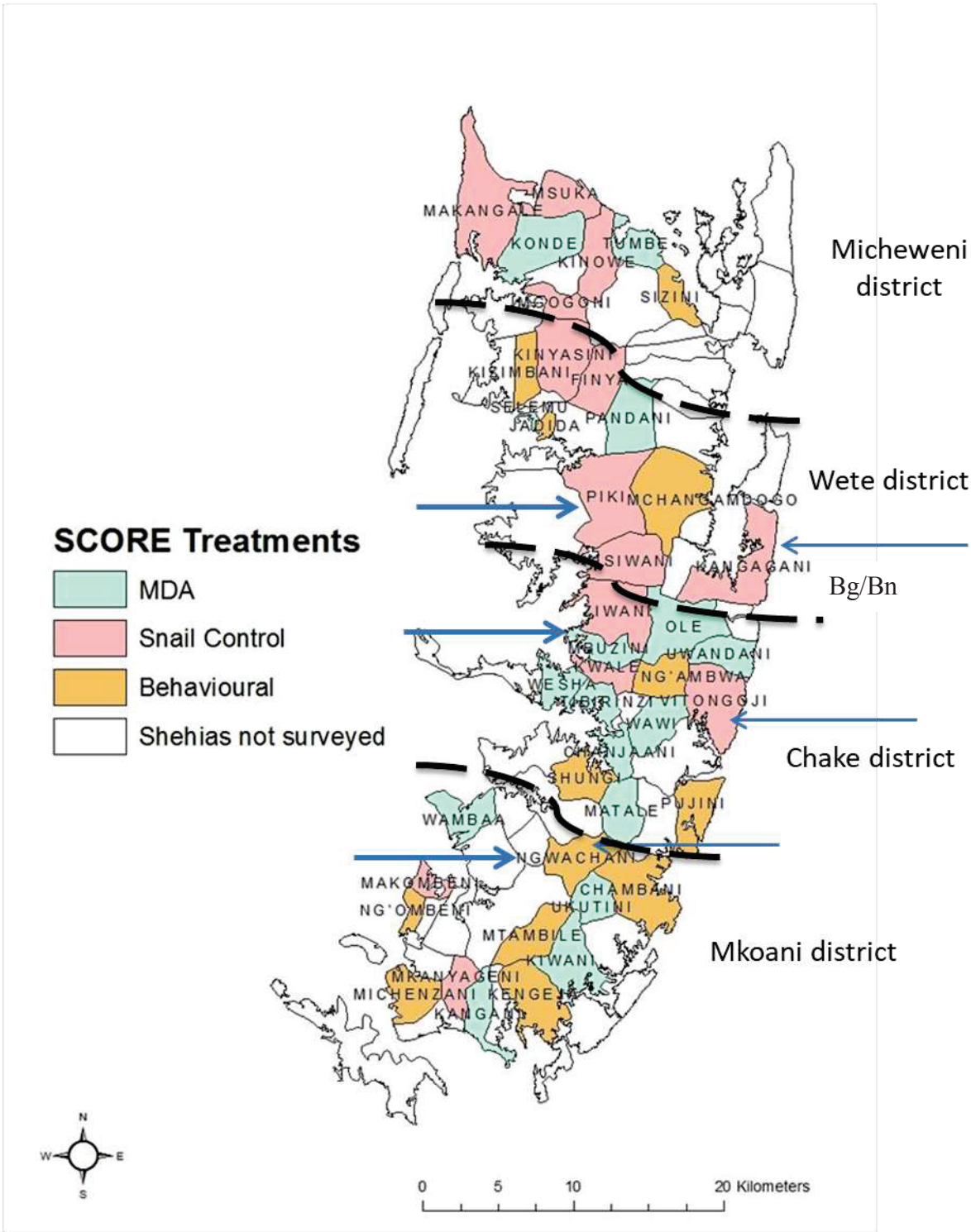
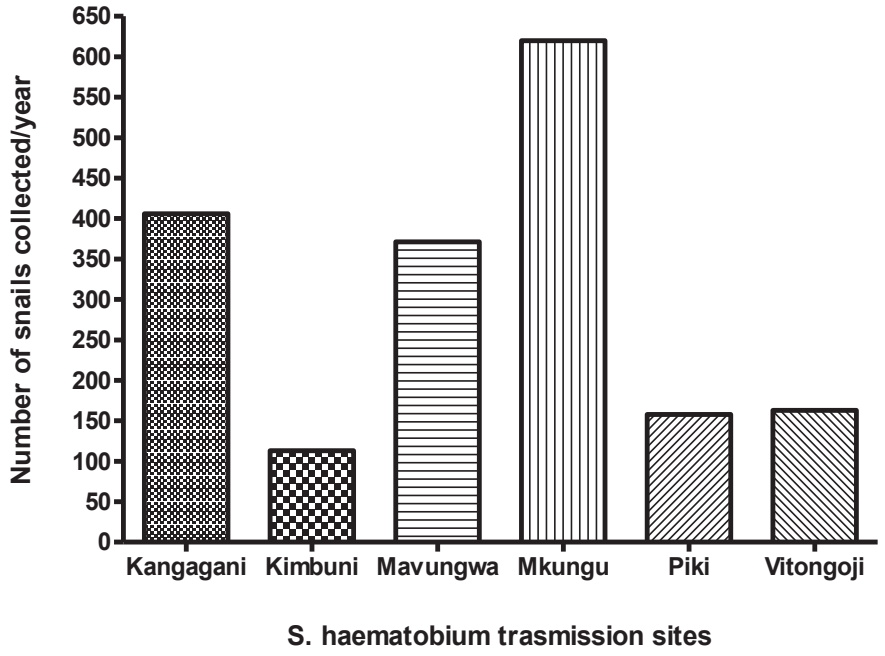


Figure 5 is replicated to allow readily visualization of shehia where snail samples were collected for more detailed analysis

The blue arrows indicate areas used for snail sampling Bg represents *Bulinus globosus* and Bn is *B. nasutus*

Figure 60: Distribution of *Bulinus* snails in different water bodies



7.2.1 Expanded survey for definition of snail sampling sites for use in the SCORE evaluation

In collaboration with Linzy Elton whose field work I supervised during her MSc project at LSHTM in 2011, additional sites were sampled to identify suitable sites for monitoring in the 15 shehias included in the snail control arm (molluscicide) of the SCORE programme. The sites visited are shown below Table 17.

In this study, a total of 52 sites were surveyed across the 15 *shehias* as shown in table 17. The human activities resulting in water contact in these sites was recorded. Bathing and washing clothes were the most common activities, occurring in 90.3% of the water bodies surveyed. 82.7% of water bodies were used for playing (children). The other notable activities were rice farming (51.9%), washing dishes (42.3%), fording (40.3%), fishing (38.4%) and ablution (30.7%). Other, less common activities included use of water for building or gardening purposes (13.4%) and washing cars or bikes and water collection (both 5.7%). The chemical characteristics of water in those areas are indicated in table 18. A total of 281 snails were found in 14 of the sites visited

(highlighted in bold in Table 7.3). Only 3 (1.1%) of the snails collected were found to shed cercariae. The snail positive sites were equally likely to be streams or lakes.

Table 17: Snail sampling sites visited in 2011

Shehia	Water bodies visited
Mgogoni	Kimbondeni, Juda, Kigenda, Makuwe Bondeni, Meno Pembe
Mkanyageni	Makorera, Kwaguni, Taasini bridge
Msuka	Mtowaungi, Kwakipande, Kwamakuku bridge, Meli 11, Kwamwanazabadi
Shumba Viamboni	Badala, Kijimbu Mali, Daragani, Kwamilango, Mtoni Msikitini
Finya	Selem, Mtatagoni, Kilima Punda, Bubujiko
Piki	Panganole, Bagoni, Tongoni, Piki Darajani
Kisiwani	Langoni, Shangafu, Fuoni, Nturuma
Makangale	Ziwalamakangale, Bondo
Mbuzini	Mavungwa, Kwamwanamwari
Makombeni	Kwakinyero
Ziwani	Ziwanimtoni, Kigogo
Kinowe	Kwamwamakuku, Kisange, Kicha
Kwale	Ziwani, Ziwakisasi, Nkibwemi, Kwakidume
Kangagani	Mboje, Mapungwi, Ziwalamkumgini, Kisiwaiwe Kubwa, Chogambwa
Vitongoji	Mkomani, Ziwe Kichanje

Table 18: Chemical characteristics of the water bodies.

Shehia	Number of sites	Temperature (°C)	pH	Salinity (%	TDS (mg/l)	Conductivity (µs)
Finya	4	26.4	7.6	0.0	36.3	58.6
Kangagani	6	26.9	7.8	0.1	81.0	169.6
Kinowe	3	27.8	7.6	0.1	95..7	199.9
Kisiwani	4	25.7	7.7	0.3	278.0	573.5
Kwale	4	26.9	7.5	0.1	141.0	292.6
Makangale	2	28.8	7.6	3.9	43.5	90.0
Makombeni	1	26.0	7.8	0.1	109.1	228.0
Mbuzini	2	27.7	7.6	0.4	216.4	447.5
Mgogoni	5	26.6	8.4	0.2	195.8	404.8
Mkanyageni	3	26.4	7.5	0.0	53.7	96.5
Msuka	5	26.6	7.6	0.1	89.1	186.1
Piki	4	27.8	7.9	0.3	263.0	542.3
Shumba Viamboni	5	26.0	7.1	0.1	78.8	126.6
Vitongoji	2	26.6	7.7	0.1	64.8	135.9
Ziwani	2	26.3	7.2	0.1	105.0	195.2

7.2.2 Identification of S. haematobium *in snails by PCR (Dra-1 gene)*

Due to cost implications and accidental loss of the labelling of tubes resulting from breakdown of snail containers during transfer of the snail samples from Pemba to London, which were fixed in ethanol; it was not be possible to examine high numbers of samples. For reference, complete data relating to all 94 of these snails is given in Appendix 7.1.

DNA was isolated from 96 snail samples of which 94 were tested further. Of these, 18 (19.1%) were snails that had shed cercariae. Figure 61 shows representative PCR results for the Dra-1 repeat (121 bp) from a subset of tested snail samples. For the positive snails the characteristic tandem repeats lead to the ladder appearance (lanes B, C and E) absent from the negative snails (F). Overall, 53 (56.4%) of the snail samples were PCR positive for *Dra*-1, 28 (29.8%) were negative and 13 (13.8%) were undetermined apparently due to high concentrations of template DNA. Of the 18 snails which had been recorded as shedding cercariae after collection, 13 were PCR positive for Dra-1, 4 were equivocal by PCR and one (snail 77 in Appendix 7.1) was negative. Ideally this negative sample 77 would have been repeated along with all equivocal samples (tested using lower DNA concentrations) but such retesting was not feasible in this study for logistical and financial reasons. Nevertheless, the Dra-1 PCR results for the "shedding" snails are not inconsistent with the PCR-defined infection status correlating pretty well with the known shedding status. Interestingly, most (12 [92.3%]) of the samples with unequivocal results were from Kangagani. Indeed 11 (91.7%) were initially found to produce cercariae in the field. Comparing the number of snail samples tested *S. haematobium*-positive by Dra-1 PCR (n= 53) with the numbers that shed cercariae in the field (n=18), clearly indicates a high proportion of snails with prepatent infection.

Figure 61: Agarose gel separation of *S. haematobium* specific Dra-1 PCR reaction products from a representative subset of tested *Bulinus* snails

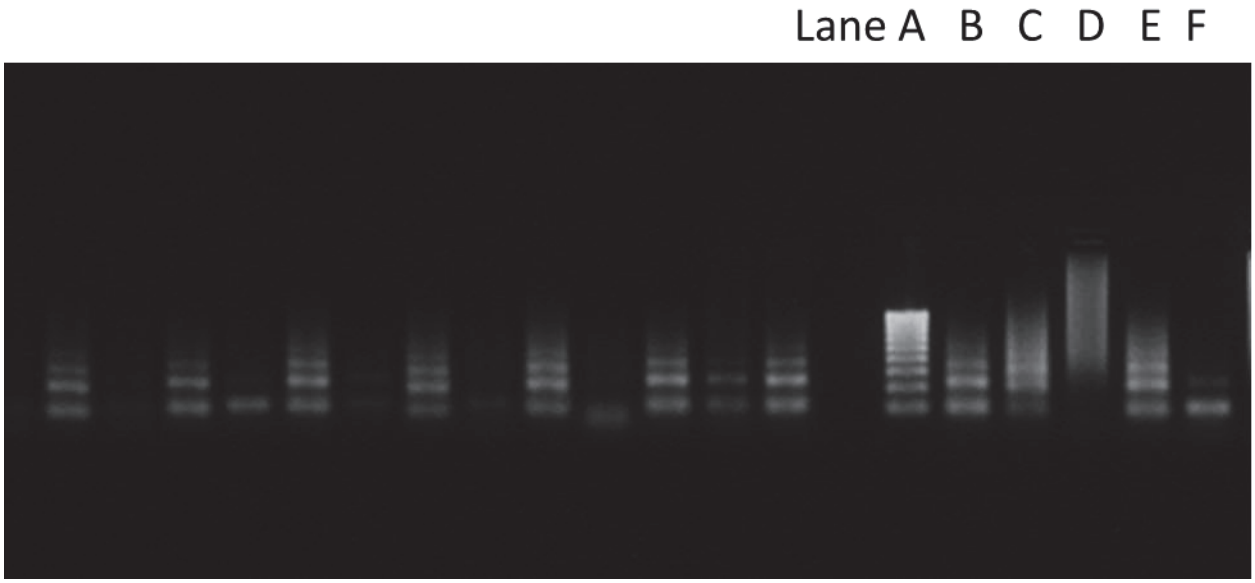


Figure 60 Figure showing a sample of the PCR amplifications of the Dra-1 gene specific for *S. haematobium* from a proportion of the snail samples.

Lanes labelled A-F show examples of A: ladder markers; B,C,E: positive reactions showing the tandemly repeated 121bp subunit indicative of *S. haematobium* infection; D an equivocal result indicating a sample that would require further dilution of test DNA; F negative reaction from uninfected snail. The DNA fragments were separated on 1.5% agarose gel in 1x TAE buffer. The gel was stained with gel-red dye and bands visualised on a UV transilluminator.

Table 19 illustrates the distribution of the PCR (*Dra*1) positive sample in relation to the site of collection. Furthermore, of the *Dra*-1 positive samples, 45(84.9%) failed to produce cercariae when isolated from the field. Only one snail sample that was negative for *S. haematobium* using the *Dra*-1 PCR assay shed cercariae. When comparing the two assessment methods for *S. haematobium* infection in snails, the use of PCR for detection of parasite DNA is far superior to assessment of cercarial production in the field (McNemar's $\chi^2 = 45$; $p = 0.00001$). The Dra-1 PCR assay is therefore valuable for detecting whether any transmission is likely to occur in the habitat under study. It is always possible that snails with pre-patent infections do not

become patent, the snails might die or the snail defence reaction might kill the developing parasite.

Table 19: Positivity rate for the Dra-1 gene specific for *S. haematobium* in snail samples collected from various transmission sites

Site	No. of snails tested	Dra- 1		No.shed cercariae		
		Neg	Pos	Unidentified	Bg	Bn
Kangagani	33	3	18	12	17	1	12
Kimbuni	12	8	4	0	0	0	0
Mavungwa	13	6	6	1	1	1	0
Mkungu	12	2	10	0	0	0	0
Piki	14	2	12	0	0	0	0
Vitongoji	10	7	3	0	0	0	0

Bg = *Bulinus globosus* and Bn = *Bulinus nasutus*

7.2.3 Confirmation of the species of snail hosts by PCR

7.2.3.1 Detection of the Cox-1 (Asmit-1) gene from snail samples:

All the 94 snail samples which were screened for *S. haematobium* using the *Dra*-1 PCR assay were also used in PCR reactions to amplify the Cox-1 gene fragment the Cox-1 gene product was generated for 92 (97.9%) of the snails. The reason for the failure to get PCR product from the other two (white arrows in Figure 61) is not known.

Figure 62: Amplification of the Cox-1 gene specific for determination of *Bulinu*s spp

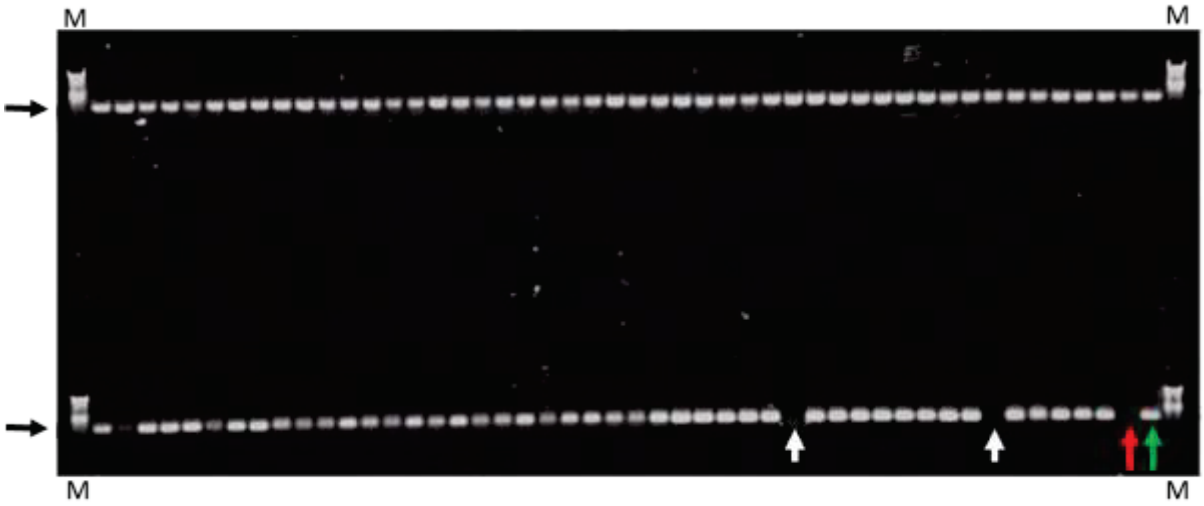


Figure 62 Amplification of the Cox-1 gene specific for determination of *Bulinu*s spp.

Gel showing PCR products derived from the 96 snail samples. Wells marked M are the DNA ladder markers. The red arrow head indicates negative control (no target DNA and the green arrow head indicates positive control snail DNA. The 300bp Cox-1 PCR product is arrowed in black. The white arrow heads are samples which did not give a PCR product. The DNA fragments were separated on 1.5% agarose gel in 1x TAE buffer. The gel was stained with gel-red dye and bands size were visualised on UV transilluminator.

7.2.3.2 DNA sequencing of the amplified Cox-1 gene products to confirm snail species.

A total of 32 DNA samples that were initially confirmed as *Bulinus* species were sequenced as described by Webster and colleagues (Webster et al., 2012b) and results are shown in Table 20 below.

Table 20: Illustration of the *Bulinus* species as identified by Sequencing

S/no	Snail #	Species identified	site collected	Infection at field
1	13	*Bulinus globosus*	Mavungwa	Not infected
2	14	*B. globosus*	Mavungwa	Not infected
3	23	*B. globosus*	Kimbuni	Not infected
4	24	*B. globosus*	Kimbuni	Not infected

5	33	*Bulinus nasutus*	Vitongoji	Not infected
6	34	*B. nasutus*	Vitongoji	Not infected
7	35	*B. nasutus*	Vitongoji	Not infected
8	41	*B. globosus*	Piki	Not infected
9	42	*B. globosus*	Piki	Not infected
10	53	*B. nasutus*	Kangagani	Not infected
11	65	*B. globosus*	Mkungu	Not infected
12	66	*B. globosus*	Mkungu	Not infected
13	78	*B. nasutus*	Kangagani	Infected
14	79	*B. globosus*	Mavungwa	Infected
15	80	*B. nasutus*	Kangagani	Infected
16	81	*B. nasutus*	Kangagani	Infected
17	84	*B. globosus*	Kangagani	Infected
18	85	*B. nasutus*	Kangagani	Infected
19	86	*B. nasutus*	Kangagani	Infected
20	87	*B. nasutus*	Kangagani	Infected
21	88	*B. nasutus*	Kangagani	Infected
22	89	*B. nasutus*	Kangagani	Infected
23	90	*B. nasutus*	Kangagani	Infected
24	93	*B. nasutus*	Kangagani	Infected
25	94	*B. nasutus*	Kangagani	Infected
26	95	*B. nasutus*	Kangagani	Infected

Of the 32 PCR products, only 26 (81.3%) were successfully sequenced or the chromatograms could be edited. Among these 16 (61.5%) were identified as *B. nasutus* and 10 (38.5%) were identified as *B. globosus*. Of the *B. nasutus* sequenced, 12 (75%) had produced cercariae in the field. Of the *B. globosus* 2 (20%) had produced cercariae in the field. Interestingly 6 (37.5%) snail samples identified as *B. nasutus* by sequencing had Dra-1 gene specific for *S. haematobium*.

Table 21: List of Shehia where snail samples were collected for the SCORE study

Shehia	No. of water bodies
Finya	14
Kangagani	19
Kinowe	6
Kisiwani	11
Kwale	5
Makangale	2
Makombeni	5
Mbuzini	5
Mgogoni	5
Mkanyageni	9
Msuka	6
Piki	15
S/viamboni	9
Vitongoji	16
Ziwani	9

7.2.3 Follow-up

7.2.3.1 Determination of the prevalence of *S. haematobium* infected snails following SCORE interventions.

The identification of the water bodies (transmission sites) in the 15 shehias involved in the snail control arm of the SCORE project carried out in July 2011 provided the necessary baseline data for the assessment of snail populations as well as for monitoring the trend of *S. haematobium* infection within the snail host. This is the work described above.

In order to monitor snail numbers and infection rates throughout SCORE, a protocol was established in which a defined area was measured and a specific time allocated for

searching snails .i.e 15 m in 15 minutes. For this 4 collectors were generally involved. This was started in the 15 shehias in the snail control areas of SCORE in August, 2012 before fully engaging with the application of niclosamide (snail control activities). A total of 136 water bodies were identified (Table 21). Overall 772 *Bulinus* snails were collected over a period of four months (Mid- July – early October). Only 4 (0.6%) of the snail collected were infected with *S. haematobium* as determined by production of cercariae in the field. Of the infected snails 3 (75%) were from Mkanyageni and 1 (25%) was from Mgogoni shehias.

7.2.3.2 Confirmation of species of snail hosts and of schistosome infection in the snails at follow-up by PCR.

As mentioned above, due to the limited availability of funds it was not possible to determine prepatent *S. haematobium* infection within the snail host, and their discrimination using molecular methods (PCR). However, a sub-sample of snails from Kangagani, where *B. nasutus* were preliminarily found to be infected with *S. haematobium*, was collected for further analysis.

7.2.3.3 Schistosoma haematobium infection in snails between 2011 and 2013.

The proportion of schistosomiasis infection rate in snail (snail shed cercariae in the field) detected in 2011 was almost two-fold higher (1.1%) than in 2013 (0.6%) after application of two rounds of niclosamide. However, the actual number of infected snails was similar between the two time points; 3 infected snails 2011 vs. 4 infected snails 2013.

7.3 Discussion

Schistosomiasis is widely spread across tropical and sub-tropical countries but transmission of the disease is focal, it only happens in places where particular snail intermediate hosts exist coupled with environmental factors and human activities associated with fresh water contact. It follows that it is of paramount importance to understand the distribution of snail intermediate hosts in any particular geographical region if the control program is to achieve successful control for schistosomiasis. In this present study, methods have been implemented in Pemba to assess distribution of *Bulinus* species, their characterization and to determine the *S. haematobium* infection in the snails. The identification of transmission sites and assessment of the distribution of *Bulinus* species was carried out in different phases and locations. However, most of the work included here is based on the initial assessment carried out in 6 transmission foci shown in Figure 6.1. In that particular study a marked variation in the distribution and number of *Bulinus* snails among the water bodies (transmission sites) was found. This variation is likely to be explained by diversity in ecological factors between the sites for example some of the transmission foci had more vegetation and/or mud than others. However, such ecological diversity is difficult to quantify and there are controversial findings related to the association of vegetation and snail abundance. For example in some places the presence of vegetation was not considered to be significantly associated with the abundance of snails (Opisa et al., 2011) but in other locations the presence of water lilies and other aquatic grasses were found to be important for the presence and abundance of snails (Kariuki et al., 2004b) and indeed removal of vegetation has been used to control snails population (Boelee, 2006). Other possible factors could be the physical nature of the transmission foci and variations in water velocity (Webbe and Jordan, 1966) and temperature during the rainy season. Low temperature has been shown to inhibit snail production (Webbe and Jordan, 1966). In the setting in Pemba the heavy rains experienced during the months of April-June have an impact on the snail population densities although the intensity of the rains varies between the years. In fact, in this survey, in some occasions, the collection of snails was carried out during the rainy season (April-early June) and no snail was found during that period in most water bodies especially in the streams (data not shown). But the number of snails steadily increased after rains stopped. This finding is consistent to what has been reported elsewhere (Kariuki et al., 2004a). In addition, some of the sites were permanent whist

others were temporary which tend to dry-out during dry season (Jan- March) which led to interruption of snail sampling. With the exception of Mkungu which is a slow flowing stream, more snails were found in large permanent ponds as compared to streams.

The distribution of the snail intermediate hosts is influenced by a range of environmental factors (De Meillon et al., 1958, Schutte and Frank, 1964). Regarding physico-chemical characteristics of the water bodies that might influence the distribution of snails all the transmission foci investigated had in average, alkaline pH, with salinity concentration ranging from 0.02-0.25% and moderate temperature (range: 27.7- 31.1^{o}C). Studies have shown high salinity concentration has a harmful effect on snail egg-masses and immature snails (Chu et al., 1968, Donnelly et al., 1983). Moreover, in experimental studies it has been shown that salinity concentration at 0.3% permitted survival of a large proportion (85%) of *B. truncatus* snails (Chu et al., 1968) but increased concentration of salinity to ≥ 5.25% was associated with significant reduction in egg hatching as well as fecundity and survival of adult snails (Donnelly et al., 1983). The harmful effect of the increased concentration of salinity towards survival and fecundity of snails might be true for any snail species including *B. globosus*. The water bodies investigated had an alkaline pH (range: 7.9- 8.4) with a varying levels of conductivity and total dissolved solids (TDS). The lowest average conductivity was recorded in Kangagani (110 µ/s) which is a permanent large pond. In fact, in this site, many of the characteristics recorded were low as compared to other sites. This complicates determination of the association of different chemical parameters with the distribution of snails.

Overall the variation in phyisico-chemical characteristics was minor and there was no obvious correlation between any such characteristics and snail number collected. Indeed, in the paper by De Meillon et al. (De Meillon et al., 1958) it was stated that there was “no evidence that the chemical composition of natural unpolluted water plays part in determining vector snails habitat” (De Meillon et al., 1958). Other characteristics such as turbidity-that indirectly affect snail populations (De Meillon et al., 1958) were not investigated in the present study and it is clear that much more extensive and wide-ranging studies would be needed to determine density and duration of snail populations in the various water bodies identified.

As mentioned previously, the understanding of the infection rate within the snail intermediate host is critical for the planning of the control program. In this present study both patent and prepatent infection with *S. haematobium* in snail intermediate hosts was investigated. Regarding patent infection, only a small proportion (1%) of the wild snails was found to be shedding cercariae when assessed microscopically. Most of these were found in Kangagani, notably during the months of October - December. Interestingly, snails that were recovered from ponds were more likely to shed cercariae than those collected from streams. Our finding of 1% infection rate in snails is somewhat lower than that observed by (Opisa et al., 2011) in Western, Kenya but the actual number of the infected snails was similar between the two studies. Nonetheless, most studies have detected similar, or even lower, proportions of shedding snails despite high prevalence of *S. haematobium* infection around the surveyed areas (Kariuki et al., 2004a, Hamburger et al., 2004). In the present studies cercarial shedding was observed following continuous shedding in natural light between 0830 - 1530hr without thermostatically control of temperature. Although experimental studies assessing the rhythm of cercarial shedding have shown that intermittent darkness stimulated emergence of cercariae from infected snails (Nojima and Sato, 1982) it is not considered that the low percentage shedding of cercariae observed here was explained by experimental methodology.

The advancement in the knowledge of molecular technology has overcome the challenges for the detection of many infectious agents. One such application is the detection of prepatent schistosome infection in snails (Rollinson et al., 2009). As indicated by (Kariuki et al., 2004b) field caught snails often fail to shed cercariae even if they are infected. In the present study we have such PCR-based techniques to detect the Dra-1 repeated sequence specific for *S. haematobium* parasite. Overall, such PCR identified many more (56.4% [n = 53]) infections from snails than conventional method of assessment of cercarial shedding (p = 0.00001). It has been shown that PCR is more sensitive and can detect infection in snails once they become infected (Hamburger et al., 1998). Our results are in agreement with those reported in Kenya by other workers (Hamburger et al., 2004) but were also higher than the 29.7% reported in Nigeria by (Akinwale et al., 2011b). The use of PCR in detecting the true level of infection in snails proved is an invaluable tool for surveillance of schistosomiasis transmission

which is critical for the success of a control program particularly when disease elimination is the goal.

Finally, in this present study, the species of *Bulinus* snails present and involved in *S. haematobium* transmission in Pemba has been determined by defining the Cox-1 gene sequences specific for *Bulinus* species. Application of PCR for the Cox-1 gene of *Bulinus* was positive for the majority (97.9%) of the snails tested indicating that they belong to *Bulinus* species. Barcode DNA sequencing of the PCR products revealed two species of *Bulinus* snails (*B. globosus* and *B. nasutus*) exist in Pemba. Based on the combination of the results for the various experiments, it is clear that both species of *Bulinus* snails play roles in the transmission of schistosome infection in Pemba in that both species were shown to be infected with schistosomes as defined by shedding cercariae and/or Dra-1 typing. It should be noted that that the Dra-1 gene can be detected in other schistosome parasites such as *S. bovis* (Hamburger et al., 1998) which is closely related to *S. haematobium*. In certain countries e.g. Kenya these two species of schistosomes sometimes co-exit and also parasitize the same snail intermediate host, *B. nasutus* (Barber et al., 2000). However, ruminant schistosomiasis has not been documented in Pemba Island to date and so it is suggested that, in this setting, both species of *Bulinus* transmit *S. haematobium*. The finding of *S. haematobium* infection in *B. nasutus* (as confirmed by sequencing) is contrary to what has been reported earlier by other workers in Unguja Island, the sister island of Zanzibar where infection was demonstrated in *B. globosus* but not in *B. nasutus* (Stothard and Rollinson, 1997a, Stothard et al., 2002). Possible explanations for this difference include the utilization of different genetic markers to define the snail species. The earlier studies sequenced the ribosomal internal transcribed spacer (ITS) region whilst the present study sequenced the mitochondrial cytochrome oxidase sub-unit -1 (Cox-1). Studies have shown that sequencing of the Cox-1 gene for characterisation of *Bulinu*s snails produced better result than use of ITS-2 (Kane et al., 2008). Another possible reason could be the different geographical locations where snails were collected i.e. Pemba vs Unguja. So it is possible that strain differences in the snail and/or *S. haematobium* itself mean that *B. nasutus* is involved in *S. haematobium* transmission in Pemba but not in Unguja. Certainly it has been demonstrated that *B. nasutus* are effective intermediate hosts for *S. haematobium* infection in Kenya (Kariuki et al., 2004a) and even some parts of Tanzania (Webbe, 1962, Lwambo, 1988).

In view of the interesting result of the present and past studies regarding the biology of the transmission of *S. haematobium* in Zanzibar, it would be necessary to carry out further intensive malacological studies to identify and characterize snail intermediate hosts and determine their transmission role. Furthermore, our results confirm the high superiority of the PCR in determining schistosomiasis infection rates in the snail host in Pemba and so could be invaluable as the tool for monitoring infection if and when elimination is achieved.

Chapter 8 The genetic diversity of *S. haematobium* in Pemba and the impact of praziquantel treatment

8.1 Introduction

Schistosomiasis is ranked second after malaria in terms of suffering caused by a parasitic disease (Webster et al., 2010, Caffrey, 2007). Furthermore, the disease is categorized as a neglected tropical disease (NTD) due to the large numbers of people requiring treatment and the relatively lack of resources for its control. Schistosomiasis is a debilitating disease responsible for some mortality and subtle pathology (Olds, 2013). Nevertheless, it is responsible for an estimated 1.7-4.5 million disability-adjusted life years (DALYs) (Utzinger et al., 2011, Chan, 1997, Fenwick, 2012, WHO Expert Committee on the Control of Schistosomiasis, 2002). Of the human schistosome species, *Schistosoma haematobium* is responsible for urogenital schistosomiasis and is predominantly found in Africa where an estimated 85% of all schistosomiasis cases exist (Steinmann et al., 2006) and where it is considered as one of the major public health problems.

The long-term consequences of urogenital schistosomiasis are well understood (Fenwick, 2012). Several control measures, including behavioural change initiatives (Mwanga and Lwambo, 2013) and transmission control have been applied against this devastating disease, often integrated with a preventive chemotherapy programme (Fenwick and Webster, 2006). However, chemotherapy-based control alone, using praziquantel, has been the main control strategy employed in recent years, primarily to reduce morbidity and many endemic countries largely depend on this strategy (Wang et al., 2012a, Doenhoff et al., 2009). There are multiple reasons towards this dependency:- 1) increasing donation of PZQ tablets from the pharmaceutical company (Fenwick, 2012); 2) observation of rapid impact on morbidity reduction, improved cognitive capacity and school attendance through provision of PZQ (Taylor-Robinson et al., 2012, Miguel and Kremer, 2004); 3) financial commitment from international development partners (Molyneux and Malecela, 2011); and 4) easy distribution of the drugs by non-medical professionals during MDA or school based treatment (Montresor et al., 2010).

On the other hand, over reliance on a chemotherapy-based control strategy, especially when delivered as monotherapy, as in the case of PZQ, has raised concern on the

potential development of drug resistance in the parasite population. Although low cure rate has been demonstrated (reviewed by Doenhoff et al., 2008) there have been no confirmed examples of schistosome parasites showing genetically stable resistance to PZQ (Doenhoff and Pica-Mattoccia, 2006) despite the earlier speculation reported elsewhere (Ismail et al., 1996, Picquet et al., 1998). The absence of well documented resistance should not discourage the effort for regular monitoring of PZQ efficacy. Resistance has been shown to develop in nematode infections of domestic livestock and one must be aware that increasing drug pressure acting on genetically diverse schistosome populations could select for resistant phenotypes (Doenhoff et al., 2009).

Periodic drug efficacy monitoring can be used to confirm maintenance of drug efficacy levels e.g. for schistosomiasis in Pemba (Guidi et al., 2010) but is labour intensive and efforts are being made to develop genetic monitoring of the parasite populations for changes which might be associated with loss of susceptibility. The advent of schistosome DNA sequencing has increased the attention and opportunity of studying the evolutionary relationships of schistosomes (Le et al., 2000) and their genetic diversity which may confer variation in disease morbidity, transmission and immunological responses (Rollinson et al., 1997). So far, two major groups of *S. haematobium* have been identified using partial sequences of the mitochondrial cytochrome oxidase sub-unit 1 gene (Cox-1). These groups are designated as group 1 (G1) that is found in mainland Africa and group 2 (G2) that is found in Indian Ocean Islands (Webster et al., 2012a). Surprisingly, in Zanzibar both groups exist (Webster et al., 2012a). Despite the understanding of the existence of the various groups within the species of *S. haematobium*, the consequence in terms of susceptibility to drug (PZQ) and its implication for the control have not yet been fully elucidated. Preliminary work suggests that genetic diversity may associate with displayed differences in schistosome susceptibility to PZQ. This possibility is supported by the fact that resistance to earlier other anti-schistosome drugs, oxamniquine and hycanthone has been associated with genomic change (Brindley et al., 1989, Brindley et al., 1991, Valentim et al., 2013). Concern about possible loss of PZQ efficacy increases worries for the control programmes particularly because PZQ is the only drug of choice for the treatment of schistosomiasis and is in large scale use for control. The genetic characterisation of schistosomes may address interesting questions: (1) what will be the alternative drug if PZQ becomes resistant to any of the existing *S. haematobium* groups? (2) Which of the

groups is highly transmissible? (3) Which of the groups displays serious diseases pathology?

The studies and control effort for schistosomiasis in Zanzibar began in 1920s (McCarthy, 1930, Cawston, 1927, Mansfield-Aders, 1927), however, there was interruption of the program as a result of lack of fund. But in recent years, as for many other schistosomiasis endemic countries, Zanzibar adopted chemotherapy based control strategy to fight against schistosomiasis and even more recently there is a change of strategy aiming for elimination of the disease (Knopp et al., 2013). Thus, there is a need to regularly monitor drug efficacy and also assess schistosome genetic diversity in response to drug treatment.

8.2 Results

8.2.1 Establishing the baseline efficacy of PZQ in 2011 (pre-SCORE)

8.2.1.1 Study site and population

The characteristic of the study site has been described elsewhere. In January 2010, all primary school-children aged from 7-13 yrs were treated with praziquantel (40 mg/kg body weight) for schistosomiasis. The treatment of the school-children was repeated in 2011 following completion of this assessment (described below).

Figure 62 illustrates the profile of the enrolled children. A total of 359 school children that were infected with schistosomiasis were followed-up to assess the efficacy of praziquantel. Of these 37 (10.3%) dropped out during the initial follow-up, 304 (84.7%) were found to be egg negative but 18 (5.0%) were still excreting eggs and were retreated. Amongst these 18, 8 (44.4%) passed live eggs (as they released miracidia). The overall cure rate (CR) at the first follow-up was 94.4% and the egg reduction rate (ERR) was 76.1%. Out of the 18 children who were egg positive after initial follow-up, only 2 (11.1%) still passed eggs on subsequent follow-up and in neither case were the eggs viable as judged by the failure to release miracidia. This resulted in 88.9% CR at this time point and a cumulative CR of 99.4%. The ERR at the second follow-up was 59.9%.

8.2.2 Follow-up efficacy of PZQ in 2013

The characteristics of the population for this assessment have been descried in section 3.3.4 above. A total of 176 school-children were treated with PZQ 40mg/kg and followed as indicated in section 2.4. During the initial follow-up 4weeks post-treatment, 154 children were cured as judged by not passing *S. haematobium* egg in their urines-giving a CR of 87.5% and ERR of 87.01%. All the 22 children that produced *S. haematobium* eggs at first follow-up were followed again at week 7 post treatment and were found negative i.e. they were not discharging eggs resulting in 100% CR cumulatively.

8.2.3 Amplification of Cox-1 gene

Initially it was essential to establish methodology and to determine the presence of the Cox-1 amplicon specific for *S. haematobium* from the miracidia collected from urine samples before investigating genetic variability as this would confirm the schistosome parasite species. A total of 160 potential miracidia collected pre- and post-treatment were tested for the presence of Cox-1 using PCR but only 89 were successfully amplified. The lack of amplification in 71 samples indicates lack of true presence of the samples rather than absence of the Cox-1 gene. Cox-1 amplicons from 89miracidia from 5 children infected with schistosomiasis pre- and post- treatment with PZQ were obtained (Figure 63). Of these, 74 amplicons were successfully sequenced. The list of children with their schools where the study was carried out is shown in Table 22.

Figure 63: Enrolment and follow-up of the children examined for the schistosomiasis and praziquantel efficacy.

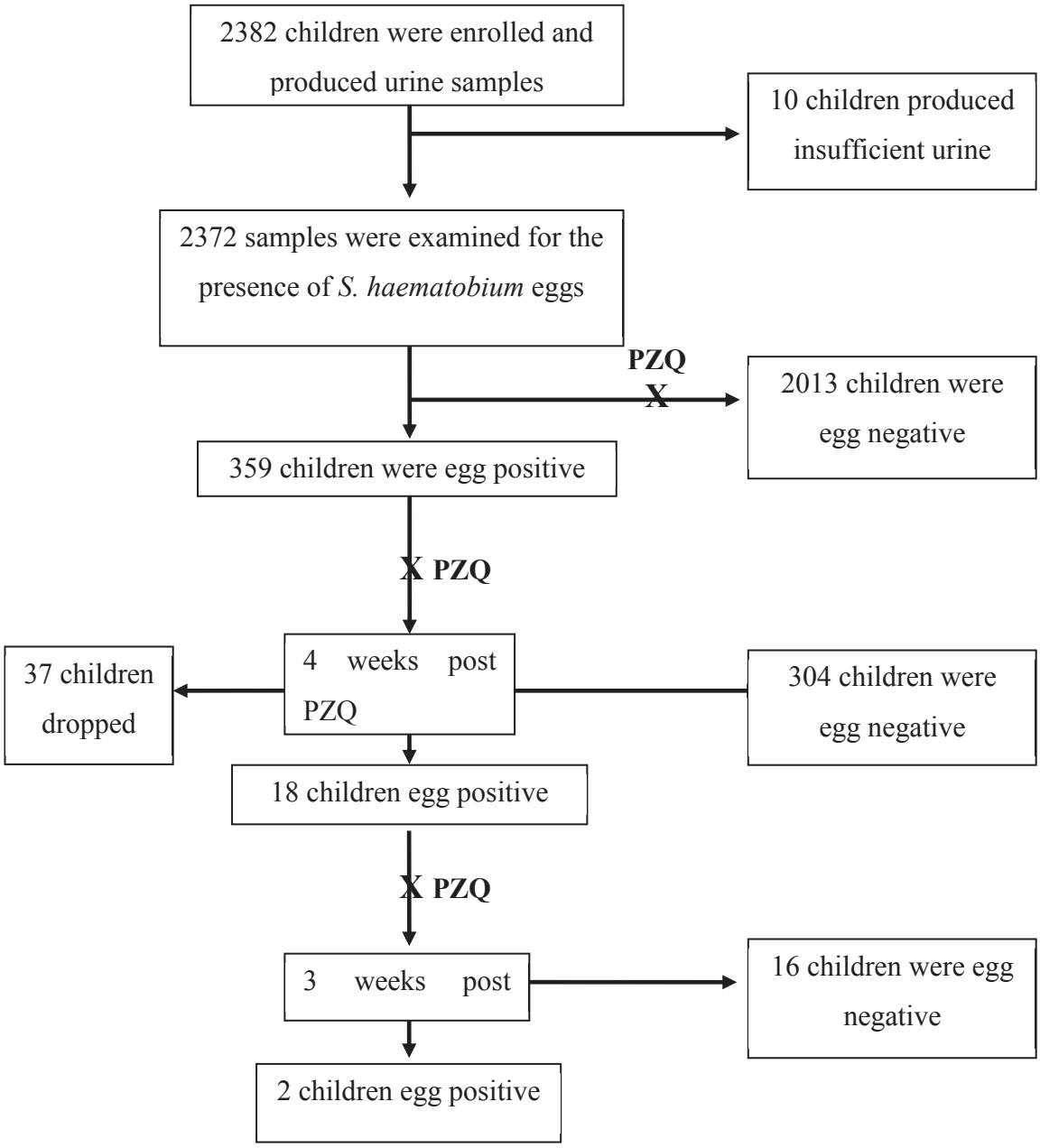


Table 22: Origin of the miracidia that were used for determination of Cox-1 gene and subsequent sequencing

ChildID	No. of miracidia		School	District
	Pre-treatment	Post-treatment (4 weeks)		
NG 92	8	8	Ng'ombeni	Mkoani
CB12	8	9	Chambani	Mkoani
CB65	10	9	Chambani	Mkoani
MT54	8	11	Mtambile	Mkoani
CM55	10	8	Chanjamjawiri	Chake

Figure 64: Amplification of the cox-1 gene from *S. haematobium* miracidia

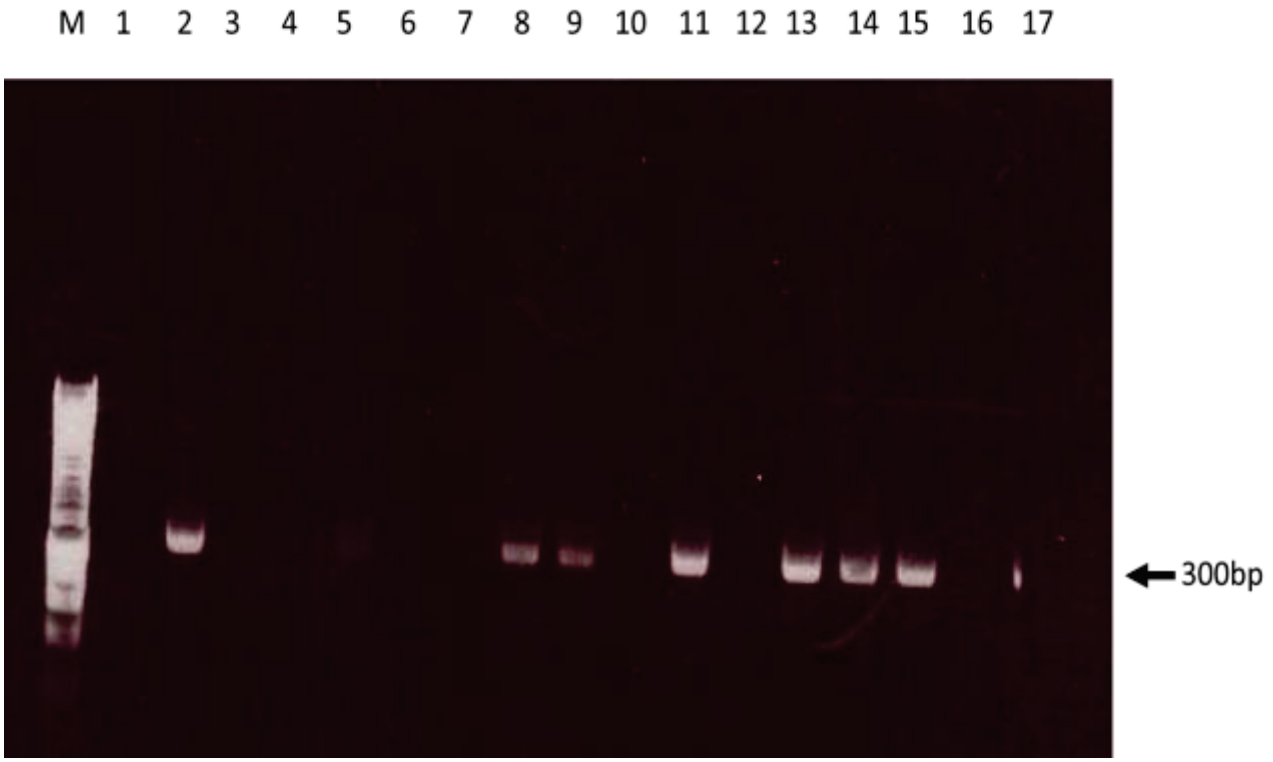


Figure 64: Amplification of the cox-1 gene from *S. haematobium* miracidia. Lane M is the ladder. Samples are numbered from 1 to 15; numbers 16 and 17 are negative and positive

controls respectively (NB the positive control strong band was off the end of the photograph). The DNA fragments were separated on 0.8% gel red agarose gel and visualised on a UV transilluminator.

8.2.4 Sequencing and haplotype Analysis

Of the 89 amplified Cox-1 genes for *S. haematobium* subjected for sequencing only 74 (83.1%) were successfully sequenced. From these sequences, 20 different haplotypes (groups) were identified. When these groups were matched with the previous published data from Webster and colleagues, (2012), (Webster et al., 2012a) both Indian Ocean Islands (Zanzibar, Madagascar and Mafia) specific haplotypes, mainland Africa specific and uniquely (not previously described) haplotypes were identified as shown in Table 23. Surprisingly some of the uniquely identified haplotypes were only found from one geographical location (Table 8.2: rows 4, 5 & 6- from Mtambile and Chambani respectively).

Table 23: Grouping of *S. haematobium* haplotypes isolated in Zanzibar

Individual Miracidia designation	Matches to previous dataset*	Group*	Currently identified haplotype number on TCS and tree
CM55.3 CM55.6 CM55.7 CM55.8 CM55.1F CB65.1F NB92.1F NB92.3F NB92.4F	Zan10	Group 1	2

CB12.2F CB65.3 MT54.1 MT54.3 MT54.2F NG92.1 NG92.3 NB92.2F NB92.6F	Zan3	Group 2	1
CM55.4F CM55.5F NG92.2 NG92.4 NG92.5 NG92.6 NG92.7 NB92.5F	Zan5	Group 2	3
MT54.6 MT54.3F MT54.5F MT54.6F MT54.7F	Unique	Group 2	4

MT54.8F MT54.9F MT54.10F			
CB12.5 CB12.6 CB12.7 CB12.4F CB65.7F	Zan12	Group 2	5
CB12.1F CB12.7F CB65.9 CB65.4F	Zan19	Group 2	6
CB65.9F MT54.4 MT54.5 MT54.1F	Zan2	Group 2	7
CB65.2F CB65.6F	Zan4	Group 1	13
CB65.6 CB65.8	Zan8	Group 1	15
CB12.1 CB12.2	Unique	Group 2	8

CB65.7			
CB12.4 CB12.3 CB12.3F	Unique	Group 2	9
CB65.1 CB65.5 CB65.3F	Unique	Group 2	10
CB65.2 CB65.4 CB65.8F	Unique	Group 2	11
CM55.1 CM55.5 CM55.2F	Unique	Group 2	12
CB12.6F	Zan11	Group 1	18
CM55.2 CB65.5F	Unique	Group 1	14
MT54.2 MT54.4F	Unique	Group 2	16
CM12.5F	Unique	Group 2	17
CM55.3F	Unique	Group 1	19
CM55.4	Unique	Group 1	20

* refers to data in Webster et al., 2012a

Interestingly within each of the previously known groups, there were haplotypes which have not been identified before ("unique"). For example, within the group of mainland Africa, initially identified as group 1 (G1) there were 7 different haplotypes (number of miracidia = 18), of which 3 haplotypes were newly observed. Similarly, within the group 2 (G2) there were 13 haplotypes (number of miracidia = 56) 8 of which were uniquely identified. Moreover, individual children shed a combination of parasite haplotypes, none of them produced single group haplotypes and the genetic diversity was high. Interestingly, some children shed eggs leading to more uniquely identified haplotypes post treatment with PZQ compared with pretreatment. Furthermore, each individual child displayed number genetic variants of *S. haematobium* pre- and post-treatment as shown in Table 24

Table 24: Genetic variants of *S. haematobium* parasites observed pre and post treatment with PZQ

Child ID group	Number of genetic variants*		Haplotype
	Pre-treatment	Post-treatment	
NG92	2	2	1
NG92		3	2
NG92	4	2	3
CB12		1	1
CB12	2	2	5
CB12		2	6
CB12	2		8
CB12	2	1	9
CB12		1	17
CB12		1	18
MT54	1	2	1

MT54		8	4
MT54	2	1	7
MT54	1	1	16
CM55	4	1	2
CM55	2	1	12
CM55	1		20
CM55		1	19
CB65		1	5
CB65	1	1	6
CB65		1	7
CB65		1	8
CB65	2	1	10
CB65	2	1	11
CB65	2	1	12
CB65		2	13
CB65	1	1	14
CB65	2		15

Table 8.3 legend: * relates to the numbers of individual miracidia sequenced

Furthermore, it was noted that some of the uniquely identified haplotypes were from eggs isolated from children that were initially uncured after an initial dose (40mg/kg) of PZQ although eggs were not detected from those children following retreatment during the subsequent follow-up 7weeks post treatment.

8.2.5 Phylogenetic analysis

As shown above, from the aligned sequence data, highly diverse haplotypes were detected which fall into the previously known two major groups. Some of those haplotypes have not been identified before. The TCS analysis revealed similar findings.

Using statistical parsimony an evolutionary network demonstrating the recognized genealogy of Cox-1 barcodes is shown in the Neighbour-joining (NJ) tree in Figure 63. Interestingly, most of the uniquely identified haplotypes of *S. haematobium* were from Mtambile, Chambani and Ng'ombeni. These schools are located in Mkoani district (Figure 64) and somewhat closer to each other indicating possibility of increased movement of children resulting in common genotypes.

Figure 65 Neighbour Joining tree incorporating all the Cox-1 haplotypes analysed in this study. The majority of the samples are closely clustered, single links and representing a single polymorphic position. G1 and G2 relate to the two groups identified in Webster et al 2012

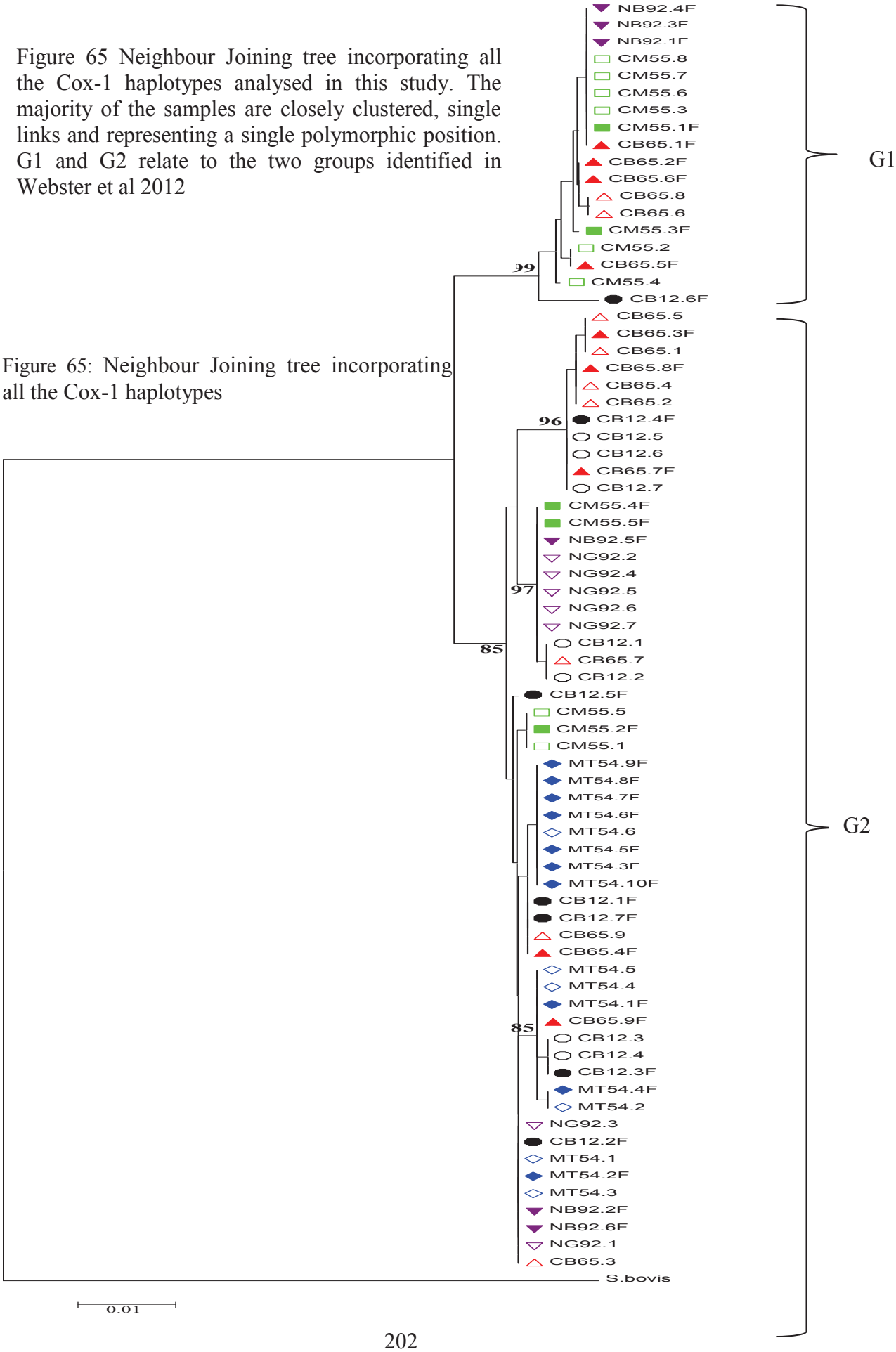


Figure 65: Neighbour Joining tree incorporating all the Cox-1 haplotypes

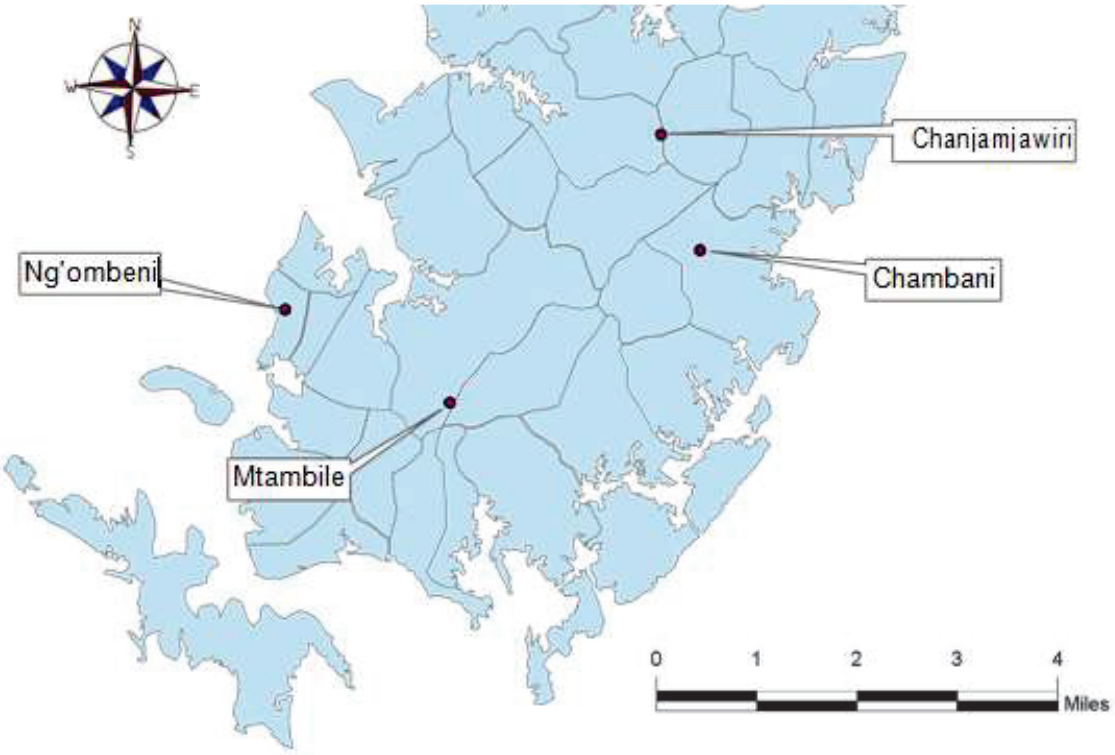


Figure 66: A section of Pemba Island indicating schools where samples for barcoding analysis were collected.

8.3 Discussion

The general goal of PC control for schistosomiasis is to mitigate the morbidity through administration of PZQ. However, as the strategy is implemented this may lead to intensive and persistent new selection pressures on the parasite population. Studies have shown that selection pressure can rapidly transform schistosomes' infectivity, virulence phenotypes and population genetics within a few generations (Davies et al., 2001). This present study was set up to apply genetic population genetic analysis to determine baseline genetic diversity of *S. haematobium* in Pemba in relation to the potential effects of the subsequent ongoing administration of PZQ in community wide treatment or SBT through the ZNCP.

Overall marked variation of *S. haematobium* genetic markers was demonstrated; with 20 different haplotypes being identified which fell into two major groups (G1 & G2) of *S. haematobium* similar to that described earlier by other workers (Webster et al., 2012a). Moreover, each individual child produced a mixture of haplotypes (table 23) though some of them were predominantly produced by certain children and hence were more common from certain geographical area. For example the haplotypes designed as Zan10 were only found in children from Chanjamjawiri and Ng'ombeni whilst Zan3 haplotypes was found in three locations (Chambani, Mtambile and Ng'ombeni). Likewise, haplotypes Zan12 and Zan19 were only observed from Chambani. It has been shown that parasite population diversity or genotype alteration is an expression of genetic exchange between parasite populations in diverse geographical locations (Norton et al., 2010). The striking observation revealed in this study is that some of the uniquely identified haplotype were only isolated from children failed to be cured with PZQ during the first follow-up at fourth week post PZQ administration.

Cure rates assessed a few weeks following a single dose of PZQ are generally in the range 70-90% (Gryseels et al., 2001a) similar to the present study. Possible explanations for the presence of eggs and therefore persisting worms following PZQ treatment 4 weeks previously include the presence of immature worms at the time of treatment which survive PZQ treatment and mature to egg production. Thus, studies have demonstrated that PZQ does not have an effect on immature schistosome worms (Caffrey, 2007, Fallon et al., 1996) as the tegument is not fully developed. Another plausible explanation is that that some children might carry both susceptible and

resistant schistosome genotypes. No such "resistance genes" have yet been identified in schistosomes and although genetic diversity was demonstrated in the present study any influence of this on PZQ susceptibility of the worms has not been established. Nevertheless, the occurrence of PZQ resistance is not expected any time soon due to various reasons: 1) the parasite's obligate diecious sexual reproduction 2) Even with high drug distribution coverage usually there are individuals who escape treatment and so creating refugia 3) the long life-cycle of the parasite (King et al., 2000) (Botros and Bennett, 2007)

Any such resistance genotypes would be subject to selection pressure and likely lead to reduced PZQ efficacy in the face of high level drug usage against the worm gene pool. Studies have suggested that MDA may lead to changes in the frequency of particular alleles and genotypes in the population which could be linked to genes involved in susceptibility to treatment (Norton et al., 2010). However, although such analysis may ultimately identify existence of genetic factors influencing PZQ efficacy, the present studies did not demonstrate loss if PZQ efficacy in practice. At baseline the CR and ERR at initial follow-up 4weeks post-treatment were 94.4% and 76.1% respectively. In the subsequent follow-up, the CR increased to 99.4% (cumulatively). An additional efficacy assessment carried out 2yrs later (2013) revealed slightly lower CR (87.5%) but higher ERR (87.01%) compared to baseline. On both occasions, efficacy was similar to that described previously by other workers in the same setting (Guidi et al., 2010). These efficacy indicators are within the acceptable ranges and comparable to the CR for S.mansoni of 70-90% (Gryseels et al., 2001a) and for S. haematobium (Kardaman et al., 1983, Gryseels et al., 1987). There was thus no evidence of loss of PZQ efficacy over time in Pemba or as a result of two years of twice-yearly MDA.

Assessment of the phylogenetic relationships of the isolates studied found that most of them are clustered together, sharing common ancestors. This indicated that there is high movement of people across Pemba Island not only because of trade but also from other social reasons including high inter-marriage and that people tend to frequently visit families, although the travel history of the children was not established.

In conclusion, high genetic diversity within *S. haematobium* across Pemba Island was identified at the start of the proposed ZNCP/SCORE initiatives. It would be valuable to

monitor stability/fluctuations in these haplotypes over the course of the continuing intensive PC but this was beyond the scope and resources of the present study. It would also be of interest to further determine *S. haematobium* population genetics using microsatellite markers so as to understand gene flow, allele frequency and accompanying mutation which might have implications for the control of the diseases. This is especially important as the preventive chemotherapy with PZQ is intensively distributed among the population which in turn increases the drug selection pressure and hence increases the potential for drug resistance.

Chapter 9 General Discussion

Preventive chemotherapy (PC) is the main strategy for the control of morbidity due to schistosomiasis and soil transmitted helminths (STH). The crucial and immediate impact of PC based strategies was acknowledged in the Fifty-fourth World Health Assembly document "WHA54.19 Schistosomiasis and soil-transmitted helminth infections" which urged schistosomiasis endemic countries to attain a minimum target of regular administration of chemotherapy to at least 75%. The Schistosomiasis Control Initiative (SCI) in particular has provided support to control programmes in several countries in Sub-Saharan Africa to tackle morbidity due to these worms through the use of both SBT as well as MDA (Fenwick, 2015, Fenwick et al., 2009b, Garba et al., 2009).

Regarding STHs the 2020 WHO roadmap for neglected tropical diseases (NTD) (WHO, 2015b) advocated implementing integrated approaches but largely due to financial constraints, the control of STH still also relies on PC to alleviate associated morbidities. Such control measures have been proved to be successful in maintaining intensity at low level and hence prevent development of subtle morbidity (Albonico et al., 2006), although a recent review by Campbell and colleagues (2016) raised concerns about the real impact of large scale chemotherapy (Campbell et al., 2016).

Implementation and impact of the control interventions – urinary schistosomiasis

In Pemba, Zanzibar, as summarized in Figure 6, intensive annual selective treatment with PZQ targeted at school-children (1986-1988) in which school-children aged between 5-19yrs were diagnosed and those found positive were treated, led to a marked decline in prevalence of haematuria as a measure of schistosomiasis from the baseline of 54.1% to around 13% (Savioli and Mott, 1989). After this there was a period (1989-1998) of school-based treatments (SBT), twice a year but later, due to the high cost and limited availability of praziquantel, only once a year and restricted to high prevalence schools was delivered between the years 1994-2002.

After this period, the prevalence had risen to 31% and following subsequent sporadic provision of SBT prevalence had rebounded by 2004 to 63% among the school-children

and 37% in the community (Mr Haji, Unpublished data). Then in 2004-2006 reintroduction of intense treatment through CWT-MDA supported by the Schistosomiasis Control Initiative (SCI) again led to marked reductions in prevalence which in 2007 had fallen to 18% based both on haemastix testing and egg detection. It is clear from Table 3.1 that selective or mass chemotherapy can have a major impact on prevalence of schistosomiasis but the impact of the targeted SBT on prevalence had not been monitored in Pemba. At the outset of this thesis it was planned to monitor the impact on transmission of the introduction of a more consistent implementation of SBT with PZQ. Delivery of MBZ was included in this SBT and so monitoring of STH levels was also undertaken. As summarized in Figure 34, sporadic use of MBZ in Pemba had not had marked effects in reducing the high prevalence of STH although MDA using ALB in 2004-5 did reduce prevalence noticeably. It should be emphasized that it is the intensity of these helminth infections which relates to pathology and disease rather than prevalence per se and chemotherapy can dramatically reduce worm burdens and so disease in individuals even though they may not be "cured" and still pass eggs. This effect is most pronounced in areas with high prevalence of infections (notably >50%) (Albonico et al., 2006, King et al., 1988) The impact of PC with PZQ had been monitored in Pemba over the years by microhaematuria, a valuable index of disease and of intensity of infection (Warren et al., 1979). Because of the importance of both prevalence and intensity of infection in monitoring effectiveness of helminth control both parameters were measured in most of the work described in this thesis.

Despite the lack of data on the effectiveness of SBT on helminth infection from Pemba this approach is widely implemented targeting treatment, with praziquantel and albendazole or mebendazole, to school-aged children, because this sub-population harbours heavy disease burdens and benefits most from improved development (Stothard et al., 2013b, Hotez et al., 2006a, Anderson and May, 1992, Brooker, 2010). So SBT was reintroduced in Pemba in 2009 although its implementation and monitoring was inconsistent until the initiation of this study and the implementation of the National Plan for Pemba in 2010 which initially involved annual SBT with ALB and PZQ.

At the outset, the work in this PhD concerned monitoring the effectiveness of this reintroduction of SBT on transmission as a baseline for this thesis. A baseline survey (in 2010), carried out in the first class of primary school children, Standard-1 (Std-1) from 24 schools, showed *Schistosoma haematobium* infection in 9.5% of children with boys

having higher prevalence and intensity than girls, although the majority of infections were light. The prevalence of any STH worm was 93.9% and the prevalence of the three common STH species was 46.7%, 87.4% and 50.5% for *Ascaris lumbricoides*, *Trichuris trichiura* and hookworms respectively. For all of the STH species, girls were more infected than boys. Following implementation of SBT in 2010, the new cohort of Std-1 children were tested in the same schools one year later (2011) and the prevalence of schistosomiasis recorded as 18.9%. The Std-3 children who had received the school-based treatment in 2010 showed a lower prevalence (11.3%). The prevalence of STH was very similar in both years (90-93% for any STH and the majority of children had light intensity infections. The impact of a further round of SBT in 2012 showed no decline in the prevalence (17.6%) or intensity of *S. haematobium* or STH. Overall the prevalence and intensity of *S. haematobium* and of STH in the Std-1 children had not been measurably improved by the reintroduction of single annual SBT. Following shifting of treatment strategy, from single SBT to twice yearly MDA, further surveys for STH were carried out in 2013 that revealed inconsistent reductions in both the overall and worm-specific prevalences. Similarly, the intensity of infections was also inconsistently reduced although the majority of infected children had light infections.

In view of the lack of effect of SBT on lowering prevalence or intensity it was proposed that the emphasis of the ZNCP be switched to use of MDA. Coincident with this the low prevalence of <10% initially recorded in the baseline survey in 2010 had alerted the attention of the SCORE programme managers. The SCORE programme secretariat based at the University of Georgia, Atlanta, United States of America, had solicited funds from the Bill and Melinda Gates Foundation in order to carry out operational research to assess strategies aimed at to elimination of schistosomiasis (Hotez and Fenwick, 2009, Colley, 2014). The SCORE secretariat – which comprised Prof Daniel Colley (Programme Director), Dr Carl Campbell, Dr Sue Binder, Mrs. Tammy Andros, Mrs. Jennifer Castleman, Mrs. Nupur Kittur and Prof Charles King initiated discussions through Prof David Rollinson of the NHM, London, in engaging the ZHCP in the fight against schistosomiasis. Given the complexity of eliminating schistosomiasis, SCORE aimed at assessing the effectiveness of combinations of various interventions and also demonstrating their feasibility and associated cost. It was acknowledged that, for various reasons including limited funds (Fenwick et al., 2009c), elimination of schistosomiasis might take time to be achieved in many settings such as Pemba and so

PC may well remain as the main control strategy. Therefore, the SCORE programme joined forces with the ZNCP by also assessing the effectiveness of MDA alone in this endeavour. This approach was consistent with WHO policy to shift focus from morbidity control to interruption of transmission and elimination where feasible (WHO, 2012, WHO, 2015b)
[http://www.who.int/neglected_diseases/London_Declaration_NTDs.pdf]
[http://www.who.int/neglected_diseases/9789241564540/en/]).

Zanzibar resolved to try to eliminate schistosomiasis as a public health problem (Zanzibar Elimination of Schistosomiasis Transmission programme, ZEST) through implementation of integrated approaches (Knopp et al., 2012). The potential for the elimination was considered feasible because: 1) only one species of schistosome, *S. haematobium*, exists in Zanzibar 2) the prevalence of infection was not high (15.1[14]% in Pemba and 8.0% in Unguja), at the time of the initiation of the project. Thus in 2010 preliminary discussions between the Ministry of Health – through ZHCP, SCORE and NHM were started and finally an agreement was reached. Based on these prevalences the project set different aims between the two Islands. For Unguja the aim was to eliminate schistosomiasis as a public health problem for 3 years and interrupt transmission in 5 years whilst for Pemba the aim was to control schistosomiasis throughout the Island and specifically, to reduce prevalence to below 10% in 3 years and eliminate it as a public health problem in 5 years (Knopp et al., 2012). The SCORE secretariat agreed to fund the project which started its implementation in 2012. In particular, the project trialled implementation of MDA alone or MDA in combination with snail control or behavioural modification in defined areas. The implementation of snail control activities which involved application of molluscicide, niclosamide, started effectively in August, 2012, two months after the heavy rains and was only carried out for 3months. The application of niclosamide was done focally i.e only in areas where there is human contact. This implementation period was deemed insufficient to effectively reduce snail population to positively interrupt schistosomiasis transmission. Hence it was agreed to increase field time to cover 8month/year and halt activities during heavy rains seasons (April-July).

[14] This prevalence was obtained in 2011 –described in detail in chapter 3 (survey_2 cohort 24)

The behavioural change strategy was considered as a bottom-up approach (Person et al., 2016) and consisted of various major (football, netball) and minor (tosse game, drama) sport games. The games were meant to engage children to move them away from risky behaviours which can lead to acquisition of schistosomiasis. Furthermore, there were series of training sessions given to school and madrasa teachers. Additionally, in some occasions the team organized "*Kichocho day*"- specific day in which children competed in different sport games. Minor sport games carried health education information –and explained dangers of engaging in risk behaviours. Nevertheless, behavioural changes evolved over the years and new approaches were incorporated based on the results of periodic surveys (Person et al., 2016). For instance, installation of washing platforms came later during the course of implementation of the strategy.

As aforementioned, in 2012, the ZHCP implemented the multi-year project aimed at eliminating schistosomiasis as a public health problem by application of MDA across the island supplemented by snail control and behavioural modification in defined areas. Prior to implementation of this MDA a new extensive baseline survey of Std-3 and Std-4 children in 2012 gave a prevalence of 8.3% of schistosomiasis. At the beginning of the project, the MDA was administered twice/year in the communities alone but following disappointing results at the first follow-up survey (2013), the strategy was changed to include twice/yearly MDA in communities and also SBT in schools. Monitoring of the schoolchildren over 2012-2014 showed a progressive reduction to 5.3% overall prevalence in 2014. However, the different interventions of ZEST revealed variable reductions in prevalence, with the Behavioural change cohort showing the most positive downward trend (Behaviour > Snail control > MDA alone). The intensity of schistosomiasis also showed downward trends in the mean egg count over the years although reduction in the proportion of children with heavy infection was inconsistent. Survey data carried out at the end of the SCORE intervention in 2017 (after completion of the work in this thesis) showed prevalences of 1.4% for MDA, 2.8% for Behaviour and 1.8% for the Snail arm suggesting that the Snail control arm may have been the most effective interventions. Regarding the fact that the Behaviour arm seemed to be the most effective during the period when this thesis data was collected (2012-2014) may be explained by the fact that the Behavioural initiatives were initiated and implemented much earlier compared to Snail control activities in this period.

Implementation of Snail control and Behavioural interventions were novel in Pemba and much of value was learnt about their use and about how this could be improved.

Washing platforms were not necessarily installed adjacent to surface water bodies- almost all were installed around taps or wells and the numbers varied in the different shehias (ranging from 2-4/shehia). The installation of washing platform was a novel idea that aimed at keeping away children and community members from risky behaviour and reducing frequent contact with surface water bodies but installation adjacent to other sources of water supplies may complicate the effectiveness of the education campaign provided. It is considered likely that individuals who used installed washing platforms were not those who used surface water bodies for domestic purposes. For various reasons, women tend to prefer using surface water bodies for washing clothes and utensils rather than tap water or wells. One reason for this is that it is perceived that less soap is required in natural water sources. The true impact of the washing platforms is also difficult to assess since they were inconsistently and progressively constructed during the course of the SCORE interventions.

Based on the results of SCORE it would seem feasible to interrupt schistosomiasis transmission and achieve elimination of schistosomiasis as a public health problem in Zanzibar. It is likely that this would require maintaining components of the various interventions trialled in SCORE although what this would need to consist of is uncertain as it should also be noted that during the project period there has also been a remarkable increase in the distribution and so availability of safe water supplies in many communities. Several countries in the world where low transmission exists or has been achieved have targeted elimination of transmission using integrated approaches. The successes in these areas and the challenges still posed have been reviewed in two workshop manuscripts (WHO, 2007, WHO, 2009)

Recommendations: Integrated interventions could be the long-term solution for interrupting transmission of schistosomiasis in Pemba and eventually lead to elimination. Continued evaluation of the washing platforms built during SCORE will provide useful insight into their value. However, the other components of the Behaviour and snail control interventions of SCORE have now ceased in Pemba and in such poor resource areas, it would be difficult to maintain such integrated control measures at the same and so focusing on PC in areas with high transmission coupled with low-cost

behavioural changes and strengthening of diagnostic capability of health facilities could be essential.

Implementation and impact of the control interventions – STH

The control measures against STH infection were solely based on PC as for schistosomiasis (initially with SBT and later with MDA) and implemented in parallel for the two infections. So the changes in the strategies imposed for schistosomiasis also affected the control of STH. Monitoring of impact of those strategies was carried out through investigation of the prevalence and intensity in the newly Std-1 children from 2010-2013. On one occasion, 2011, Std-3 children were also sampled. This allowed comparison of worm burden between younger (Std-1) and older aged children (Std-3) and provided opportunity to assess the effect of the treatment in the community. At baseline (2010), the prevalence of any STH worm was 93.9% and the prevalence of the three common STH species was 46.7%, 87.4% and 50.5% for *Ascaris lumbricoides*, *Trichuris trichiura* and hookworms respectively. For all of the STH species, girls were more infected than boys. A substantial proportion of children had polyparasitoses- with double (20.8% had *T.trichiura* and hookworm and 18.2% has *A. lumbricoide* and *T. trichiura*) or triple (25%) infections. The survey carried out in 2011, after implementation of one round of annual SBT, overall, showed slightly lower mean prevalence (89.79%) compared to baseline. Std-1 (younger) children who were not treated in 2010 were more infected (90.71%) than Std-3 (88.86%) (older) children who received treatment in 2010. This indicates that single annual SBT did not interrupt transmission of STH infection in the communities but had some impact in the older children.

As explained above, there was a shift of strategy – from single SBT to twice yearly MDA which was carried out in 2012. As for SBT, the monitoring of impact was carried out by sampling newly Std-1 children in 2013. The prevalence of any STH worm was a little lower at 86.2% and again a substantial proportion of children had either double or triple infections. On all occasions, the majority of the infected children had light intensity infections with any of the three worms (*A. lumbricoides*, *T. trichiura* or hookworm), although, there was no consistent change in intensity over the years. The observation of mixed infection with STH and significant prevalences of all three species in this setting was not a surprising finding as it has been previously reported in other

studies in Pemba (Albonico et al., 1997, Albonico et al., 1993). A similar situation was reported in Unguja (Knopp et al., 2009) where epidemiological and environmental factors are very similar. The occurrence of multiple parasites has been reported elsewhere, though at varying levels (Gabrielli et al., 2005, de Gier et al., 2016, Odiere et al., 2011, Tchuem Tchuente et al., 2003) although many of the studies carried out in the other parts of Tanzania have shown low prevalences of infections with sometimes only one or two species of the STH worms detected (Mugono et al., 2014, Siza et al., 2015, Kaatano et al., 2015). This consistently high prevalence of STH infection in Pemba could be explained by the diversity of ecological niches facilitating the different life cycles with the peridomestic setting (around the houses/schools) maybe favouring the transmission of *Ascaris* and *Trichuris* and the high level of rural agriculture with which children from a young age help, facilitating hookworm. In this present study the effect of multiple parasites in terms of development of anaemia or other morbidity indicators was not established but it is apparent that occurrence of poly-parasites has adverse effect on the health of the infected individuals especially young children (de Gier et al., 2016).

Recommendations: As advocated in the 2020 WHO roadmap for neglected tropical diseases (NTD) (WHO, 2015b) helminth control is integrated in many endemic countries. As demonstrated in a limited number of schistosomiasis and STH co-endemic countries, elimination of these infections can be achieved through implementation of integrated control measures such as snail control with molluscicide, environmental modification education aimed at behavioural changes and chemotherapy supplemented with WASH strategy. However, for many reasons, concurrent implementation of these strategies cannot be realized in most endemic countries and PC is advocated (Gabrielli et al., 2011, Utzinger et al., 2003, WHO, 2006, WHO, 2002b) especially following endorsement of WHA 54.19 (Savioli et al., 2009, Stothard et al., 2009a) The PC approach has proved effective in reducing and/or preventing development of disease sequel resulting from schistosomiasis or STH (Webster et al., 2014, Fenwick et al., 2009a). The data in this thesis from Pemba shows that MDA/SBT proved to be useful strategies in sustaining infection intensity at low level (light intensity) and so reducing morbidity related to STH. Thus, such PC should be continued along with low cost health education to improve the “H” (hygiene)

component of WASH to target reduction in the transmission of STHs. The use of SBT or MDA will be dependent on available funding.

MDA coverage and compliance

Evaluation of drug coverage and compliance during the above MDA had not been carried out previously for PZQ and Albendazole in Pemba. It showed ~80% of the population received the drugs, although the therapeutic coverage was uncertain as direct observed therapy was not ensured. Nevertheless, 10.2% of the surveyed population were non-compliant to praziquantel and this differed between the districts. Albendazole compliance was better with a very low proportion (2.5%) of individuals non-compliant, these being similar in the different age-groups.

Recommendations: For any future MDA careful thought needs to be given concerning the variation in compliance observed across the different areas of Pemba. There is a need to intensify sensitization meetings in the communities with discussion of the safety and also the potential side effects which may develop. There should be engagement of religious and other influential leaders during sensitization meetings. Ideally the number of personnel should be increased so as to ensure DOT is observed and to follow-up absentees.

S. haematobium infection levels in <6yr old children

A survey was carried out prior to the above implementation of MDA to assess the contribution of transmission of schistosomiasis in pre-school children (3-5yrs). This revealed 7.04% to be infected, some of them (21.9%) with severe infection. As a result, the above MDA was administered to >3yr olds. A questionnaire to mothers/guardians of such infants investigated the risk factors for such infection, notably water contact. The importance of infection in younger children has been emphasized by others (Sousa-Figueiredo et al., 2010b) (Stothard et al., 2011) and requires attention for implementation of concrete control measures and appropriate treatment strategies to prevent progressive development of disease pathology during childhood.

Recommendations: In order to deliver treatment to younger children, safety data together with modification of the dose pole has been published describing its use for the delivery of PZQ (Sousa-Figueiredo et al., 2010b). Until a PZQ paediatric formulation for use in community wide treatment is available, effort should be made to ensure there

are adequate PZQ supplies in health facilities to treat pre-school aged children. This can be supported by strengthening of the respective health facilities. The government should be ready to register and order paediatric formulation once it becomes available.

Schistosome transmission dynamics

To improve understanding of transmission dynamics of schistosomiasis in Pemba, infection in snail intermediate hosts was initially monitored by cercarial shedding. A total of 1831 *Bulinus* snails were collected over 1year (2010-2011) from 6 sites. Only ~1% shed cercariae, all from only 2 transmission sites. Confirmation of infection within the snails collected as well as identification of the *Bulinus* species was investigated using polymerase chain reaction (PCR) and DNA sequencing techniques in a sub-population of the snail samples. This revealed that 56.4% of the *Bulinus* snails were positive for the Dra-1 gene of *S. haematobium*. Furthermore, partial sequencing of the cytochrome oxidase sub-unit 1 gene (Cox-1) of the *Bulinus* snails indicated the existence of *B. nasutus* and *B. globosus* and indicated that both species were implicated in the transmission of *S. haematobium* infection in Pemba. Of particular interest was the detection of Dra-1 gene characteristic of *S. haematobium* in *B. nasutus* as demonstrated by PCR and subsequent sequencing of Cox-1 gene; as well as the existence of *B. globosus* and *B. nasutus* in the same water body, (Kangagani). Studies have shown these two snail species are usually allopatric in nature (Stothard et al., 2000). Moreover, the previous studies carried out in a similar setting found that *B. nasutus* is not responsible for the transmission of *S. haematobium* infection and was also refractory to this schistosome species in experimental conditions (Stothard et al., 2000). Despite that, in the neighbouring country, Kenya, it has been shown that *B. nasutus* can transmit *S. bovis* (Barber et al., 2000) as well as *S. haematobium* infection (Kariuki et al., 2004a). At the time of the present study, *S. bovis* infection had not been demonstrated in Pemba. It should be noted that some detection systems such as PCR and enzyme-linked immunosorbent assay (ELISA) have shown cross-reactivity between *S. bovis* and other schistosome species which infect humans (Pardo et al., 2004, Hamburger et al., 2001). Thus the apparent finding of the *S. haematobium* infection in *B. nasutus* in Pemba is novel and warrants further research to elucidate the real transmission role of the *B. nasutus* with this parasite.

Recommendations: Further studies are needed to confirm the potential role of *B. nasutus* in the transmission of urogenital schistosomiasis in Pemba. This could be investigated by experimental exposure of morphologically characterised *B. nasutus* with miracidia of *S haematobium* derived from eggs passed in the urine of patients in Pemba in order to establish if they are susceptible to the human parasite (Stothard et al., 2000). Infection of the snails would be monitored by cercarial shedding and/or molecular techniques undertaken to confirm the schistosome and snail species. Similar methods could be applied to confirming the existence of *B. globosus* and *B. nasutus* in the same water body, (Kangagani) and other water bodies in Pemba.

Schistosome population dynamics (genetic diversity)

Analysis of population genetic diversity of *S. haematobium* in relation to treatment pressure was initiated by PCR and partial sequencing of the Cox-1 gene from miracidia recovered from subjects. This revealed the existence and circulation in the communities, of highly diverse haplotypes of *S. haematobium* some of which had not been described previously but which fell into the two major groups (G1 & G2) of *S. haematobium* as described earlier (Webster et al., 2012a). However, PZQ efficacy in terms of cure rate and egg reduction rate were comparable at baseline and after two years of biannual MDA with PZQ and also comparable with earlier assessments of efficacy. This is an encouraging finding as currently PZQ is the only approved drug used for the treatment of schistosomiasis at individual level as well as for morbidity control. Furthermore, it is believed that development of PZQ resistance may take longer, if happens, because of the diecious nature and longevity of the parasite along with the existence of refugia from drug coverage (King et al., 2000, Botros and Bennett, 2007).

Recommendations: Praziquantel should still be used for the treatment of schistosomiasis at the individual level and community wide or SBT. More studies are needed to assess the significance of the different parasite haplotypes and the G1 and G2 groups on *S. haematobium* biology and notably possible influence on susceptibility to PZQ

Concluding statement

Overall this work has helped establishment, implementation and monitoring of diverse strategies for improved control of helminth infections in Pemba and provided early evaluation of their relative efficacy. Completion of the ZNCP/SCORE initiative should help consolidate this work. There were a number of interesting other findings which merit follow-up.

Reference

AAGAARD-HANSEN, J., MWANGA, J. R. & BRUUN, B. 2009. Social science perspectives on schistosomiasis control in Africa: past trends and future directions. *Parasitology,* 136**,** 1747-58.

ABBASI, I., KING, C. H., STURROCK, R. F., KARIUKI, C., MUCHIRI, E. & HAMBURGER, J. 2007. Differentiation of *Schistosoma haematobium* from related schistosomes by PCR amplifying an inter-repeat sequence. *Am J Trop Med Hyg,* 76**,** 950-5.

ABD ELAZIZ, K. M., EL-SETOUHY, M., BRADLEY, M. H., RAMZY, R. M. & WEIL, G. J. 2013. Knowledge and practice related to compliance with mass drug administration during the Egyptian national filariasis elimination program. *Am J Trop Med Hyg,* 89**,** 260-4.

ABD ELLAH, O. H., ZAYTOUN, S., AHMED, A. E., HUSSEIN, A. N. & AHMED, A. M. 2015. Schistosomiasis in Nag Hammady City, Relationship between Infection and Anemia among Children and Youth, Qena Governorate, Egypt. *J Egypt Soc Parasitol,* 45**,** 397-402.

AHMED, A. M., ABBAS, H., MANSOUR, F. A., GASIM, G. I. & ADAM, I. 2012. *Schistosoma haematobium* infections among schoolchildren in central Sudan one year after treatment with praziquantel. *Parasit Vectors,* 5**,** 108.

AKINWALE, O. P., KANE, R. A., ROLLINSON, D., STOTHARD, J. R., AJAYI, M. B., AKANDE, D. O., OGUNGBEMI, M. O., DUKER, C., GYANG, P. V. & ADELEKE, M. A. 2011a. Molecular approaches to the identification of *Bulinus* species in south-west Nigeria and observations on natural snail infections with schistosomes. *J Helminthol,* 85**,** 283-93.

AKINWALE, O. P., KANE, R. A., ROLLINSON, D., STOTHARD, J. R., AJAYI, M. B., AKANDE, D. O., OGUNGBEMI, M. O., DUKER, C., GYANG, P. V. & ADELEKE, M. A. 2011b. Molecular approaches to the identification of Bulinus species in south-west Nigeria and observations on natural snail infections with schistosomes. *Journal of Helminthology,* 85**,** 283-293.

ALBONICO, M., BICKLE, Q., RAMSAN, M., MONTRESOR, A., SAVIOLI, L. & TAYLOR, M. 2003. Efficacy of mebendazole and levamisole alone or in combination against intestinal nematode infections after repeated targeted mebendazole treatment in Zanzibar. *Bull World Health Organ,* 81**,** 343-52.

ALBONICO, M., CARNERI, I. D., MATTEO, L. D., GHLGLIETTI, R., TOSCANO, P., ULEDI, M. & SAVIOLI, L. 1993. Intestinal parasitic infections of urban and rural children on Pemba Island: implications for control. *Annals of Tropical Medicine & Parasitology,* 87**,** 579-583.

ALBONICO, M., CHWAYA, H. M., MONTRESOR, A., STOLFZFUS, R. J., TIELSCH, J. M., ALAWI, K. S. & SAVIOLI, L. 1997. Parasitic infections in Pemba Island school children. *East Afr Med J,* 74**,** 294-8.

ALBONICO, M., ENGELS, D. & SAVIOLI, L. 2004a. Monitoring drug efficacy and early detection of drug resistance in human soil-transmitted nematodes: a pressing public health agenda for helminth control. *Int J Parasitol,* 34**,** 1205-10.

ALBONICO, M., MONTRESOR, A., CROMPTON, D. W. & SAVIOLI, L. 2006. Intervention for the control of soil-transmitted helminthiasis in the community. *Adv Parasitol,* 61**,** 311-48.

ALBONICO, M., SMITH, P. G., HALL, A., CHWAYA, H. M., ALAWI, K. S. & SAVIOLI, L. 1994. A randomized controlled trial comparing mebendazole and albendazole against *Ascaris*, *Trichuris* and hookworm infections. *Trans R Soc Trop Med Hyg,* 88**,** 585-9.

ALBONICO, M., WRIGHT, V. & BICKLE, Q. 2004b. Molecular analysis of the [beta]-tubulin gene of human hookworms as a basis for possible benzimidazole resistance on Pemba Island. *Molecular and Biochemical Parasitology,* 134**,** 281-284.

ALBONICO, M., WRIGHT, V. & BICKLE, Q. 2004c. Molecular analysis of the Î²-tubulin gene of human hookworms as a basis for possible benzimidazole resistance on Pemba Island. *Molecular and Biochemical Parasitology,* 134**,** 281-284.

ALBONICO, M., WRIGHT, V., RAMSAN, M., HAJI, H. J., TAYLOR, M., SAVIOLI, L. & BICKLE, Q. 2005. Development of the egg hatch assay for detection of anthelminthic resistance in human hookworms. *Int J Parasitol,* 35**,** 803-11.

ALLAN, F., ROLLINSON, D., SMITH, J. E. & DUNN, A. M. 2009. Host choice and penetration by *Schistosoma haematobium* miracidia. *Journal of Helminthology,* 83**,** 33-38.

AMARIR, F., EL MANSOURI, B., FELLAH, H., SEBTI, F., MOHAMMED, L., HANDALI, S., WILKINS, P., EL IDRISSI, A. L., SADAK, A. & RHAJAOUI, M. 2011. National serologic survey of Haematobium schistosomiasis in Morocco: evidence for elimination. *Am J Trop Med Hyg,* 84**,** 15-9.

ANDERSON, R., TRUSCOTT, J. & HOLLINGSWORTH, T. D. 2014. The coverage and frequency of mass drug administration required to eliminate persistent transmission of soil-transmitted helminths. *Philos Trans R Soc Lond B Biol Sci,* 369**,** 20130435.

ANDERSON, R. M. & MAY, R. M. 1992. *Infectious diseases of humans: dynamics and control*, Oxford university press.

BABU, B. V. & KAR, S. K. 2004. Coverage, compliance and some operational issues of mass drug administration during the programme to eliminate lymphatic filariasis in Orissa, India. *Trop Med Int Health,* 9**,** 702-9.

BALASCH, J., MARTINEZ-ROMAN, S., CREUS, M., CAMPO, E., FORTUNY, A. & VANRELL, J. A. 1995. Schistosomiasis: an unusual cause of tubal infertility. *Hum Reprod,* 10**,** 1725-7.

BARBER, K. E., MKOJI, G. M. & LOKER, E. S. 2000. PCR-RFLP analysis of the ITS2 region to identify *Schistosoma haematobium* and *S. bovis* from Kenya. *Am J Trop Med Hyg,* 62**,** 434-40.

BARKIA, H., BARKIA, A., YACOUBI, R., ALEMAD, A., EL KHARIM, K. & BELGHYTI, D. 2014. Contribution of Mobile Teams to Efforts to Eliminate Schistosomiasis at *Schistosoma haematobium* in Morocco- Narrative Review Article. *Iran J Public Health,* 43**,** 1167-75.

BASANEZ, M. G., MCCARTHY, J. S., FRENCH, M. D., YANG, G. J., WALKER, M., GAMBHIR, M., PRICHARD, R. K. & CHURCHER, T. S. 2012. A research agenda for helminth diseases of humans: modelling for control and elimination. *PLoS Negl Trop Dis,* 6**,** e1548.

BERHE, N., GUNDERSEN, S. G., ABEBE, F., BIRRIE, H., MEDHIN, G. & GEMETCHU, T. 1999. Praziquantel side effects and efficacy related to *Schistosoma mansoni* egg loads and morbidity in primary school children in north-east Ethiopia. *Acta Tropica,* 72**,** 53-63.

BETHONY, J., BROOKER, S., ALBONICO, M., GEIGER, S. M., LOUKAS, A., DIEMERT, D. & HOTEZ, P. J. 2006. Soil-transmitted helminth infections: *ascariasis*, *trichuriasis*, and hookworm. *Lancet,* 367**,** 1521-32.

BETHONY, J. M., SIMON, G., DIEMERT, D. J., PARENTI, D., DESROSIERS, A., SCHUCK, S., FUJIWARA, R., SANTIAGO, H. & HOTEZ, P. J. 2008. Randomized, placebo-controlled, double-blind trial of the Na-ASP-2 hookworm vaccine in unexposed adults. *Vaccine,* 26**,** 2408-17.

BETSON, M., SOUSA-FIGUEIREDO, J. C., ROWELL, C., KABATEREINE, N. B. & STOTHARD, J. R. 2010. Intestinal schistosomiasis in mothers and young children in Uganda: investigation of field-applicable markers of bowel morbidity. *Am J Trop Med Hyg,* 83**,** 1048-55.

BLACK, C. L., STEINAUER, M. L., MWINZI, P. N., EVAN SECOR, W., KARANJA, D. & COLLEY, D. G. 2009. Impact of intense, longitudinal retreatment with praziquantel on cure rates of schistosomiasis mansoni in a cohort of occupationally exposed adults in western Kenya. *Tropical Medicine & International Health,* 14**,** 450-457.

BOELEE, E. 2006. Irrigation and schistosomiasis in Africa: ecological aspects. 99.

BORKOW, G. & BENTWICH, Z. 2004. Chronic immune activation associated with chronic helminthic and human immunodeficiency virus infections: role of hyporesponsiveness and anergy. *Clin Microbiol Rev,* 17**,** 1012-30, table of contents.

BOTROS, S., SAYED, H., AMER, N., EL-GHANNAM, M., BENNETT, J. L. & DAY, T. A. 2005. Current status of sensitivity to praziquantel in a focus of potential drug resistance in Egypt. *Int J Parasitol,* 35**,** 787-91.

BOTROS, S. S. & BENNETT, J. L. 2007. Praziquantel resistance. *Expert Opin Drug Discov,* 2**,** S35-40.

BRIEGER, W. R., OKEIBUNOR, J. C., ABIOSE, A. O., WANJI, S., ELHASSAN, E., NDYOMUGYENYI, R. & AMAZIGO, U. V. 2011. Compliance with eight years of annual ivermectin treatment of onchocerciasis in Cameroon and Nigeria. *Parasit Vectors,* 4**,** 152.

BRINDLEY, P. J., HEATH, S., WATERS, A. P., MCCUTCHAN, T. F. & SHER, A. 1991. Characterization of a programmed alteration in an 18S ribosomal gene that accompanies the experimental induction of drug resistance in *Schistosoma mansoni*. *Proc Natl Acad Sci U S A,* 88**,** 7754-8.

BRINDLEY, P. J., STRAND, M., NORDEN, A. P. & SHER, A. 1989. Role of host antibody in the chemotherapeutic action of praziquantel against *Schistosoma mansoni*: identification of target antigens. *Mol Biochem Parasitol,* 34**,** 99-108.

BROADHURST, M. J., LEUNG, J. M., KASHYAP, V., MCCUNE, J. M., MAHADEVAN, U., MCKERROW, J. H. & LOKE, P. 2010. IL-22+ CD4+ T cells are associated with therapeutic *trichuris trichiura* infection in an ulcerative colitis patient. *Sci Transl Med,* 2**,** 60ra88.

BROADHURST, M. J., LEUNG, J. M., KASHYAP, V., MCCUNE, J. M., MAHADEVAN, U., MCKERROW, J. H. & LOKE, P. 2012. IL-22+ CD4+ T cells are associated with therapeutic *trichuris trichiura* infection in an ulcerative colitis patient. *Sci Transl Med,* 2**,** 60ra88.

BROOKER, S. 2010. Estimating the global distribution and disease burden of intestinal nematode infections: adding up the numbers–a review. *International journal for parasitology,* 40**,** 1137-1144.

BROOKER, S., CLEMENTS, A. C. & BUNDY, D. A. 2006a. Global epidemiology, ecology and control of soil-transmitted helminth infections. *Adv Parasitol,* 62**,** 221 - 261.

BROOKER, S., CLEMENTS, A. C. & BUNDY, D. A. 2006b. Global epidemiology, ecology and control of soil-transmitted helminth infections. *Adv Parasitol,* 62**,** 221-61.

BROOKER, S., HOTEZ, P. J. & BUNDY, D. A. 2008. Hookworm-related anaemia among pregnant women: a systematic review. *PLoS Negl Trop Dis,* 2**,** e291.

BROOKER, S., WHAWELL, S., KABATEREINE, N. B., FENWICK, A. & ANDERSON, R. M. 2004. Evaluating the epidemiological impact of national control programmes for helminths. *Trends in Parasitology,* 20**,** 537-545.

BUTLER, S. E., MUOK, E. M., MONTGOMERY, S. P., ODHIAMBO, K., MWINZI, P. M., SECOR, W. E. & KARANJA, D. M. 2012. Mechanism of anemia in *Schistosoma mansoni*-infected school children in Western Kenya. *Am J Trop Med Hyg,* 87**,** 862-7.

CAFFREY, C. R. 2007. Chemotherapy of schistosomiasis: present and future. *Current Opinion in Chemical Biology,* 11**,** 433-439.

CANTEY, P. T., RAO, G., ROUT, J. & FOX, L. M. 2010. Predictors of compliance with a mass drug administration programme for lymphatic filariasis in Orissa State, India 2008. *Trop Med Int Health,* 15**,** 224-31.

CANTRELL, M. A. 1981. Bilharzia snails and water level fluctuations in a tropical swamp. *Oikos***,** 226-232.

CAO, C. L., BAO, Z. P., CHEN, L., WANG, D. H., MENG, X. H., WANG, L., ZHANG, Y. Y., WANG, H., ZHONG, B., ZHAO, G. M. & GUO, J. G. 2011. [Compliance of film-coated praziquantel tablets in schistosomiasis transmission-controlled areas]. *Zhongguo Xue Xi Chong Bing Fang Zhi Za Zhi,* 23**,** 659-63.

CAO, L., HU, G., GUO, J., ZHANG, J., CAO, C., LI, D., YU, Q. & LIN, H. 2002. Compliance study on mass chemotherapy with praziquantel in schistosomiasis hyper-endemic area of Poyang Lake region for successive 6 years. *Parasitoses and Infectious Diseases,* 1**,** 153-155.

CARABIN, H., CHAN, M. S. & GUYATT, H. L. 2000. A population dynamic approach to evaluating the impact of school attendance on the unit cost and effectiveness of school-based schistosomiasis chemotherapy programmes. *Parasitology,* 121 (Pt 2)**,** 171-83.

CARVALHO, S., CALDEIRA, R. L., SIMPSON, A. J. & VIDIGAL, T. H. 2001. Genetic variability and molecular identification of Brazilian Biomphalaria species (Mollusca: Planorbidae). *Parasitology,* 123 Suppl**,** S197-209.

CASMO, V., AUGUSTO, G., NALA, R., SABONETE, A. & CARVALHO-COSTA, F. A. 2014. The effect of hookworm infection and urinary schistosomiasis on blood hemoglobin concentration of schoolchildren living in northern Mozambique. *Rev Inst Med Trop Sao Paulo,* 56**,** 219-24.

CAWSTON, F. 1927. The snail host for bilharzia in Zanzibar. *Journal of Tropical Medicine and Hygiene,* 30**,** 209.

CHAN, M. S. 1997. The global burden of intestinal nematode infections--fifty years on. *Parasitol Today,* 13**,** 438-43.

CHAN, M. S., MONTRESOR, A., SAVIOLI, L. & BUNDY, D. A. 1999. Planning chemotherapy based schistosomiasis control: validation of a mathematical model using data on *Schistosoma haematobium* from Pemba, Tanzania. *Epidemiol Infect,* 123**,** 487-97.

CHANDIWANA, S. K. & CHRISTENSEN, N. O. 1988. Analysis of the dynamics of transmission of human schistosomiasis in the highveld region of Zimbabwe. A review. *Trop Med Parasitol,* 39**,** 187-93.

CHANDIWANA, S. K., CHRISTENSEN, N. O. & FRANDSEN, F. 1987. Seasonal patterns in the transmission of *Schistosoma haematobium*, *S. mattheei* and *S. mansoni* in the highveld region of Zimbabwe. *Acta Trop,* 44**,** 433-44.

CHANDRAWATHANI, P., ADNAN, M. & WALLER, P. J. 1999. Anthelmintic resistance in sheep and goat farms on Peninsular Malaysia. *Veterinary Parasitology,* 82**,** 305-310.

CHEN, Y. Y., LIU, J. B., HUANG, X. B., CAI, S. X., SU, Z. M., ZHONG, R., ZOU, L. & MIAO, X. P. 2014. New integrated strategy emphasizing infection source control to curb *Schistosomiasis japonica* in a marshland area of Hubei Province, China: findings from an eight-year longitudinal survey. *PLoS One,* 9**,** e89779.

CHIARAMONTE, M. G., DONALDSON, D. D., CHEEVER, A. W. & WYNN, T. A. 1999. An IL-13 inhibitor blocks the development of hepatic fibrosis during a T-helper type 2–dominated inflammatory response. *The Journal of clinical investigation,* 104**,** 777-785.

CHRISTENSEN, D. B. 1978. Drug-taking compliance: a review and synthesis. *Health Serv Res,* 13**,** 171-87.

CHU, K. Y., MASSOUD, J. & ARFAA, F. 1968. Distribution and ecology of *Bulinus truncatus* in Khuzestan, Iran. *Bull World Health Organ,* 39**,** 607-37.

CHU, T. B., LIAO, C. W., D'LAMINI, P., CHANG, P. W., CHIU, W. T., DU, W. Y. & FAN, C. K. 2010. Prevalence of *Schistosoma haematobium* infection among inhabitants of Lowveld, Swaziland, an endemic area for the disease. *Trop Biomed,* 27**,** 337-42.

CIOLI, D., BOTROS, S. S., WHEATCROFT-FRANCKLOW, K., MBAYE, A., SOUTHGATE, V., TCHUENTÉ, L.-A. T., PICA-MATTOCCIA, L., TROIANI, A. R., EL-DIN, S. H. S. & SABRA, A.-N. A. 2004. Determination of ED 50 values for praziquantel in praziquantel-resistant and-susceptible *Schistosoma mansoni* isolates. *International journal for parasitology,* 34**,** 979-987.

COLLEY, D. G. 2014. Morbidity control of schistosomiasis by mass drug administration: how can we do it best and what will it take to move on to elimination? *Trop Med Health,* 42**,** 25-32.

COOPER, E., WHYTE-ALLENG, C., FINZI-SMITH, J. & MACDONALD, T. 1992. Intestinal nematode infections in children: the pathophysiological price paid. *Parasitology,* 104**,** S91-S103.

COOPER, P. J., CHICO, M. E., LOSONSKY, G., SANDOVAL, C., ESPINEL, I., SRIDHARA, R., AGUILAR, M., GUEVARA, A., GUDERIAN, R. H., LEVINE, M. M., GRIFFIN, G. E. & NUTMAN, T. B. 2000. Albendazole treatment of children with ascariasis enhances the vibriocidal antibody response to the live attenuated oral cholera vaccine CVD 103-HgR. *J Infect Dis,* 182**,** 1199-206.

CORACHAN, M. 2002. Schistosomiasis and international travel. *Clin Infect Dis,* 35**,** 446-50.

COURA, J. R. 1995. Control of schistosomiasis in Brazil: perspectives and proposals. *Mem Inst Oswaldo Cruz,* 90**,** 257-60.

DABO, A., BADAWI, H. M., BARY, B. & DOUMBO, O. K. 2011. Urinary schistosomiasis among preschool-aged children in Sahelian rural communities in Mali. *Parasit Vectors,* 4**,** 21.

DAVIES, C. M., WEBSTER, J. P. & WOOLHOUS, M. E. 2001. Trade-offs in the evolution of virulence in an indirectly transmitted macroparasite. *Proc Biol Sci,* 268**,** 251-7.

DE GIER, B., NGA, T. T., WINICHAGOON, P., DIJKHUIZEN, M. A., KHAN, N. C., VAN DE BOR, M., PONCE, M. C., POLMAN, K. & WIERINGA, F. T. 2016. Species-Specific Associations Between Soil-Transmitted Helminths and Micronutrients in Vietnamese Schoolchildren. *Am J Trop Med Hyg,* 95**,** 77-82.

DE MEILLON, B., FRANK, G. H. & ALLANSON, B. R. 1958. Some aspects of snail ecology in South Africa; a preliminary report. *Bull World Health Organ,* 18**,** 771-83.

DE MOIRA, A. P., FULFORD, A. J., KABATEREINE, N. B., OUMA, J. H., BOOTH, M. & DUNNE, D. W. 2010. Analysis of complex patterns of human exposure and immunity to *Schistosomiasis mansoni*: the influence of age, sex, ethnicity and IgE. *PLoS Negl Trop Dis,* 4**,** e820.

DE SILVA, N. R., BROOKER, S., HOTEZ, P. J., MONTRESOR, A., ENGELS, D. & SAVIOLI, L. 2003. Soil-transmitted helminth infections: updating the global picture. *Trends Parasitol,* 19**,** 547-51.

DENNIS, E., VORKPOR, P., HOLZER, B., HANSON, A., SALADIN, B., SALADIN, K. & DEGREMONT, A. 1983. Studies on the epidemiology of schistosomiasis in Liberia: the prevalence and intensity of schistosomal infections in Bong County and the bionomics of the snail intermediate hosts. *Acta Trop,* 40**,** 205-29.

DIAWARA, A., DRAKE, L. J., SUSWILLO, R. R., KIHARA, J., BUNDY, D. A., SCOTT, M. E., HALPENNY, C., STOTHARD, J. R. & PRICHARD, R. K. 2009. Assays to detect beta-tubulin codon 200 polymorphism in *Trichuris trichiura* and *Ascaris lumbricoides*. *PLoS Negl Trop Dis,* 3**,** e397.

DOENHOFF, M. J., HAGAN, P., CIOLI, D., SOUTHGATE, V., PICA-MATTOCCIA, L., BOTROS, S., COLES, G., TCHUEM TCHUENTE, L. A., MBAYE, A. & ENGELS, D. 2009. Praziquantel: its use in control of schistosomiasis in sub-Saharan Africa and current research needs. *Parasitology,* 136**,** 1825-35.

DOENHOFF, M. J. & PICA-MATTOCCIA, L. 2006. Praziquantel for the treatment of schistosomiasis: its use for control in areas with endemic disease and prospects for drug resistance. *Expert Rev Anti Infect Ther,* 4**,** 199-210.

DONNELLY, F. A., APPLETON, C. C. & SCHUTTE, C. H. 1983. The influence of salinity on certain aspects of the biology of *Bulinus* (Physopsis) *africanus*. *Int J Parasitol,* 13**,** 539-45.

ENGELS, D., CHITSULO, L., MONTRESOR, A. & SAVIOLI, L. 2002. The global epidemiological situation of schistosomiasis and new approaches to control and research. *Acta Tropica,* 82**,** 139-146.

ERKO, B., DEGAREGE, A., TADESSE, K., MATHIWOS, A. & LEGESSE, M. 2012. Efficacy and side effects of praziquantel in the treatment of *Schistosomiasis mansoni* in schoolchildren in Shesha Kekele Elementary School, Wondo Genet, Southern Ethiopia. *Asian Pac J Trop Biomed,* 2**,** 235-9.

ESREY, S. A., POTASH, J. B., ROBERTS, L. & SHIFF, C. 1991. Effects of improved water supply and sanitation on ascariasis, diarrhoea, dracunculiasis, hookworm infection, schistosomiasis, and trachoma. *Bull World Health Organ,* 69**,** 609-21.

ETARD, J. E. 2004. [Modelling sensitivity, specificity and predictive values of hematuria testing using reagent sticks in the diagnosis of *Schistosoma haematobium* infection]. *Bull Soc Pathol Exot,* 97**,** 24-8.

ETARD, J. F., AUDIBERT, M. & DABO, A. 1995. Age-acquired resistance and predisposition to reinfection with *Schistosoma haematobium* after treatment with praziquantel in Mali. *Am J Trop Med Hyg,* 52**,** 549-58.

FENWICK, A. 2012. The global burden of neglected tropical diseases. *Public Health,* 126**,** 233-236.

FENWICK, A. & JOURDAN, P. 2016. Schistosomiasis elimination by 2020 or 2030? *Int J Parasitol,* 46**,** 385-8.

FENWICK, A., ROLLINSON, D. & SOUTHGATE, V. 2006a. Implementation of human schistosomiasis control: Challenges and prospects. *Adv Parasitol,* 61**,** 567-622.

FENWICK, A., ROLLINSON, D., SOUTHGATE, V. & DAVID, H. M. 2006b. Implementation of Human Schistosomiasis Control: Challenges and Prospects. *Advances in Parasitology.* Academic Press.

FENWICK, A. & WEBSTER, J. P. 2006. Schistosomiasis: challenges for control, treatment and drug resistance. *Curr Opin Infect Dis,* 19**,** 577-82.

FENWICK, A., WEBSTER, J. P., BOSQUE-OLIVA, E., BLAIR, L., FLEMING, F., ZHANG, Y., GARBA, A., STOTHARD, J., GABRIELLI, A. F. & CLEMENTS, A. 2009. The Schistosomiasis Control Initiative (SCI): rationale, development and implementation from 2002–2008. *Parasitology,* 136**,** 1719-1730.

FIRMO, J. O., LIMA COSTA, M. F., GUERRA, H. L. & ROCHA, R. S. 1996. Urban schistosomiasis: morbidity, sociodemographic characteristics and water contact patterns predictive of infection. *Int J Epidemiol,* 25**,** 1292-300.

FLEMING, F. M., FENWICK, A., TUKAHEBWA, E. M., LUBANGA, R. G., NAMWANGYE, H., ZARAMBA, S. & KABATEREINE, N. B. 2009. Process evaluation of schistosomiasis control in Uganda, 2003 to 2006: perceptions, attitudes and constraints of a national programme. *Parasitology,* 136**,** 1759-69.

FORSYTH, D. M. & MACDONALD, G. 1965. Urological Complications of Endemic Schistosomiasis in School-Children. I. Usagara School. *Trans R Soc Trop Med Hyg,* 59**,** 171-8.

FRIEDMAN, J. F., KANZARIA, H. K., ACOSTA, L. P., LANGDON, G. C., MANALO, D. L., WU, H., OLVEDA, R. M., MCGARVEY, S. T. & KURTIS, J. D. 2005a. Relationship between *Schistosoma japonicum* and nutritional status among children and young adults in Leyte, the Philippines. *Am J Trop Med Hyg,* 72**,** 527-33.

FRIEDMAN, J. F., KANZARIA, H. K. & MCGARVEY, S. T. 2005b. Human schistosomiasis and anemia: the relationship and potential mechanisms. *Trends Parasitol,* 21**,** 386-92.

FROBERG, G., JORNHAGEN, L., MORRIS, U., SHAKELY, D., MSELLEM, M. I., GIL, J. P., BJORKMAN, A. & MARTENSSON, A. 2012. Decreased prevalence of *Plasmodium falciparum* resistance markers to amodiaquine despite its wide scale use as ACT partner drug in Zanzibar. *Malar J,* 11**,** 321.

GABRIELLI, A.-F., MONTRESOR, A., CHITSULO, L., ENGELS, D. & SAVIOLI, L. 2011. Preventive chemotherapy in human helminthiasis: theoretical and operational aspects. *Transactions of the Royal Society of Tropical Medicine and Hygiene,* 105**,** 683-693.

GABRIELLI, A. F., RAMSAN, M., NAUMANN, C., TSOGZOLMAA, D., BOJANG, B., KHOSHAL, M. H., CONNOLLY, M., STOTHARD, J. R., MONTRESOR, A. & SAVIOLI, L. 2005. Soil-transmitted helminths and haemoglobin status among Afghan children in World Food Programme assisted schools. *J Helminthol,* 79**,** 381-4.

GARBA, A., BARKIRE, N., DJIBO, A., LAMINE, M. S., SOFO, B., GOUVRAS, A. N., BOSQUE-OLIVA, E., WEBSTER, J. P., STOTHARD, J. R., UTZINGER, J. & FENWICK, A. 2010. Schistosomiasis in infants and preschool-aged children: Infection in a single *Schistosoma haematobium* and a mixed S. haematobium-S. mansoni foci of Niger. *Acta Trop,* 115**,** 212-9.

GARBA, A., LAMINE, M. S., DJIBO, A., TAHIROU, A., AOUAMI, M. A., ALFARI, A., PHILLIPS, A. E., FENWICK, A. & UTZINGER, J. 2013. Safety and efficacy of praziquantel syrup (Epiquantel(R)) against *Schistosoma haematobium* and *Schistosoma mansoni* in preschool-aged children in Niger. *Acta Trop,* 128**,** 318-25.

GEARY, T. G., WOO, K., MCCARTHY, J. S., MACKENZIE, C. D., HORTON, J., PRICHARD, R. K., DE SILVA, N. R., OLLIARO, P. L., LAZDINS-HELDS, J. K., ENGELS, D. A. & BUNDY, D. A. 2009. Unresolved issues in anthelmintic pharmacology for helminthiases of humans. *Int J Parasitol,* 40**,** 1-13.

GENTA, R. M. 1993. Diarrhea in helminthic infections. *Clin Infect Dis,* 16 Suppl 2**,** S122-9.

GILLES, H. M., WILLIAMS, E. J. & BALL, P. A. 1964. Hookworm Infection and Anaemia. An Epidemiological, Clinical, and Laboratory Study. *Q J Med,* 33**,** 1-24.

GOODMAN, D., HAJI, H. J., BICKLE, Q. D., STOLTZFUS, R. J., TIELSCH, J. M., RAMSAN, M., SAVIOLI, L. & ALBONICO, M. 2007. A comparison of methods for detecting the eggs of *Ascaris*, *Trichuris,* and hookworm in infant stool, and the epidemiology of infection in Zanzibari infants. *The American journal of tropical medicine and hygiene,* 76**,** 725-731.

GREENBERG, R. M. 2005. Are Ca2+ channels targets of praziquantel action? *International Journal for Parasitology,* 35**,** 1-9.

GRYSEELS, B., MBAYE, A., DE VLAS, S. J., STELMA, F. F., GUISSE, F., VAN LIESHOUT, L., FAYE, D., DIOP, M., LY, A., TCHUEM-TCHUENTE, L. A., ENGELS, D. & POLMAN, K. 2001a. Are poor responses to praziquantel for the treatment of *Schistosoma mansoni* infections in Senegal due to resistance? An overview of the evidence. *Trop Med Int Health,* 6**,** 864-73.

GRYSEELS, B., MBAYE, A., DE VLAS, S. J., STELMA, F. F., GUISSÉ, F., VAN LIESHOUT, L., FAYE, D., DIOP, M., LY, A., TCHUEM-TCHUENTÉ, L. A., ENGELS, D. & POLMAN, K. 2001b. Are poor responses to praziquantel for the treatment of *Schistosoma mansoni* infections in Senegal due to resistance? An overview of the evidence. *Tropical Medicine & International Health,* 6**,** 864-873.

GUERRA-SILVEIRA, F. & ABAD-FRANCH, F. 2013. Sex bias in infectious disease epidemiology: patterns and processes. *PLoS One,* 8**,** e62390.

GUIDI, A., ANDOLINA, C., MAKAME AME, S., ALBONICO, M., CIOLI, D. & JUMA HAJI, H. 2010. Praziquantel efficacy and long-term appraisal of schistosomiasis control in Pemba Island. *Trop Med Int Health,* 15**,** 614-8.

HAMBURGER, J., ABBASI, I., KARIUKI, C., WANJALA, A., MZUNGU, E., MUNGAI, P., MUCHIRI, E. & KING, C. H. 2013. Evaluation of loop-mediated isothermal amplification suitable for molecular monitoring of schistosome-infected snails in field laboratories. *Am J Trop Med Hyg,* 88**,** 344-51.

HAMBURGER, J., HE, N., ABBASI, I., RAMZY, R. M., JOURDANE, J. & RUPPEL, A. 2001. Polymerase chain reaction assay based on a highly repeated sequence of *Schistosoma haematobium*: a potential tool for monitoring schistosome-infested water. *Am J Trop Med Hyg,* 65**,** 907-11.

HAMBURGER, J., HE, N., XIN, X. Y., RAMZY, R. M., JOURDANE, J. & RUPPEL, A. 1998. A polymerase chain reaction assay for detecting snails infected with bilharzia parasites (Schistosoma mansoni) from very early prepatency. *Am J Trop Med Hyg,* 59**,** 872-6.

HAMBURGER, J., HOFFMAN, O., KARIUKI, H. C., MUCHIRI, E. M., OUMA, J. H., KOECH, D. K., STURROCK, R. F. & KING, C. H. 2004. Large-scale, polymerase chain reaction-based surveillance of *Schistosoma haematobium* DNA in snails from transmission sites in

coastal Kenya: a new tool for studying the dynamics of snail infection. *Am J Trop Med Hyg,* 71**,** 765-73.

HARRIS, J. B., PODOLSKY, M. J., BHUIYAN, T. R., CHOWDHURY, F., KHAN, A. I., LAROCQUE, R. C., LOGVINENKO, T., KENDALL, J., FARUQUE, A. S., NAGLER, C. R., RYAN, E. T., QADRI, F. & CALDERWOOD, S. B. 2009. Immunologic responses to Vibrio cholerae in patients co-infected with intestinal parasites in Bangladesh. *PLoS Negl Trop Dis,* 3**,** e403.

HICKS, R. M. 1983. The canopic worm: role of bilharziasis in the aetiology of human bladder cancer. *J R Soc Med,* 76**,** 16-22.

HOTEZ, P. J., BROOKER, S., BETHONY, J. M., BOTTAZZI, M. E., LOUKAS, A. & XIAO, S. 2004. Hookworm infection. *N Engl J Med,* 351**,** 799-807.

HOTEZ, P. J., BUNDY, D. A. P., BEEGLE, K., BROOKER, S., DRAKE, L., DE SILVA, N., MONTRESOR, A., ENGELS, D., JUKES, M., CHITSULO, L., CHOW, J., LAXMINARAYAN, R., MICHAUD, C., BETHONY, J., CORREA-OLIVEIRA, R., SHUHUA, X., FENWICK, A. & SAVIOLI, L. 2006a. Helminth Infections: Soil-transmitted Helminth Infections and Schistosomiasis. *In:* JAMISON, D. T., BREMAN, J. G., MEASHAM, A. R., ALLEYNE, G., CLAESON, M., EVANS, D. B., JHA, P., MILLS, A. & MUSGROVE, P. (eds.) *Disease Control Priorities in Developing Countries.* 2nd ed. Washington (DC).

HOTEZ, P. J., BUNDY, D. A. P., BEEGLE, K., BROOKER, S., DRAKE, L., DE SILVA, N., MONTRESOR, A., ENGELS, D., JUKES, M., CHITSULO, L., CHOW, J., LAXMINARAYAN, R., MICHAUD, C., BETHONY, J., OLIVEIRA, R., XIAO, S. H., FENWICK, A. & SAVIOLI, L. 2006b. Helminth Infections: soil-transmitted helminth infections and schistosomiasis. *Disease Control Priorities in Developing Countries***,** 467 - 497.

HOTEZ, P. J. & FENWICK, A. 2009. Schistosomiasis in Africa: an emerging tragedy in our new global health decade. *PLoS Negl Trop Dis,* 3**,** e485.

HOTEZ, P. J., FENWICK, A., SAVIOLI, L. & MOLYNEUX, D. H. 2009. Rescuing the bottom billion through control of neglected tropical diseases. *Lancet,* 373**,** 1570-5.

HOTEZ, P. J., ZHAN, B., BETHONY, J. M., LOUKAS, A., WILLIAMSON, A., GOUD, G. N., HAWDON, J. M., DOBARDZIC, A., DOBARDZIC, R., GHOSH, K., BOTTAZZI, M. E., MENDEZ, S., ZOOK, B., WANG, Y., LIU, S., ESSIET-GIBSON, I., CHUNG-DEBOSE, S., XIAO, S., KNOX, D., MEAGHER, M., INAN, M., CORREA-OLIVEIRA, R., VILK, P., SHEPHERD, H. R., BRANDT, W. & RUSSELL, P. K. 2003. Progress in the development of a recombinant vaccine for human hookworm disease: the Human Hookworm Vaccine Initiative. *Int J Parasitol,* 33**,** 1245-58.

ISMAIL, M., METWALLY, A., FARGHALY, A., BRUCE, J., TAO, L. F. & BENNETT, J. L. 1996. Characterization of isolates of *Schistosoma mansoni* from Egyptian villagers that tolerate high doses of praziquantel. *Am J Trop Med Hyg,* 55**,** 214-8.

JACKSON, J. A., TURNER, J. D., RENTOUL, L., FAULKNER, H., BEHNKE, J. M., HOYLE, M., GRENCIS, R. K., ELSE, K. J., KAMGNO, J., BRADLEY, J. E. & BOUSSINESQ, M. 2004. Cytokine response profiles predict species-specific infection patterns in human GI nematodes. *Int J Parasitol,* 34**,** 1237-44.

JORDAN, P. 1985. *Schistosomiasis-the St Lucia Projectcontinued*, Cambridge University Press.

JORGENSEN, A., STOTHARD, J. R., MADSEN, H., NALUGWA, A., NYAKAANA, S. & ROLLINSON, D. 2013. The ITS2 of the genus *Bulinus*: novel secondary structure among freshwater snails and potential new taxonomic markers. *Acta Trop,* 128**,** 218-25.

JOSEPH, N., SUBBA, S., JAIN, A., UNNIKRISHNAN, B., NAGARAJ, K. & KOTIAN, S. 2011. Awareness of health personnel about lymphatic filariasis and mass drug administration in Karnataka state of South India. *Australas Med J,* 4**,** 87-93.

KAATANO, G. M., SIZA, J. E., MWANGA, J. R., MIN, D. Y., YONG, T. S., CHAI, J. Y., KO, Y., CHANG, S. Y., KULLAYA, C. M., RIM, H. J., CHANGALUCHA, J. M. & EOM, K. S. 2015. Integrated Schistosomiasis and Soil-Transmitted Helminthiasis Control over Five Years on Kome Island, Tanzania. *Korean J Parasitol,* 53**,** 535-43.

KABATEREINE, N. B., BROOKER, S., KOUKOUNARI, A., KAZIBWE, F., TUKAHEBWA, E. M., FLEMING, F. M., ZHANG, Y., WEBSTER, J. P., STOTHARD, J. R. & FENWICK, A. 2007. Impact of a national helminth control programme on infection and morbidity in Ugandan schoolchildren. *Bull World Health Organ,* 85**,** 91 - 99.

KABATEREINE, N. B., TUKAHEBWA, E. M., KAZIBWE, F., TWA-TWA, J. M., BARENZI, J. F., ZARAMBA, S., STOTHARD, J. R., FENWICK, A. & BROOKER, S. 2005. Soil-transmitted helminthiasis in Uganda: epidemiology and cost of control. *Trop Med Int Health,* 10**,** 1187-9.

KABATEREINE, N. B., VENNERVALD, B. J., OUMA, J. H., KEMIJUMBI, J., BUTTERWORTH, A. E., DUNNE, D. W. & FULFORD, A. J. 1999. Adult resistance to schistosomiasis mansoni: age-dependence of reinfection remains constant in communities with diverse exposure patterns. *Parasitology,* 118 (Pt 1)**,** 101-5.

KAMAL, S. M. & EL SAYED KHALIFA, K. 2006. Immune modulation by helminthic infections: worms and viral infections. *Parasite Immunol,* 28**,** 483-96.

KANDIL, O. M., MAHMOUD, M. S., ALLAM, N. & NAMAKY, A. 2010. Mitochondrial cytochrome c oxidase subunit 1 (cox 1) gene sequence of the Hymenolepis species. *J Am Sci,* 6**,** 640-647.

KANE, R. A., STOTHARD, J. R., EMERY, A. M. & ROLLINSON, D. 2008. Molecular characterization of freshwater snails in the genus Bulinus: a role for barcodes? *Parasit Vectors,* 1**,** 15.

KANE, R. A., STOTHARD, J. R., ROLLINSON, D., LECLIPTEUX, T., EVRAERTS, J., STANDLEY, C. J., ALLAN, F., BETSON, M., KABA, R., MERTENS, P. & LAURENT, T. 2013. Detection and quantification of schistosome DNA in freshwater snails using either fluorescent probes in real-time PCR or oligochromatographic dipstick assays targeting the ribosomal intergenic spacer. *Acta Trop,* 128**,** 241-9.

KARIUKI, H. C., CLENNON, J. A., BRADY, M. S., KITRON, U., STURROCK, R. F., OUMA, J. H., NDZOVU, S. T., MUNGAI, P., HOFFMAN, O., HAMBURGER, J., PELLEGRINI, C., MUCHIRI, E. M. & KING, C. H. 2004a. Distribution patterns and cercarial shedding of *Bulinus nasutus* and other snails in the Msambweni area, Coast Province, Kenya. *Am J Trop Med Hyg,* 70**,** 449-56.

KARIUKI, H. C., CLENNON, J. A., BRADY, M. S., KITRON, U., STURROCK, R. F., OUMA, J. H., NDZOVU, S. T. M., MUNGAI, P., HOFFMAN, O. & HAMBURGER, J. 2004b. Distribution patterns and cercarial shedding of *Bulinus nasutus* and other snails in the Msambweni area, Coast Province, Kenya. *American Journal of Tropical Medicine and Hygiene,* 70**,** 449-456.

KASINATHAN, R. S., GORONGA, T., MESSERLI, S. M., WEBB, T. R. & GREENBERG, R. M. 2010. Modulation of a *Schistosoma mansoni* multidrug transporter by the antischistosomal drug praziquantel. *The FASEB Journal,* 24**,** 128-135.

KATZ, N. 1998. Schistosomiasis control in Brazil. *Mem Inst Oswaldo Cruz,* 93 Suppl 1**,** 33-5.

KEISER, J. & UTZINGER, J. 2008. Efficacy of current drugs against soil-transmitted helminth infections: systematic review and meta-analysis. *JAMA,* 299**,** 1937 - 1948.

KING, C. H., DICKMAN, K. & TISCH, D. J. 2005. Reassessment of the cost of chronic helmintic infection: a meta-analysis of disability-related outcomes in endemic schistosomiasis. *Lancet,* 365**,** 1561-9.

KING, C. H., KEATING, C. E., MURUKA, J. F., OUMA, J. H., HOUSER, H., SIONGOK, T. K. & MAHMOUD, A. A. 1988. Urinary tract morbidity in *schistosomiasis haematobia*: associations with age and intensity of infection in an endemic area of Coast Province, Kenya. *Am J Trop Med Hyg,* 39**,** 361-8.

KING, C. H., MUCHIRI, E. M. & OUMA, J. H. 2000. Evidence against rapid emergence of praziquantel resistance in *Schistosoma haematobium*, Kenya. *Emerg Infect Dis,* 6**,** 585-94.

KIRKWOOD, B. & STERNE, J. 2003. *Essential Medical Statistics*, Wiley.

KJETLAND, E. F., POGGENSEE, G., HELLING-GIESE, G., RICHTER, J., SJAASTAD, A., CHITSULO, L., KUMWENDA, N., GUNDERSEN, S. G., KRANTZ, I. & FELDMEIER, H. 1996. Female genital schistosomiasis due to *Schistosoma haematobium*. Clinical and parasitological findings in women in rural Malawi. *Acta Trop,* 62**,** 239-55.

KNOPP, S., MGENI, A. F., KHAMIS, I. S., STEINMANN, P., STOTHARD, J. R., ROLLINSON, D., MARTI, H. & UTZINGER, J. 2008. Diagnosis of soil-transmitted helminths in the era of preventive chemotherapy: effect of multiple stool sampling and use of different diagnostic techniques. *PLoS Negl Trop Dis,* 2**,** e331.

KNOPP, S., MOHAMMED, K. A., ALI, S. M., KHAMIS, I. S., AME, S. M., ALBONICO, M., GOUVRAS, A., FENWICK, A., SAVIOLI, L., COLLEY, D. G., UTZINGER, J., PERSON, B. & ROLLINSON, D. 2012. Study and implementation of urogenital schistosomiasis elimination in Zanzibar (Unguja and Pemba islands) using an integrated multidisciplinary approach. *BMC Public Health,* 12**,** 930.

KNOPP, S., MOHAMMED, K. A., ROLLINSON, D., STOTHARD, J. R., KHAMIS, I. S., UTZINGER, J. & MARTI, H. 2009. Changing patterns of soil-transmitted helminthiases in Zanzibar in the context of national helminth control programs. *Am J Trop Med Hyg,* 81**,** 1071-8.

KNOPP, S., PERSON, B., AME, S. M., MOHAMMED, K. A., ALI, S. M., KHAMIS, I. S., RABONE, M., ALLAN, F., GOUVRAS, A., BLAIR, L., FENWICK, A., UTZINGER, J. & ROLLINSON, D. 2013. Elimination of schistosomiasis transmission in Zanzibar: baseline findings before the onset of a randomized intervention trial. *PLoS Negl Trop Dis,* 7**,** e2474.

KOTB, M. M., SHOUMAN, A. E., HUSSEIN, H. M., KHELA, A. K. & KANDIL, S. K. 1996. Evaluation of the effectiveness of dipstick haematuria and proteinuria in screening *Schistosoma haematobium* infection among school children in upper Egypt. *J Egypt Public Health Assoc,* 71**,** 353-67.

KOUKOUNARI, A., GABRIELLI, A. F., TOURE, S., BOSQUE-OLIVA, E., ZHANG, Y., SELLIN, B., DONNELLY, C. A., FENWICK, A. & WEBSTER, J. P. 2007. *Schistosoma haematobium* infection and morbidity before and after large-scale administration of praziquantel in Burkina Faso. *J Infect Dis,* 196**,** 659-69.

KUMAGAI, T., FURUSHIMA-SHIMOGAWARA, R., OHMAE, H., WANG, T. P., LU, S., CHEN, R., WEN, L. & OHTA, N. 2010. Detection of early and single infections of *Schistosoma japonicum* in the intermediate host snail, Oncomelania hupensis, by PCR and loop-mediated isothermal amplification (LAMP) assay. *Am J Trop Med Hyg,* 83**,** 542-8.

KUNG'U, J. K., GOODMAN, D., HAJI, H. J., RAMSAN, M., WRIGHT, V. J., BICKLE, Q. D., TIELSCH, J. M., RAYNES, J. G. & STOLTZFUS, R. J. 2009. Early helminth infections are inversely related to anemia, malnutrition, and malaria and are not associated with inflammation in 6- to 23-month-old Zanzibari children. *Am J Trop Med Hyg,* 81**,** 1062-70.

LAAMRANI, H., KHALLAAYOUNE, K., MADSEN, H., MAHJOUR, J. & GRYSEELS, B. 2000a. New challenges in schistosomiasis control in Morocco. *Acta Trop,* 77**,** 61-7.

LAAMRANI, H., MAHJOUR, J., MADSEN, H., KHALLAAYOUNE, K. & GRYSEELS, B. 2000b. *Schistosoma haematobium* in Morocco: moving from control to elimination. *Parasitol Today,* 16**,** 257-60.

LE HESRAN, J.-Y., AKIANA, J., NDIAYE, E. H. M., DIA, M., SENGHOR, P. & KONATE, L. 2004. Severe malaria attack is associated with high prevalence of *Ascaris lumbricoides* infection among children in rural Senegal. *Transactions of the Royal Society of Tropical Medicine and Hygiene,* 98**,** 397-399.

LE, T. H., BLAIR, D. & MCMANUS, D. P. 2000. Mitochondrial DNA sequences of human schistosomes: the current status. *Int J Parasitol,* 30**,** 283-90.

LEE, K. L. & LEWIS, E. R. 1977. Prediction of schistosome cercarial shedding with a physiological - time model. *Int J Epidemiol,* 6**,** 161-7.

LESLIE, J., GARBA, A., OLIVA, E. B., BARKIRE, A., TINNI, A. A., DJIBO, A., MOUNKAILA, I. & FENWICK, A. 2011. Schistosomiasis and soil-transmitted helminth control in Niger: cost

effectiveness of school based and community distributed mass drug administration [corrected]. *PLoS Negl Trop Dis,* 5**,** e1326.

LIMA E COSTA, M. F., MAGALHAES, M. H., ROCHA, R. S., ANTUNES, C. M. & KATZ, N. 1987. Water-contact patterns and socioeconomic variables in the epidemiology of *schistosomiasis mansoni* in an endemic area in Brazil. *Bull World Health Organ,* 65**,** 57-66.

LIU, L. X. & WELLER, P. F. 1996. Antiparasitic drugs. *N Engl J Med,* 334**,** 1178-84.

LO, N. C., BOGOCH, II, BLACKBURN, B. G., RASO, G., N'GORAN, E. K., COULIBALY, J. T., BECKER, S. L., ABRAMS, H. B., UTZINGER, J. & ANDREWS, J. R. 2015. Comparison of community-wide, integrated mass drug administration strategies for schistosomiasis and soil-transmitted helminthiasis: a cost-effectiveness modelling study. *Lancet Glob Health,* 3**,** e629-38.

LWAMBO, N. J. S. 1988. Transmission of urinary schistosomiasis in Sukumaland, Tanzania. 1. Snail infection rates and incidence of infection in school children. *Journal of Helminthology,* 62**,** 213-217.

MAGNUSSEN, P., MUCHIRI, E., MUNGAI, P., NDZOVU, M., OUMA, J. & TOSHA, S. 1997. A school-based approach to the control of urinary schistosomiasis and intestinal helminth infections in children in Matuga, Kenya: impact of a two-year chemotherapy programme on prevalence and intensity of infections. *Trop Med Int Health,* 2**,** 825-31.

MAHMOUD, A. & WOODRUFF, A. 1972. Mechanisms involved in the anaemia of schistosomiasis. *Transactions of the Royal Society of Tropical Medicine and Hygiene,* 66**,** 75-84.

MAIZELS, R. M. & YAZDANBAKHSH, M. 2003. Immune Regulation by helminth parasites: cellular and molecular mechanisms. *Nat Rev Immunol,* 3**,** 733-744.

MANGAL, T. D., PATERSON, S. & FENTON, A. 2008. Predicting the impact of long-term temperature changes on the epidemiology and control of schistosomiasis: a mechanistic model. *PLoS One,* 3**,** e1438.

MANSFIELD-ADERS, W. 1927. IX. Scientific. *Zanzibar Protectorate: Annual Report on the Medical, Sanitary and Biological Divisions for the Year***,** 49-78.

MARTENS, W. J. M., JETTEN, T. H., ROTMANS, J. & NIESSEN, L. W. 1995. Climate change and vector-borne diseases: A global modelling perspective. *Global Environmental Change,* 5**,** 195-209.

MAS-COMA, S., VALERO, M. A. & BARGUES, M. D. 2009. Climate change effects on trematodiases, with emphasis on zoonotic fascioliasis and schistosomiasis. *Vet Parasitol,* 163**,** 264-80.

MASSA, K., MAGNUSSEN, P., SHESHE, A., NTAKAMULENGA, R., NDAWI, B. & OLSEN, A. 2009a. Community perceptions on the community-directed treatment and school-based approaches for the control of schistosomiasis and soil-transmitted helminthiasis among school-age children in Lushoto District, Tanzania. *J Biosoc Sci,* 41**,** 89-105.

MASSA, K., OLSEN, A., SHESHE, A., NTAKAMULENGA, R., NDAWI, B. & MAGNUSSEN, P. 2009b. Can coverage of schistosomiasis and soil transmitted helminthiasis control programmes targeting school-aged children be improved? New approaches. *Parasitology,* 136**,** 1781-8.

MATHIEU, E., LAMMIE, P. J., RADDAY, J., BEACH, M. J., STREIT, T., WENDT, J. & ADDISS, D. G. 2004. Factors associated with participation in a campaign of mass treatment against lymphatic filariasis, in Leogane, Haiti. *Ann Trop Med Parasitol,* 98**,** 703-14.

MCCARTHY, D. 1930. Medical notes from Weti, Pemba. *Transactions of the Royal Society of Tropical Medicine and Hygiene,* 23**,** 401-412.

MCCULLOUGH, F. 1959. The susceptibility and resistance of *Bulinus* (Physopsis) *globosus* and *Bulinus* (Bulinus) *truncatus* rohlfsi to two strains of *Schistosoma haematobium* in Ghana. *Bull World Health Organ,* 20**,** 75-85.

MCCULLOUGH, F. S., GAYRAL, P., DUNCAN, J. & CHRISTIE, J. D. 1980. Molluscicides in schistosomiasis control. *Bull World Health Organ,* 58**,** 681-9.

MCMANUS, D. P., GRAY, D. J., LI, Y., FENG, Z., WILLIAMS, G. M., STEWART, D., REY-LADINO, J. & ROSS, A. G. 2010. Schistosomiasis in the People's Republic of China: the era of the Three Gorges Dam. *Clin Microbiol Rev,* 23**,** 442-66.

MIGUEL, E. & KREMER, M. 2004. Worms: Identifying Impacts on Education and Health in the Presence of Treatment Externalities. *Econometrica,* 72**,** 159-217.

MOHAMMED, K. A., HAJI, H. J., GABRIELLI, A. F., MUBILA, L., BISWAS, G., CHITSULO, L., BRADLEY, M. H., ENGELS, D., SAVIOLI, L. & MOLYNEUX, D. H. 2008. Triple co-administration of ivermectin, albendazole and praziquantel in zanzibar: a safety study. *PLoS Negl Trop Dis,* 2**,** e171.

MOHAMMED, K. A., MOLYNEUX, D. H., ALBONICO, M. & RIO, F. 2006. Progress towards eliminating lymphatic filariasis in Zanzibar: a model programme. *Trends Parasitol,* 22**,** 340-4.

MOLYNEUX, D. H. & MALECELA, M. N. 2011. Neglected tropical diseases and the millennium development goals: why the "other diseases" matter: reality versus rhetoric. *Parasit Vectors,* 4**,** 234.

MONTRESOR, A., CROMPTON, D. W., HALL, A., BUNDY, D. & SAVIOLI, L. 1998. Guidelines for the evaluation of soil-transmitted helminthiasis and schistosomiasis at community level. *Geneva: World Health Organization***,** 1-49.

MONTRESOR, A., CROMPTON, D. W. T., GYORKOS, T. W. & SAVIOLI, L. 2002. *Helminth control in school-age children: a guide for managers of control programmes*, World Health Organization.

MONTRESOR, A., GABRIELLI, A. F., DIARRA, A. & ENGELS, D. 2010. Estimation of the cost of large-scale school deworming programmes with benzimidazoles. *Trans R Soc Trop Med Hyg,* 104**,** 129-32.

MONTRESOR, A., ZIN, T. T., PADMASIRI, E., ALLEN, H. & SAVIOLI, L. 2004. Soil-transmitted helminthiasis in Myanmar and approximate costs for countrywide control. *Tropical Medicine & International Health,* 9**,** 1012-1015.

MORGAN, U. M., REYNOLDSON, J. A. & THOMPSON, R. C. 1993. Activities of several benzimidazoles and tubulin inhibitors against Giardia spp. in vitro. *Antimicrob Agents Chemother,* 37**,** 328-31.

MUCHIRI, E. M., OUMA, J. H. & KING, C. H. 1996. Dynamics and control of *Schistosoma haematobium* transmission in Kenya: an overview of the Msambweni Project. *Am J Trop Med Hyg,* 55**,** 127-34.

MUGONO, M., KONJE, E., KUHN, S., MPOGORO, F. J., MORONA, D. & MAZIGO, H. D. 2014. Intestinal schistosomiasis and geohelminths of Ukara Island, North-Western Tanzania: prevalence, intensity of infection and associated risk factors among school children. *Parasit Vectors,* 7**,** 612.

MUHUMUZA, S., OLSEN, A., KATAHOIRE, A. & NUWAHA, F. 2015. Reduced uptake of mass treatment for schistosomiasis control in absence of food: Beyond a randomized trial. *BMC infectious diseases,* 15**,** 1.

MUSGROVE, P. & HOTEZ, P. J. 2009. Turning neglected tropical diseases into forgotten maladies. *Health Aff (Millwood),* 28**,** 1691-706.

MUTAPI, F., RUJENI, N., BOURKE, C., MITCHELL, K., APPLEBY, L., NAUSCH, N., MIDZI, N. & MDULUZA, T. 2011. *Schistosoma haematobium* treatment in 1-5 year old children: safety and efficacy of the antihelminthic drug praziquantel. *PLoS Negl Trop Dis,* 5**,** e1143.

MWANDAWIRO, C. S., NIKOLAY, B., KIHARA, J. H., OZIER, O., MUKOKO, D. A., MWANJE, M. T., HAKOBYAN, A., PULLAN, R. L., BROOKER, S. J. & NJENGA, S. M. 2013. Monitoring and

evaluating the impact of national school-based deworming in Kenya: study design and baseline results. *Parasit Vectors,* 6**,** 198.

MWANGA, J. R. & LWAMBO, N. J. S. 2013. Pre- and post-intervention perceptions and water contact behaviour related to schistosomiasis in north-western Tanzania. *Acta Tropica,* 128**,** 391-398.

MWINZI, P. N., MONTGOMERY, S. P., OWAGA, C. O., MWANJE, M., MUOK, E. M., AYISI, J. G., LASERSON, K. F., MUCHIRI, E. M., SECOR, W. E. & KARANJA, D. M. 2012. Integrated community-directed intervention for schistosomiasis and soil transmitted helminths in western Kenya - a pilot study. *Parasit Vectors,* 5**,** 182.

NACHER, M., GAY, F., SINGHASIVANON, P., KRUDSOOD, S., TREEPRASERTSUK, S., MAZIER, D., VOULDOUKIS, I. & LOOAREESUWAN, S. 2000. *Ascaris lumbricoides* infection is associated with protection from cerebral malaria. *Parasite Immunol,* 22**,** 107-13.

NANDHA, B., KRISHNAMOORTHY, K. & JAMBULINGAM, P. 2013. Towards elimination of lymphatic filariasis: social mobilization issues and challenges in mass drug administration with anti-filarial drugs in Tamil Nadu, South India. *Health Educ Res,* 28**,** 591-8.

NAVARATNAM, A. M., SOUSA-FIGUEIREDO, J. C., STOTHARD, J. R., KABATEREINE, N. B., FENWICK, A. & MUTUMBA-NAKALEMBE, M. J. 2012. Efficacy of praziquantel syrup versus crushed praziquantel tablets in the treatment of intestinal schistosomiasis in Ugandan preschool children, with observation on compliance and safety. *Trans R Soc Trop Med Hyg,* 106**,** 400-7.

NOJIMA, H. & SATO, A. 1982. Schistosoma mansoni and *Schistosoma haematobium*: Emergence of schistosome cercariae from snails with darkness and illumination. *Experimental Parasitology,* 53**,** 189-198.

NORTON, A. J., GOWER, C. M., LAMBERTON, P. H., WEBSTER, B. L., LWAMBO, N. J., BLAIR, L., FENWICK, A. & WEBSTER, J. P. 2010. Genetic consequences of mass human chemotherapy for *Schistosoma mansoni*: population structure pre- and post-praziquantel treatment in Tanzania. *Am J Trop Med Hyg,* 83**,** 951-7.

NUJUM, Z. T., REMADEVI, S., NIRMALA, C., RAJMOHANAN, K., INDU, P. & NAIR, S. M. 2012. Factors determining noncompliance to mass drug administration for lymphatic filariasis elimination. *Trop Parasitol,* 2**,** 109-15.

ODIERE, M. R., OPISA, S., ODHIAMBO, G., JURA, W. G., AYISI, J. M., KARANJA, D. M. & MWINZI, P. N. 2011. Geographical distribution of schistosomiasis and soil-transmitted helminths among school children in informal settlements in Kisumu City, Western Kenya. *Parasitology,* 138**,** 1569-77.

OLDS, G. R. 2013. Deworming the world. *Trans Am Clin Climatol Assoc,* 124**,** 265-74.

OPISA, S., ODIERE, M. R., JURA, W. G., KARANJA, D. M. & MWINZI, P. N. 2011. Malacological survey and geographical distribution of vector snails for schistosomiasis within informal settlements of Kisumu City, western Kenya. *Parasit Vectors,* 4**,** 226.

OQUEKA, T., SUPALI, T., ISMID, I. S., PURNOMO, RUCKERT, P., BRADLEY, M. & FISCHER, P. 2005. Impact of two rounds of mass drug administration using diethylcarbamazine combined with albendazole on the prevalence of Brugia timori and of intestinal helminths on Alor Island, Indonesia. *Filaria J,* 4**,** 5.

PARDO, J., CARRANZA, C., TURRIENTES, M., ARELLANO, J. P., VÉLEZ, R. L., RAMAJO, V. & MURO, A. 2004. Utility of Schistosoma bovis adult worm antigens for diagnosis of human schistosomiasis by enzyme-linked immunosorbent assay and electroimmunotransfer blot techniques. *Clinical and diagnostic laboratory immunology,* 11**,** 1165-1170.

PARKER, M. & ALLEN, T. 2011. Does mass drug administration for the integrated treatment of neglected tropical diseases really work? Assessing evidence for the control of schistosomiasis and soil-transmitted helminths in Uganda. *Health Res Policy Syst,* 9**,** 3.

PARKER, M. & ALLEN, T. 2013. Will mass drug administration eliminate lymphatic filariasis? Evidence from northern coastal Tanzania. *J Biosoc Sci,* 45**,** 517-45.

PARKER, M., ALLEN, T. & HASTINGS, J. 2008. Resisting control of neglected tropical diseases: dilemmas in the mass treatment of schistosomiasis and soil-transmitted helminths in north-west Uganda. *J Biosoc Sci,* 40**,** 161-81.

PEARCE, E. J. & MACDONALD, A. S. 2002. The immunobiology of schistosomiasis. *Nat Rev Immunol,* 2**,** 499-511.

PEARSON, M. S., TRIBOLET, L., CANTACESSI, C., PERIAGO, M. V., VALERO, M. A., JARIWALA, A. R., HOTEZ, P., DIEMERT, D., LOUKAS, A. & BETHONY, J. 2012. Molecular mechanisms of hookworm disease: stealth, virulence, and vaccines. *J Allergy Clin Immunol,* 130**,** 13-21.

PERSON, B., KNOPP, S., ALI, S. M., A'KADIR F, M., KHAMIS, A. N., ALI, J. N., LYMO, J. H., MOHAMMED, K. A. & ROLLINSON, D. 2016. Community Co-Designed Schistosomiasis Control Interventions for School-Aged Children in Zanzibar. *J Biosoc Sci,* 48 Suppl 1**,** S56-73.

PICQUET, M., VERCRUYSSE, J., SHAW, D. J., DIOP, M. & LY, A. 1998. Efficacy of praziquantel against *Schistosoma mansoni* in northern Senegal. *Trans R Soc Trop Med Hyg,* 92**,** 90-3.

PINOT DE MOIRA, A., FULFORD, A. J., KABATEREINE, N. B., OUMA, J. H., BOOTH, M. & DUNNE, D. W. 2010. Analysis of complex patterns of human exposure and immunity to *Schistosomiasis mansoni*: the influence of age, sex, ethnicity and IgE. *PLoS Negl Trop Dis,* 4.

PRICHARD, R. K. 2007. Markers for benzimidazole resistance in human parasitic nematodes? *Parasitology,* 134**,** 1087-92.

PRICHARD, R. K., BASANEZ, M. G., BOATIN, B. A., MCCARTHY, J. S., GARCIA, H. H., YANG, G. J., SRIPA, B. & LUSTIGMAN, S. 2012. A research agenda for helminth diseases of humans: intervention for control and elimination. *PLoS Negl Trop Dis,* 6**,** e1549.

PULLAN, R. L., SMITH, J. L., JASRASARIA, R. & BROOKER, S. J. 2014. Global numbers of infection and disease burden of soil transmitted helminth infections in 2010. *Parasites & vectors,* 7**,** 1.

RICHTER, J. 2003. The impact of chemotherapy on morbidity due to schistosomiasis. *Acta Trop,* 86**,** 161-83.

ROBINSON, E., PICON, D., STURROCK, H. J., SABASIO, A., LADO, M., KOLACZINSKI, J. & BROOKER, S. 2009. The performance of haematuria reagent strips for the rapid mapping of urinary schistosomiasis: field experience from Southern Sudan. *Trop Med Int Health,* 14**,** 1484-7.

ROBINSON, M. W., MCFERRAN, N., TRUDGETT, A., HOEY, L. & FAIRWEATHER, I. 2004. A possible model of benzimidazole binding to beta-tubulin disclosed by invoking an inter-domain movement. *J Mol Graph Model,* 23**,** 275-84.

ROLLINSON, D., KAUKAS, A., JOHNSTON, D. A., SIMPSON, A. J. G. & TANAKA, M. 1997. Some molecular insights into Schistosome evolution. *International Journal for Parasitology,* 27**,** 11-28.

ROLLINSON, D., STOTHARD, J. R. & SOUTHGATE, V. R. 2001. Interactions between intermediate snail hosts of the genus Bulinus and schistosomes of the *Schistosoma haematobium* group. *Parasitology,* 123 Suppl**,** S245-60.

ROLLINSON, D., WEBSTER, J. P., WEBSTER, B., NYAKAANA, S., JORGENSEN, A. & STOTHARD, J. R. 2009. Genetic diversity of schistosomes and snails: implications for control. *Parasitology,* 136**,** 1801-11.

RUDGE, J. W., STOTHARD, J. R., BASANEZ, M. G., MGENI, A. F., KHAMIS, I. S., KHAMIS, A. N. & ROLLINSON, D. 2008. Micro-epidemiology of urinary schistosomiasis in Zanzibar: Local risk factors associated with distribution of infections among schoolchildren and relevance for control. *Acta Trop,* 105**,** 45-54.

SANGSTER, N. C. & GILL, J. 1999. Pharmacology of Anthelmintic Resistance. *Parasitology Today,* 15**,** 141-146.

SAVIOLI, L., ALBONICO, M., ENGELS, D. & MONTRESOR, A. 2004a. Progress in the prevention and control of schistosomiasis and soil-transmitted helminthiasis. *Parasitology International,* 53**,** 103-113.

SAVIOLI, L., DIXON, H., KISUMKU, U. M. & MOTT, K. E. 1989a. Control of morbidity due to *Schistosoma haematobium* on Pemba Island: programme organization and management. *Trop Med Parasitol,* 40**,** 189-94.

SAVIOLI, L., DIXON, H., KISUMKU, U. M. & MOTT, K. E. 1989b. Control of morbidity due to *Schistosoma haematobium* on Pemba island; selective population chemotherapy of schoolchildren with haematuria to identify high-risk localities. *Trans R Soc Trop Med Hyg,* 83**,** 805-10.

SAVIOLI, L., ENGELS, D., ROUNGOU, J. B., FENWICK, A. & ENDO, H. 2004b. Schistosomiasis control. *Lancet,* 363**,** 658.

SAVIOLI, L., HATZ, C., DIXON, H., KISUMKU, U. M. & MOTT, K. E. 1990. Control of morbidity due to *Schistosoma haematobium* on Pemba Island: egg excretion and hematuria as indicators of infection. *Am J Trop Med Hyg,* 43**,** 289-95.

SAVIOLI, L. & MOTT, K. E. 1989. Urinary schistosomiasis on Pemba Island: low-cost diagnosis for control in a primary health care setting. *Parasitol Today,* 5**,** 333-7.

SCHWENKENBECHER, J. M., ALBONICO, M., BICKLE, Q. & KAPLAN, R. M. 2007. Characterization of beta-tubulin genes in hookworms and investigation of resistance-associated mutations using real-time PCR. *Mol Biochem Parasitol,* 156**,** 167-74.

SCOTT, J. T., DIAKHATE, M., VEREECKEN, K., FALL, A., DIOP, M., LY, A., DE CLERCQ, D., DE VLAS, S. J., BERKVENS, D., KESTENS, L. & GRYSEELS, B. 2003. Human water contacts patterns in *Schistosoma mansoni* epidemic foci in northern Senegal change according to age, sex and place of residence, but are not related to intensity of infection. *Trop Med Int Health,* 8**,** 100-8.

SECOR, W. E. 2006. Interactions between schistosomiasis and infection with HIV-1. *Parasite Immunol,* 28**,** 597-603.

SHAPIRO, A. E., TUKAHEBWA, E. M., KASTEN, J., CLARKE, S. N. E., MAGNUSSEN, P., OLSEN, A., KABATEREINE, N. B., NDYOMUGYENYI, R. & BROOKER, S. 2005. Epidemiology of helminth infections and their relationship to clinical malaria in southwest Uganda. *Transactions of the Royal Society of Tropical Medicine and Hygiene,* 99**,** 18-24.

SHEBEL, H. M., ELSAYES, K. M., ABOU EL ATTA, H. M., ELGUINDY, Y. M. & EL-DIASTY, T. A. 2012. Genitourinary schistosomiasis: life cycle and radiologic-pathologic findings. *Radiographics,* 32**,** 1031-46.

SHIFF, C. J., COUTTS, W. C., YIANNAKIS, C. & HOLMES, R. W. 1979. Seasonal patterns in the transmission of *Schistosoma haematobium* in Rhodesia, and its control by winter application of molluscicide. *Trans R Soc Trop Med Hyg,* 73**,** 375-80.

SIMEON, D. T., GRANTHAM-MCGREGOR, S. M. & WONG, M. S. 1995. *Trichuris trichiura* infection and cognition in children: results of a randomized clinical trial. *Parasitology,* 110 (Pt 4)**,** 457-64.

SIZA, J. E., KAATANO, G. M., CHAI, J. Y., EOM, K. S., RIM, H. J., YONG, T. S., MIN, D. Y., CHANG, S. Y., KO, Y. & CHANGALUCHA, J. M. 2015. Prevalence of Schistosomes and Soil-Transmitted Helminths and Morbidity Associated with Schistosomiasis among Adult Population in Lake Victoria Basin, Tanzania. *Korean J Parasitol,* 53**,** 525-33.

SMITS, H. L. 2009. Prospects for the control of neglected tropical diseases by mass drug administration. *Expert Rev Anti Infect Ther,* 7**,** 37-56.

SOUSA-FIGUEIREDO, J. C., BASANEZ, M. G., MGENI, A. F., KHAMIS, I. S., ROLLINSON, D. & STOTHARD, J. R. 2008. A parasitological survey, in rural Zanzibar, of pre-school children and their mothers for urinary schistosomiasis, soil-transmitted helminthiases and

malaria, with observations on the prevalence of anaemia. *Ann Trop Med Parasitol,* 102**,** 679-92.

SOUSA-FIGUEIREDO, J. C., DAY, M., BETSON, M., KABATEREINE, N. B. & STOTHARD, J. R. 2010a. An inclusive dose pole for treatment of schistosomiasis in infants and preschool children with praziquantel. *Trans R Soc Trop Med Hyg,* 104**,** 740-2.

SOUSA-FIGUEIREDO, J. C., DAY, M., BETSON, M., KABATEREINE, N. B. & STOTHARD, J. R. 2010b. An inclusive dose pole for treatment of schistosomiasis in infants and preschool children with praziquantel☆. *Transactions of the Royal Society of Tropical Medicine and Hygiene,* 104**,** 740-742.

SOUSA-FIGUEIREDO, J. C., PLEASANT, J., DAY, M., BETSON, M., ROLLINSON, D., MONTRESOR, A., KAZIBWE, F., KABATEREINE, N. B. & STOTHARD, J. R. 2010c. Treatment of intestinal schistosomiasis in Ugandan preschool children: best diagnosis, treatment efficacy and side-effects, and an extended praziquantel dosing pole. *International Health,* 2**,** 103-113.

SOW, S., DE VLAS, S. J., STELMA, F., VEREECKEN, K., GRYSEELS, B. & POLMAN, K. 2011. The contribution of water contact behavior to the high *Schistosoma mansoni* Infection rates observed in the Senegal River Basin. *BMC Infect Dis,* 11**,** 198.

SPEICH, B., AME, S. M., ALI, S. M., ALLES, R., HUWYLER, J., HATTENDORF, J., UTZINGER, J., ALBONICO, M. & KEISER, J. 2014. Oxantel pamoate-albendazole for Trichuris trichiura infection. *N Engl J Med,* 370**,** 610-20.

STEINMANN, P., KEISER, J., BOS, R., TANNER, M. & UTZINGER, J. 2006. Schistosomiasis and water resources development: systematic review, meta-analysis, and estimates of people at risk. *Lancet Infect Dis,* 6**,** 411-25.

STEPHENSON, L. S. & HOLLAND, C. 1987. *The impact of helminth infections on human nutrition : schistosomes and soil-transmitted helminths,* London, Taylor & Francis.

STOLTZFUS, R. J., ALBONICO, M., TIELSCH, J. M., CHWAYA, H. M. & SAVIOLI, L. 1997. School-based deworming program yields small improvement in growth of Zanzibari school children after one year. *J Nutr,* 127**,** 2187-93.

STOLTZFUS, R. J., CHWAY, H. M., MONTRESOR, A., TIELSCH, J. M., JAPE, J. K., ALBONICO, M. & SAVIOLI, L. 2004. Low dose daily iron supplementation improves iron status and appetite but not anemia, whereas quarterly anthelminthic treatment improves growth, appetite and anemia in Zanzibari preschool children. *J Nutr,* 134**,** 348-56.

STOLTZFUS, R. J., CHWAYA, H. M., MONTRESOR, A., ALBONICO, M., SAVIOLI, L. & TIELSCH, J. M. 2000. Malaria, hookworms and recent fever are related to anemia and iron status indicators in 0- to 5-y old Zanzibari children and these relationships change with age. *J Nutr,* 130**,** 1724-33.

STOTHARD, I. R. & ROLLINSON, D. 1997a. Molecular characterization of *Bulinus globosus* and *B. nasutus* on Zanzibar, and an investigation of their roles in the epidemiology of *Schistosoma haematobium*. *Trans R Soc Trop Med Hyg,* 91**,** 353-7.

STOTHARD, J. R., AMERI, H., KHAMIS, I. S., BLAIR, L., NYANDINDI, U. S., KANE, R. A., JOHNSTON, D. A., WEBSTER, B. L. & ROLLINSON, D. 2013a. Parasitological and malacological surveys reveal urogenital schistosomiasis on Mafia Island, Tanzania to be an imported infection. *Acta Tropica,* 128**,** 326-333.

STOTHARD, J. R., LOXTON, N., ROLLINSON, D., MGENI, A. F., KHAMIS, S., AMERI, H., RAMSAN, M. & SAVIOLI, L. 2000. The transmission status of *Bulinus* on Zanzibar Island (Unguja), with implications for control of urinary schistosomiasis. *Ann Trop Med Parasitol,* 94**,** 87-94.

STOTHARD, J. R., MGENI, A. F., KHAMIS, S., SETO, E., RAMSAN, M., HUBBARD, S. J., KRISTENSEN, T. K. & ROLLINSON, D. 2002. New insights into the transmission biology of urinary schistosomiasis in Zanzibar. *Trans R Soc Trop Med Hyg,* 96**,** 470-5.

STOTHARD, J. R. & ROLLINSON, D. 1997b. Partial DNA sequences from the mitochondrial cytochrome oxidase subunit I (COI) gene can differentiate the intermediate snail hosts *Bulinus globosus* and *B. nasutus* (Gastropoda: Planorbidae). *Journal of Natural History,* 31**,** 727-737.

STOTHARD, J. R., ROLLINSON, D., IMISON, E. & KHAMIS, I. S. 2009. A spot-check of the efficacies of albendazole or levamisole, against soil-transmitted helminthiases in young Ungujan children, reveals low frequencies of cure. *Ann Trop Med Parasitol,* 103**,** 357-60.

STOTHARD, J. R., SOUSA-FIGUEIREDO, J. C., BETSON, M., GREEN, H. K., SETO, E. Y., GARBA, A., SACKO, M., MUTAPI, F., VAZ NERY, S., AMIN, M. A., MUTUMBA-NAKALEMBE, M., NAVARATNAM, A., FENWICK, A., KABATEREINE, N. B., GABRIELLI, A. F. & MONTRESOR, A. 2011. Closing the praziquantel treatment gap: new steps in epidemiological monitoring and control of schistosomiasis in African infants and preschool-aged children. *Parasitology,* 138**,** 1593-606.

STOTHARD, J. R., SOUSA-FIGUEIREDO, J. C. & NAVARATNAM, A. M. 2013b. Advocacy, policies and practicalities of preventive chemotherapy campaigns for African children with schistosomiasis. *Expert Rev Anti Infect Ther,* 11**,** 733-52.

STURROCK, R. F. 1995. Current concepts of snail control. *Mem Inst Oswaldo Cruz,* 90**,** 241-8.

STURROCK, R. F. 2001. Schistosomiasis epidemiology and control: how did we get here and where should we go? *Mem Inst Oswaldo Cruz,* 96 Suppl**,** 17-27.

SUNISH, I. P., SHRIRAM, A. N., SIVAN, A., KARTICK, C., SAHA, B. P. & VIJAYACHARI, P. 2013. Lymphatic filariasis elimination programme in Andaman and Nicobar Islands, India: drug coverage and compliance post eight rounds of MDA. *Trop Doct,* 43**,** 30-2.

SUPALI, T., DJUARDI, Y., BRADLEY, M., NOORDIN, R., RUCKERT, P. & FISCHER, P. U. 2013. Impact of six rounds of mass drug administration on Brugian filariasis and soil-transmitted helminth infections in eastern Indonesia. *PLoS Negl Trop Dis,* 7**,** e2586.

SWAI, B., POGGENSEE, G., MTWEVE, S. & KRANTZ, I. 2006. Female genital schistosomiasis as an evidence of a neglected cause for reproductive ill-health: a retrospective histopathological study from Tanzania. *BMC Infect Dis,* 6**,** 134.

TALLO, V. L., CARABIN, H., ALDAY, P. P., BALOLONG, E., JR., OLVEDA, R. M. & MCGARVEY, S. T. 2008. Is mass treatment the appropriate schistosomiasis elimination strategy? *Bull World Health Organ,* 86**,** 765-71.

TAYLOR-ROBINSON, D. C., MAAYAN, N., SOARES-WEISER, K., DONEGAN, S. & GARNER, P. 2012. Deworming drugs for soil-transmitted intestinal worms in children: effects on nutritional indicators, haemoglobin and school performance. *Cochrane Database Syst Rev,* 11**,** CD000371.

TCHUEM TCHUENTE, L. A., BEHNKE, J. M., GILBERT, F. S., SOUTHGATE, V. R. & VERCRUYSSE, J. 2003. Polyparasitism with *Schistosoma haematobium* and soil-transmitted helminth infections among school children in Loum, Cameroon. *Trop Med Int Health,* 8**,** 975-86.

TEMPLETON, A. R., CRANDALL, K. A. & SING, C. F. 1992. A cladistic analysis of phenotypic associations with haplotypes inferred from restriction endonuclease mapping and DNA sequence data. III. Cladogram estimation. *Genetics,* 132**,** 619-33.

TOHON, Z. B., MAINASSARA, H. B., GARBA, A., MAHAMANE, A. E., BOSQUE-OLIVA, E., IBRAHIM, M. L., DUCHEMIN, J. B., CHANTEAU, S. & BOISIER, P. 2008. Controlling schistosomiasis: significant decrease of anaemia prevalence one year after a single dose of praziquantel in Nigerian schoolchildren. *PLoS Negl Trop Dis,* 2**,** e241.

TOLENTINO, K. & FRIEDMAN, J. F. 2007. An update on anemia in less developed countries. *Am J Trop Med Hyg,* 77**,** 44-51.

TOURE, S., ZHANG, Y., BOSQUE-OLIVA, E., KY, C., OUEDRAOGO, A., KOUKOUNARI, A., GABRIELLI, A. F., BERTRAND, S., WEBSTER, J. P. & FENWICK, A. 2008. Two-year impact

of single praziquantel treatment on infection in the national control programme on schistosomiasis in Burkina Faso. *Bull World Health Organ,* 86**,** 780-7, A.

TRUSCOTT, J., HOLLINGSWORTH, T. D. & ANDERSON, R. 2014a. Modeling the interruption of the transmission of soil-transmitted helminths by repeated mass chemotherapy of school-age children. *PLoS Negl Trop Dis,* 8**,** e3323.

TRUSCOTT, J. E., HOLLINGSWORTH, T. D., BROOKER, S. J. & ANDERSON, R. M. 2014b. Can chemotherapy alone eliminate the transmission of soil transmitted helminths? *Parasit Vectors,* 7**,** 266.

TURNER, H. C., TRUSCOTT, J. E., FLEMING, F. M., HOLLINGSWORTH, T. D., BROOKER, S. J. & ANDERSON, R. M. 2016. Cost-effectiveness of scaling up mass drug administration for the control of soil-transmitted helminths: a comparison of cost function and constant costs analyses. *Lancet Infect Dis*.

USEH, M. & EJEZIE, G. 1999. Modification of behaviour and attitude in the control of schistosomiasis. 1. Observations on water-contact patterns and perception of infection. *Annals of Tropical Medicine & Parasitology,* 93**,** 711-720.

UTZINGER, J., BERGQUIST, R., SHU-HUA, X., SINGER, B. H. & TANNER, M. 2003. Sustainable schistosomiasis control—the way forward. *The Lancet,* 362**,** 1932-1934.

UTZINGER, J., N'GORAN E, K., CAFFREY, C. R. & KEISER, J. 2011. From innovation to application: social-ecological context, diagnostics, drugs and integrated control of schistosomiasis. *Acta Trop,* 120 Suppl 1**,** S121-37.

UTZINGER, J., XIAO, S., KEISER, J., CHEN, M., ZHENG, J. & TANNER, M. 2001. Current progress in the development and use of artemether for chemoprophylaxis of major human schistosome parasites. *Curr Med Chem,* 8**,** 1841-60.

VALENTIM, C. L., CIOLI, D., CHEVALIER, F. D., CAO, X., TAYLOR, A. B., HOLLOWAY, S. P., PICA-MATTOCCIA, L., GUIDI, A., BASSO, A., TSAI, I. J., BERRIMAN, M., CARVALHO-QUEIROZ, C., ALMEIDA, M., AGUILAR, H., FRANTZ, D. E., HART, P. J., LOVERDE, P. T. & ANDERSON, T. J. 2013. Genetic and molecular basis of drug resistance and species-specific drug action in schistosome parasites. *Science,* 342**,** 1385-9.

VAN RIET, E., HARTGERS, F. C. & YAZDANBAKHSH, M. 2007. Chronic helminth infections induce immunomodulation: Consequences and mechanisms. *Immunobiology,* 212**,** 475-490.

VIDIGAL, T. H., DIAS NETO, E., SPATZ, L., NUNES, D. N., PIRES, E. R., SIMPSON, A. J. & CARVALHO, O. S. 1998. Genetic variability and identification of the intermediate snail hosts of *Schistosoma mansoni*. *Mem Inst Oswaldo Cruz,* 93 Suppl 1**,** 103-10.

WANG, W., WANG, L. & LIANG, Y.-S. 2012a. Susceptibility or resistance of praziquantel in human schistosomiasis: a review. *Parasitology Research,* 111**,** 1871-1877.

WANG, X., GURARIE, D., MUNGAI, P. L., MUCHIRI, E. M., KITRON, U. & KING, C. H. 2012b. Projecting the long-term impact of school- or community-based mass-treatment interventions for control of Schistosoma infection. *PLoS Negl Trop Dis,* 6**,** e1903.

WARREN, K. S. 1981. The control of helminths: nonreplicating infectious agents of man. *Annu Rev Public Health,* 2**,** 101-5.

WARREN, K. S., MAHMOUD, A. A., MURUKA, J. F., WHITTAKER, L. R., OUMA, J. H. & ARAP SIONGOK, T. K. 1979. *Schistosomiasis haematobia* in coast province Kenya. Relationship between egg output and morbidity. *Am J Trop Med Hyg,* 28**,** 864-70.

WEBBE, G. 1962. The transmission of *Schistosoma haematobium* in an area of Lake Province, Tanganyika. *Bull World Health Organ,* 27**,** 59-85.

WEBBE, G. & JORDAN, P. 1966. Recent advances in knowledge of schistosomiasis in East Africa. *Transactions of the Royal Society of Tropical Medicine and Hygiene,* 60**,** 279-306.

WEBSTER, B. L., EMERY, A. M., WEBSTER, J. P., GOUVRAS, A., GARBA, A., DIAW, O., SEYE, M. M., TCHUENTE, L. A., SIMOONGA, C., MWANGA, J., LANGE, C., KARIUKI, C., MOHAMMED, K. A., STOTHARD, J. R. & ROLLINSON, D. 2012a. Genetic diversity within

Schistosoma haematobium: DNA barcoding reveals two distinct groups. *PLoS Negl Trop Dis,* 6**,** e1882.

WEBSTER, B. L., EMERY, A. M., WEBSTER, J. P., GOUVRAS, A., GARBA, A., DIAW, O., SEYE, M. M., TCHUENTE, L. A. T., SIMOONGA, C. & MWANGA, J. 2012b. Genetic diversity within *Schistosoma haematobium*: DNA barcoding reveals two distinct groups. *PLoS neglected tropical diseases,* 6**,** e1882.

WEBSTER, J. P., KOUKOUNARI, A., LAMBERTON, P. H., STOTHARD, J. R. & FENWICK, A. 2009. Evaluation and application of potential schistosome-associated morbidity markers within large-scale mass chemotherapy programmes. *Parasitology,* 136**,** 1789-99.

WEBSTER, J. P., OLIVIERA, G., ROLLINSON, D. & GOWER, C. M. 2010. Schistosome genomes: a wealth of information. *Trends in parasitology,* 26**,** 103-106.

WHO 2002. Geneva: World Health Organization.

WHO EXPERT COMMITTEE ON THE CONTROL OF SCHISTOSOMIASIS, W. 2002. *Prevention and control of schistosomiasis and soil-transmitted helminthiasis: report of a WHO expert committee*, Who.

WHO, W. H. O. 1983. *The role of chemotherapy in schistosomiasis control*, WHO Parasitic Diseases Programme.

WHO, W. H. O. 1994. Bench aids for the diagnosis of intestinal parasites.

WHO, W. H. O. 2001. Iron deficiency anaemia: assessment, prevention and control: a guide for programme managers.

WHO, W. H. O. 2006. *Preventive chemotherapy in human helminthiasis: coordinated use of anthelminthic drugs in control interventions: a manual for health professionals and programme managers*, World Health Organization.

WHO, W. H. O. 2010. Monitoring drug coverage for preventive chemotherapy.

WHO, W. H. O. 2011a. Assuring safety of preventive chemotherapy interventions for the control of neglected tropical diseases. *Geneva, Switzerland*.

WHO, W. H. O. 2011b. *Helminth control in school-age children : a guide for managers of control programmes,* Geneva, World Health Organization.

WHO, W. H. O. 2015. Haemoglobin concentrations for the diagnosis of anaemia and assessment of severity. 2011. *Download from: http://www. who. int/vmnis/indicators/haemoglobin. pdf*.

WOELFLE, M., SEERDEN, J. P., DE GOOIJER, J., POUWER, K., OLLIARO, P. & TODD, M. H. 2011. Resolution of praziquantel. *PLoS Negl Trop Dis,* 5**,** e1260.

WORRELL, C. & MATHIEU, E. 2012. Drug coverage surveys for neglected tropical diseases: 10 years of field experience. *Am J Trop Med Hyg,* 87**,** 216-22.

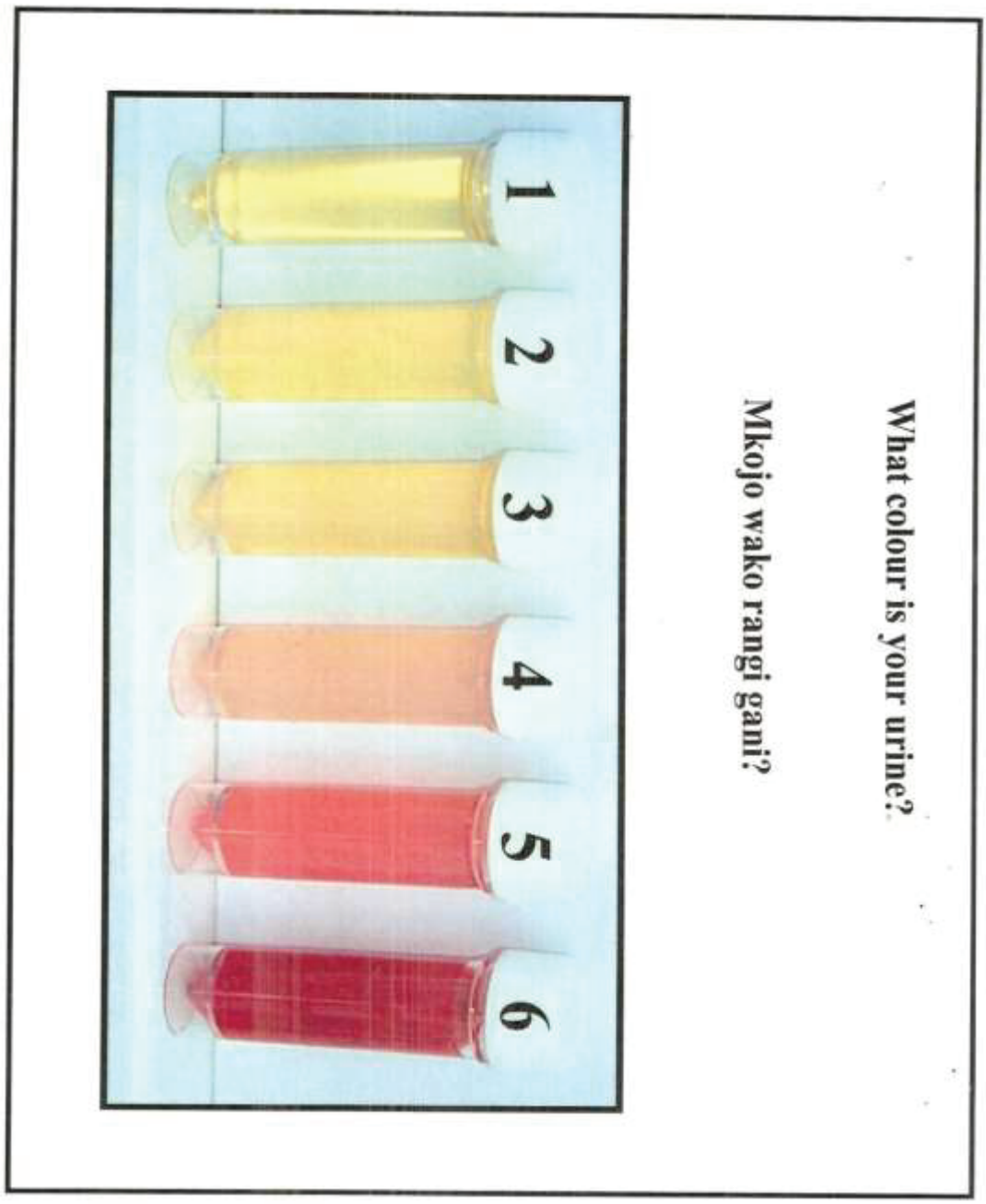
XIANYI, C., LIYING, W., JIMING, C., XIAONONG, Z., JIANG, Z., JIAGANG, G., XIAOHUA, W., ENGELS, D. & MINGGANG, C. 2005a. Schistosomiasis control in China: the impact of a 10-year World Bank Loan Project (1992-2001). *Bull World Health Organ,* 83**,** 43-8.

XIANYI, C., LIYING, W., JIMING, C., XIAONONG, Z., JIANG, Z., JIAGANG, G., XIAOHUA, W., ENGELS, D. & MINGGANG, C. 2005b. Schistosomiasis control in China: the impact of a 10-year World Bank Loan Project (1992-2001). *Bulletin of the World Health Organization,* 83**,** 43-48.

XIAO, S., ZHAN, B., XUE, J., GOUD, G. N., LOUKAS, A., LIU, Y., WILLIAMSON, A., LIU, S., DEUMIC, V. & HOTEZ, P. 2008. The evaluation of recombinant hookworm antigens as vaccines in hamsters (Mesocricetus auratus) challenged with human hookworm, *Necator americanus*. *Experimental Parasitology,* 118**,** 32-40.

Appendices

Appendix 2.1: Urine colour chart developed by WHO



Appendix 3.1 List of the schools in schistosomiasis assessment under the auspice of the SCORE initiative in 2012

S/no	School Name	Status		Baseline (2012) Prevalence	Intervention
		Old (Among cohort 24)	New		
1	Chambani	Yes		28.5	Behaviour
2	Chanjamjawiri	Yes		3.8	MDA
3	Daya		Yes	0	Behaviour
4	Finya		Yes	9.2	Snail control
5	Jadida		Yes	4.4	Behaviour
6	Kangagani	Yes		16.9	Snail control
7	Kangani		Yes	2.6	MDA
8	Kengeja		Yes	3.9	Behaviour
9	Kinowe	Yes		11.7	Snail control
10	Kinyasini		Yes	32.5	Behaviour
11	Kisiwani		Yes	15.3	Snail control
12	Kiwani		Yes	3	MDA
13	Kizimbani	Yes		28.8	Behaviour
14	Konde A	Yes		6.7	MDA
15	Kwale		Yes	16.3	Snail control
16	Madungu		Yes	0	MDA
17	Makangale		Yes	3.6	Snail control
18	Makombeni		Yes	0	Snail control
19	Mbuzini		Yes	4	Snail control
20	Mchangamdogo		Yes	2	Behaviour
21	Mgogoni		Yes	16.9	Snail control
22	Michenzani		Yes	3.8	Behaviour
23	Miti ulaya		Yes	1.5	MDA
24	Mkanyageni	Yes		8.9	Snail control
25	Msuka		Yes	1.5	Snail control
26	Mtambile	Yes		8.3	Behaviour
27	Ng'ambwa		Yes	0.7	Behaviour
28	Ng'ombeni	Yes		0	Behaviour
29	Ngwachani	Yes		11.7	Behaviour
30	Ole		Yes	5.8	MDA
31	Pandani		Yes	6	MDA
32	Piki	Yes		23.8	Snail control
33	Pondeani		Yes	2.2	MDA
34	Pujini	Yes		20.8	Behaviour
35	Shumba Viamboni	Yes		26.4	Snail control
36	Shungi		Yes	5.5	Behaviour
37	Sizini		Yes	2.7	Behaviour
38	Tumbe	Yes		3.2	MDA
39	Ukutini		Yes	17.4	MDA
40	Uwandani	Yes		28.7	MDA
41	Vitongoji		Yes	20	Snail control
42	Wambaa		Yes	17.5	MDA
43	Wawi		Yes	2.1	MDA
44	Wesha	Yes		3.3	MDA
45	Ziwani	Yes		13.5	Snail control

Appendix 3.2 Analysis of the score test for trend of schistosomiasis in Std-1 children in cohort 24 schools from 2010-13

```
. mhodds schisresult year, by(School)

Score test for trend of odds with year
by School

(The Odds Ratio estimate is an approximation to the odds ratio
for a one unit increase in year)
```

School	Odds Ratio	chi2(1)	P>chi2	[95% Conf.	Interval]
Bagamoyo	0.565204	1.95	0.1627	0.25371	1.25915
Chambani	0.746502	5.97	0.0145	0.59045	0.94380
Chanjamj	0.788257	1.16	0.2805	0.51168	1.21433
K/minung	0.855593	1.11	0.2925	0.63995	1.14389
Kangagan	1.460451	5.90	0.0152	1.07584	1.98256
Kinowe	1.399912	2.16	0.1416	0.89392	2.19232
Kizimban	0.651761	3.54	0.0601	0.41715	1.01833
Konde A	1.142604	0.21	0.6505	0.64188	2.03392
Konde B	1.004639	0.00	0.9795	0.70599	1.42963
Michakai	0.631539	0.38	0.5383	0.14614	2.72922
Mizingan	0.654175	7.66	0.0056	0.48440	0.88345
Mkanyage	0.637883	4.24	0.0396	0.41571	0.97880
Mtambile	0.591267	7.65	0.0057	0.40746	0.85798
Mzambara	0.763881	0.76	0.3829	0.41714	1.39885
Ng'omben	0.827007	0.57	0.4503	0.50504	1.35423
Ngwachan	0.594007	11.14	0.0008	0.43748	0.80655
Piki	0.733603	5.21	0.0224	0.56231	0.95708
Pujini	0.945776	0.13	0.7225	0.69524	1.28659
S/viambo	1.041557	0.06	0.8076	0.75055	1.44539
Tumbe	1.475318	0.76	0.3830	0.61587	3.53412
Uwandani	0.907808	0.64	0.4220	0.71691	1.14953
Wesha	0.856926	0.08	0.7769	0.29452	2.49330
Wingwi	1.081337	0.12	0.7320	0.69121	1.69166
Ziwani	0.592494	10.52	0.0012	0.43182	0.81296

Mantel-Haenszel estimate controlling for School

Odds Ratio	chi2(1)	P>chi2	[95% Conf.	Interval]
0.825898	25.24	0.0000	0.766509	0.889889

```
Test of homogeneity of ORs (approx): chi2(23)  =   46.67
                                     Pr>chi2   =  0.0025

.
```

Appendix 3.3A Score test for trend of Odds of infection in schools subjected to repeated treatment from 2010-2014

```
. xi:mhodds schisresult year, by(school)

Score test for trend of odds with year
by school

(The Odds Ratio estimate is an approximation to the odds ratio
for a one unit increase in year)
```

school	Odds Ratio	chi2(1)	P>chi2	[95% Conf.	Interval]
Chanjamj	0.715026	1.92	0.1658	0.44491	1.14915
Konde A	1.143323	0.28	0.5935	0.69914	1.86971
Tumbe	1.405533	0.76	0.3842	0.65292	3.02569
Uwandani	0.893601	0.88	0.3489	0.70619	1.13075
Wesha	0.979491	0.00	0.9590	0.44416	2.16005

Mantel-Haenszel estimate controlling for school

Odds Ratio	chi2(1)	P>chi2	[95% Conf.	Interval]
0.922286	0.75	0.3857	0.768202	1.107275

```
Test of homogeneity of ORs (approx): chi2(4)   =    3.09
                                     Pr>chi2   =  0.5429
```

Appendix 3.3B Score test for trend of Odds of infection in schools subjected to repeated treatment from 2010-2014

```
. xi: mhodds schisto_infection year, by(school)

Score test for trend of odds with year
by school

(The Odds Ratio estimate is an approximation to the odds ratio
for a one unit increase in year)
```

school	Odds Ratio	chi2(1)	P>chi2	[95% Conf.	Interval]
Chanjamj	0.382085	12.39	0.0004	0.22363	0.65281
Konde A	0.813155	0.64	0.4232	0.49017	1.34897
Tumbe	0.568437	1.39	0.2386	0.22218	1.45432
Uwandani	1.010851	0.01	0.9400	0.76304	1.33914
Wesha	0.542170	1.58	0.2082	0.20898	1.40661

Mantel-Haenszel estimate controlling for school

Odds Ratio	chi2(1)	P>chi2	[95% Conf.	Interval]
0.786834	4.92	0.0266	0.636567	0.972572

```
Test of homogeneity of ORs (approx): chi2(4)   =   11.10
                                     Pr>chi2   =  0.0255
```

Appendix 3.5 Score test for trend of schistosomiasis for combined Std-3&4 schoolchildren from 2012- 2014 in different schools (cohort 45) in Pemba.

```
. xi: mhodds schistoso_infect year, by(school)

Score test for trend of odds with year
by school

note: only 44 of the 45 strata formed in this analysis contribute
      information about the effect of the explanatory variable

(The Odds Ratio estimate is an approximation to the odds ratio
for a one unit increase in year)
```

school	Odds Ratio	chi2(1)	P>chi2	[95% Conf.	Interval]
CHAMBANI	0.685104	4.54	0.0332	0.48374	0.97030
CHANJAMJ	0.659723	0.90	0.3424	0.27955	1.55692
DAYA	2.942315	2.08	0.1491	0.67923	12.74569
FINYA	0.354139	7.22	0.0072	0.16605	0.75530
JADIDA	1.314118	0.60	0.4390	0.65789	2.62493
KANGAGAN	1.051407	0.10	0.7492	0.77317	1.42977
KANGANI	1.136274	0.07	0.7880	0.44787	2.88279
KENGEJA	0.587966	1.16	0.2819	0.22347	1.54698
KINOWE	1.303461	1.03	0.3101	0.78136	2.17444
KINYASIN	0.707082	4.68	0.0305	0.51653	0.96793
KISIWANI	0.681033	1.66	0.1973	0.37979	1.22123
KIWANI	0.900150	0.02	0.9018	0.16922	4.78839
KIZIMBAN	0.743962	3.43	0.0640	0.54404	1.01735
KONDE A	0.864975	0.24	0.6213	0.48650	1.53789
KWALE	0.200505	32.20	0.0000	0.11510	0.34927
M/MDOGO	0.204236	1.66	0.1970	0.01829	2.28109
MADUNGU	.	.	.	.	.
MAKANGAL	0.335544	3.54	0.0598	0.10764	1.04594
MAKOMBEN	3.832475	1.23	0.2668	0.35784	41.04559
MBUZINI	2.169663	4.04	0.0444	1.01951	4.61733
MGOGONI	1.151351	0.58	0.4473	0.80048	1.65602
MICHENZA	0.520814	1.42	0.2334	0.17810	1.52299
MITI ULA	1.173453	0.17	0.6827	0.54498	2.52668
MKANYAGE	0.582661	3.63	0.0567	0.33429	1.01555
MSUKA	0.224793	3.06	0.0803	0.04219	1.19760
MTAMBILE	0.332204	10.05	0.0015	0.16809	0.65656
NG'AMBWA	0.200138	1.67	0.1961	0.01746	2.29399
NG'OMBEN	2.235676	0.89	0.3448	0.42128	11.86445
NGWACHAN	0.542856	3.68	0.0550	0.29085	1.01323
OLE	0.933191	0.03	0.8518	0.45170	1.92794
PANDANI	0.466197	2.58	0.1084	0.18363	1.18358
PIKI	0.624196	6.37	0.0116	0.43292	0.89998
PONDEANI	0.343355	2.29	0.1301	0.08602	1.37053
PUJINI	0.691262	2.72	0.0991	0.44575	1.07201
SHU/VYAM	0.773928	2.60	0.1069	0.56674	1.05686
SHUNGI	0.633010	1.58	0.2086	0.31038	1.29100
SIZINI	0.427064	2.32	0.1279	0.14281	1.27708
TUMBE	0.505778	1.44	0.2297	0.16631	1.53818
UKUTINI	0.881560	0.33	0.5651	0.57374	1.35452
UWANDANI	0.906233	0.36	0.5460	0.65832	1.24751
VITONGOJ	1.387868	3.67	0.0553	0.99266	1.94042
WAMBAA	1.403028	2.01	0.1563	0.87853	2.24067
WAWI	1.665499	1.86	0.1724	0.80050	3.46519
WESHA	0.324121	4.99	0.0255	0.12059	0.87116
ZIWANI	0.635310	5.34	0.0208	0.43243	0.93337

Mantel-Haenszel estimate controlling for school

Odds Ratio	chi2(1)	P>chi2	[95% Conf.	Interval]
0.798806	29.23	0.0000	0.736329	0.866584

```
Test of homogeneity of ORs (approx): chi2(43)  =  106.85
                                     Pr>chi2   =  0.0000
```

Appendix 3.4A Mantel-Haenzsel Score test for trends for schistosomiasis infection in different interventions in individual Stds (3 and 4) in cohort 45 schools over the years (2012-2014)

```
. xi: mhodds schistoso_infect year, by(intervention standard )

Score test for trend of odds with year
by intervention standard

(The Odds Ratio estimate is an approximation to the odds ratio
for a one unit increase in year)
```

interv~n	standard	Odds Ratio	chi2(1)	P>chi2	[95% Conf.	Interval]
Behaviou	3	0.680544	11.68	0.0006	0.54575	0.84863
Behaviou	4	0.713778	13.66	0.0002	0.59691	0.85353
MDA	3	1.036365	0.09	0.7588	0.82505	1.30181
MDA	4	0.814017	2.94	0.0864	0.64338	1.02991
Snail_co	3	0.794336	7.24	0.0071	0.67170	0.93936
Snail_co	4	0.818260	5.87	0.0154	0.69570	0.96241

Mantel-Haenszel estimate controlling for intervention and standard

Odds Ratio	chi2(1)	P>chi2	[95% Conf.	Interval]
0.795149	32.83	0.0000	0.735182	0.860008

```
Test of homogeneity of ORs (approx): chi2(5)   =    8.65
                                     Pr>chi2   =  0.1237
```

Appendix 3.4B MH Score test for trends for schistosomiasis infection in different interventions in cohort 45 schools over the years (2012-2014)

```
. xi: mhodds schistoso_infect year, by(intervention)

Score test for trend of odds with year
by intervention

(The Odds Ratio estimate is an approximation to the odds ratio
for a one unit increase in year)
```

interv~n	Odds Ratio	chi2(1)	P>chi2	[95% Conf.	Interval]
Behaviou	0.709000	23.91	0.0000	0.61770	0.81379
MDA	0.921807	0.95	0.3300	0.78252	1.08589
Snail_co	0.808073	12.90	0.0003	0.71936	0.90772

Mantel-Haenszel estimate controlling for intervention

Odds Ratio	chi2(1)	P>chi2	[95% Conf.	Interval]
0.798382	31.91	0.0000	0.738382	0.863257

```
Test of homogeneity of ORs (approx): chi2(2)   =    5.85
                                     Pr>chi2   =  0.0537
```

Appendix 3.5A: Manten-Haenzsel analysis indicating the Odds of *S. haematobium* infection between 2010 and 2011 for Std-1 schoolchildren in different schools (cohort 24) in Pemba.

```
. xi:mhodds schisresult year, by( School)

Maximum likelihood estimate of the odds ratio
Comparing year==2011 vs. year==2010
by School
```

School	Odds Ratio	chi2(1)	P>chi2	[95% Conf.	Interval]
Bagamoyo	1.980000	0.35	0.5569	0.19390	20.21848
Chambani	2.333333	4.27	0.0387	1.01976	5.33894
Chanjamj	4.571429	3.96	0.0466	0.88217	23.68916
Kangagan	1.534091	0.40	0.5286	0.40124	5.86546
Kinowe	.	4.13	0.0423	.	.
Kiuyu Mi	2.479167	3.74	0.0532	0.95639	6.42653
Kizimban	5.684211	5.47	0.0193	1.09597	29.48092
Konde A	2.177778	0.41	0.5235	0.18752	25.29182
Konde B	9.800000	6.68	0.0098	1.16181	82.66381
Michakai	.	1.56	0.2113	.	.
Mizingan	2.100000	2.46	0.1164	0.81410	5.41701
Mkanyage	1.559322	0.48	0.4889	0.43849	5.54511
Mtambile	4.040541	5.68	0.0171	1.16506	14.01298
Mzambara	1.600000	0.21	0.6465	0.21069	12.15046
Ng'omben	12.782609	7.84	0.0051	1.27253	128.40182
Ngwachan	0.833333	0.14	0.7087	0.31982	2.17133
Piki	1.319444	0.37	0.5409	0.54101	3.21794
Pujini	0.816667	0.13	0.7188	0.27070	2.46377
Shumba V	3.916667	4.29	0.0384	0.97093	15.79949
Tumbe	.	1.02	0.3124	.	.
Uwandani	2.373626	4.20	0.0404	1.01202	5.56720
Wesha	0.000000	0.98	0.3222	.	.
Wingwi	.	7.61	0.0058	.	.
Ziwani	2.303571	2.91	0.0879	0.85901	6.17742

Mantel-Haenszel estimate controlling for School

Odds Ratio	chi2(1)	P>chi2	[95% Conf.	Interval]
2.346808	44.22	0.0000	1.811117	3.040945

```
Test of homogeneity of ORs (approx): chi2(23)  =   27.61
                                     Pr>chi2   =  0.2309
```

Appendix 3.5B Score test for trend of schistosomiasis in Std-1 schoolchildren of from 2010- 2012 for Std-1 schoolchildren in different schools (cohort 24/17) in Pemba.

```
. xi:mhodds schisresult year, by( school)

Score test for trend of odds with year
by school

(The Odds Ratio estimate is an approximation to the odds ratio
for a one unit increase in year)
```

school	Odds Ratio	chi2(1)	P>chi2	[95% Conf.	Interval]
Chambani	0.838531	1.29	0.2565	0.61859	1.13667
Chanjamj	0.647817	2.39	0.1218	0.37380	1.12271
Kangagan	1.369685	2.31	0.1283	0.91317	2.05442
Kinowe	2.502214	9.88	0.0017	1.41229	4.43327
Kizimban	2.036019	15.30	0.0001	1.42575	2.90750
Konde A	1.444666	1.32	0.2508	0.77110	2.70661
Mkanyage	0.987506	0.00	0.9604	0.60101	1.62253
Mtambile	0.798555	0.94	0.3334	0.50625	1.25963
Ng'omben	0.371016	4.79	0.0287	0.15265	0.90175
Ngwachan	0.634183	4.77	0.0290	0.42143	0.95434
Piki	1.016522	0.01	0.9261	0.71916	1.43683
Pujini	1.331160	2.19	0.1388	0.91145	1.94415
S/vyambo	1.870537	11.73	0.0006	1.30716	2.67673
Tumbe	1.669089	1.14	0.2854	0.65207	4.27230
Uwandani	0.932741	0.19	0.6606	0.68358	1.27272
Wesha	1.546129	0.81	0.3695	0.59699	4.00430
Ziwani	0.840525	0.76	0.3844	0.56824	1.24328

Mantel-Haenszel estimate controlling for school

Odds Ratio	chi2(1)	P>chi2	[95% Conf.	Interval]
1.102855	3.39	0.0657	0.993674	1.224033

```
Test of homogeneity of ORs (approx): chi2(16)  =   56.42
                                     Pr>chi2   =  0.0000
```

Appendix 4a: Mantel-Haenszel analysis of the Score test for trend of the odds of *A. lumbricoide* infection over the years (2010-2012) following two rounds of school based treatment with Albendazole

```
. mhodds  asc_infection year, by( school)

Score test for trend of odds with year
by school

(The Odds Ratio estimate is an approximation to the odds ratio
for a one unit increase in year)
```

school	Odds Ratio	chi2(1)	P>chi2	[95% Conf.	Interval]
Bagamoyo	1.543894	3.73	0.0534	0.99372	2.39867
Chambani	0.752017	1.95	0.1628	0.50397	1.12214
Chanjamj	1.174643	0.61	0.4351	0.78405	1.75982
K/minung	1.824485	8.39	0.0038	1.21460	2.74062
Kangagan	1.394677	2.32	0.1278	0.90891	2.14007
Kinowe	1.175126	0.50	0.4790	0.75169	1.83708
Kizimban	1.379897	2.21	0.1370	0.90270	2.10935
Konde A	1.185483	0.75	0.3880	0.80561	1.74447
Konde B	1.638013	6.15	0.0131	1.10903	2.41931
Michakai	0.526627	6.12	0.0134	0.31681	0.87540
Mizingan	0.868967	0.53	0.4680	0.59466	1.26980
Mkanyage	0.903479	0.23	0.6329	0.59575	1.37017
Mtambile	1.049766	0.06	0.8120	0.70348	1.56651
Mzambara	0.581614	6.76	0.0093	0.38657	0.87507
Ng'omben	0.759751	1.75	0.1853	0.50593	1.14091
Ngwachan	1.825337	2.26	0.1327	0.83312	3.99926
Piki	1.134522	0.33	0.5675	0.73609	1.74861
Pujini	0.557005	9.02	0.0027	0.38023	0.81597
S/viambo	1.431237	3.50	0.0615	0.98293	2.08402
Tumbe	2.713646	17.93	0.0000	1.70953	4.30755
Uwandani	0.929212	0.13	0.7140	0.62744	1.37612
Wesha	1.509940	3.83	0.0504	0.99934	2.28143
Wingwi	1.317962	1.73	0.1880	0.87380	1.98789
Ziwani	1.299868	1.27	0.2602	0.82346	2.05190

Mantel-Haenszel estimate controlling for school

Odds Ratio	chi2(1)	P>chi2	[95% Conf.	Interval]
1.115510	6.26	0.0123	1.023966	1.215237

```
Test of homogeneity of ORs (approx): chi2(23)  =   75.79
                                     Pr>chi2   =  0.0000
```

Appendix 4b: Mantel-Haenszel analysis of the Score test for trend of the odds of hookworm infection over the years (2010-2012) following two rounds of school based treatment with Albendazole

```
. mhodds   hwk_infection year, by( school)

Score test for trend of odds with year
by school

(The Odds Ratio estimate is an approximation to the odds ratio
for a one unit increase in year)
```

school	Odds Ratio	chi2(1)	P>chi2	[95% Conf.	Interval]
Bagamoyo	0.982419	0.01	0.9418	0.61035	1.58129
Chambani	0.796540	1.04	0.3079	0.51439	1.23346
Chanjamj	0.992676	0.00	0.9713	0.66480	1.48226
K/minung	0.866239	0.50	0.4784	0.58241	1.28840
Kangagan	0.739169	1.71	0.1906	0.47007	1.16231
Kinowe	3.059759	24.45	0.0000	1.96417	4.76645
Kizimban	0.632821	4.05	0.0440	0.40537	0.98788
Konde A	1.516183	4.44	0.0352	1.02934	2.23328
Konde B	1.580841	5.24	0.0221	1.06795	2.34006
Michakai	0.362547	11.73	0.0006	0.20288	0.64788
Mizingan	0.776170	1.62	0.2037	0.52512	1.14725
Mkanyage	0.975684	0.01	0.9091	0.63938	1.48887
Mtambile	0.981104	0.01	0.9271	0.65184	1.47668
Mzambara	1.354112	2.17	0.1412	0.90427	2.02773
Ng'omben	0.327259	23.81	0.0000	0.20895	0.51256
Ngwachan	0.889289	0.09	0.7702	0.40471	1.95407
Piki	1.173118	0.62	0.4309	0.78850	1.74535
Pujini	0.555765	8.87	0.0029	0.37759	0.81803
S/viambo	1.843186	8.28	0.0040	1.21542	2.79520
Tumbe	1.858327	6.37	0.0116	1.14834	3.00729
Uwandani	0.853542	0.64	0.4231	0.57938	1.25744
Wesha	0.717492	2.69	0.1010	0.48252	1.06690
Wingwi	1.839199	9.15	0.0025	1.23933	2.72942
Ziwani	0.958326	0.04	0.8371	0.63868	1.43794

Mantel-Haenszel estimate controlling for school

Odds Ratio	chi2(1)	P>chi2	[95% Conf.	Interval]
1.003056	0.00	0.9451	0.919689	1.093979

```
Test of homogeneity of ORs (approx): chi2(23)  =  117.54
                                     Pr>chi2   =  0.0000

.
```

Appendix 4c: Mantel-Haenszel analysis of the Score test for trend of the odds of *T. trichiura* infection over the years (2010-2012) following two rounds of school based treatment with Albendazole.

```
. mhodds    tri_infection year, by( school)

Score test for trend of odds with year
by school

(The Odds Ratio estimate is an approximation to the odds ratio
for a one unit increase in year)
```

school	Odds Ratio	chi2(1)	P>chi2	[95% Conf.	Interval]
Bagamoyo	1.175705	0.13	0.7169	0.49007	2.82057
Chambani	0.977408	0.01	0.9428	0.52349	1.82491
Chanjamj	0.909733	0.13	0.7207	0.54162	1.52803
K/minung	1.197277	0.40	0.5248	0.68742	2.08530
Kangagan	2.008956	2.82	0.0933	0.88946	4.53745
Kinowe	2.375401	8.85	0.0029	1.34349	4.19990
Kizimban	0.634017	3.34	0.0675	0.38900	1.03335
Konde A	1.158972	0.40	0.5295	0.73174	1.83564
Konde B	1.112086	0.12	0.7304	0.60767	2.03521
Michakai	0.681824	2.83	0.0926	0.43634	1.06542
Mizingan	0.470875	2.76	0.0966	0.19370	1.14469
Mkanyage	1.226408	0.25	0.6194	0.54819	2.74372
Mtambile	1.406382	1.49	0.2222	0.81342	2.43159
Mzambara	1.176244	0.33	0.5643	0.67738	2.04249
Ng'omben	0.338223	17.54	0.0000	0.20364	0.56174
Ngwachan	0.129868	2.06	0.1510	0.00801	2.10636
Piki	0.891931	0.14	0.7048	0.49360	1.61170
Pujini	1.055392	0.03	0.8567	0.58795	1.89448
S/viambo	0.919946	0.11	0.7364	0.56599	1.49527
Tumbe	1.600530	0.87	0.3507	0.59601	4.29807
Uwandani	0.931262	0.05	0.8157	0.51174	1.69470
Wesha	3.516381	9.89	0.0017	1.60592	7.69961
Wingwi	1.477414	1.84	0.1750	0.84059	2.59669
Ziwani	1.171127	0.31	0.5801	0.66924	2.04940

Mantel-Haenszel estimate controlling for school

Odds Ratio	chi2(1)	P>chi2	[95% Conf.	Interval]
1.020288	0.10	0.7467	0.903232	1.152514

```
Test of homogeneity of ORs (approx): chi2(23)  =   56.60
                                     Pr>chi2   =  0.0001
```

Appendix 4.2d: Mantel-Haenszel analysis of the Score test for trend of the odds of *T. trichiura* infection over the years (2010-2012) following two rounds of school based treatment with Albendazole across the districts

```
. mhodds  tri_infection year, by(district)

Score test for trend of odds with year
by district

(The Odds Ratio estimate is an approximation to the odds ratio
for a one unit increase in year)
```

district	Odds Ratio	chi2(1)	P>chi2	[95% Conf.	Interval]
Chake-ch	1.052769	0.21	0.6504	0.84285	1.31497
Michewen	1.261887	3.97	0.0462	1.00391	1.58616
Mkoani	0.677814	7.25	0.0071	0.51073	0.89957
Wete	1.027760	0.05	0.8269	0.80406	1.31370

Mantel-Haenszel estimate controlling for district

Odds Ratio	chi2(1)	P>chi2	[95% Conf.	Interval]
1.016013	0.07	0.7968	0.900314	1.146580

```
Test of homogeneity of ORs (approx): chi2(3)   =   11.41
                                     Pr>chi2   =  0.0097
```

Appendix 4.2e: Mantel-Haenszel analysis of the Score test for trend of the odds of hookworm infection over the years (2010-2012) following two rounds of school based treatment with Albendazole across the districts

```
. mhodds   hwk_infection year, by(district)

Score test for trend of odds with year
by district

(The Odds Ratio estimate is an approximation to the odds ratio
for a one unit increase in year)
```

district	Odds Ratio	chi2(1)	P>chi2	[95% Conf.	Interval]
Chake-ch	0.778704	8.95	0.0028	0.66103	0.91733
Michewen	1.781759	46.38	0.0000	1.50888	2.10399
Mkoani	0.780763	8.06	0.0045	0.65816	0.92621
Wete	0.986692	0.02	0.8761	0.83372	1.16773

Mantel-Haenszel estimate controlling for district

Odds Ratio	chi2(1)	P>chi2	[95% Conf.	Interval]
1.018576	0.19	0.6662	0.936848	1.107434

```
Test of homogeneity of ORs (approx): chi2(3)   =   63.23
                                     Pr>chi2   =  0.0000
```

Appendix 4.2f: Mantel-Haenszel analysis of the Score test for trend of the odds of *A. lumbricoides* infection over the years (2010-2012) following two rounds of school based treatment with Albendazole across the districts

```
. mhodds    asc_infection year, by(district)

Score test for trend of odds with year
by district

(The Odds Ratio estimate is an approximation to the odds ratio
for a one unit increase in year)
```

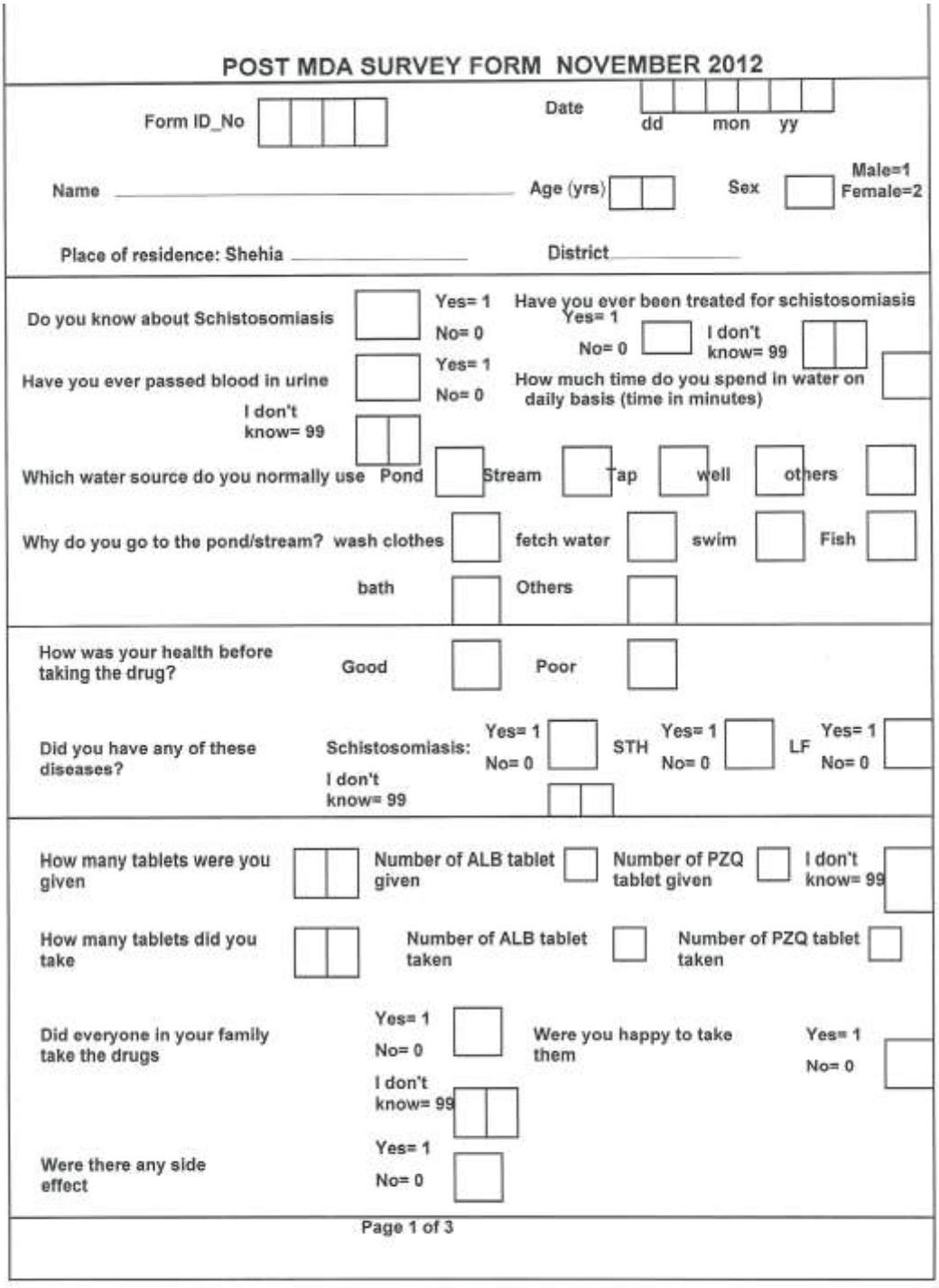
district	Odds Ratio	chi2(1)	P>chi2	[95% Conf.	Interval]
Chake-ch	0.970767	0.12	0.7244	0.82316	1.14485
Michewen	1.424582	17.62	0.0000	1.20763	1.68051
Mkoani	0.904422	1.33	0.2485	0.76257	1.07266
Wete	1.235676	5.90	0.0151	1.04170	1.46577

Mantel-Haenszel estimate controlling for district

Odds Ratio	chi2(1)	P>chi2	[95% Conf.	Interval]
1.116703	6.65	0.0099	1.026829	1.214444

```
Test of homogeneity of ORs (approx): chi2(3)   =   18.33
                                     Pr>chi2   =  0.0004
```

Appendix 5.1 Questionnaire used for assessing compliance for drugs administered in MDA compaigns



POST MDA SURVEY FORM NOVEMBER 2012

Form ID_No

Date dd mon yy

Name _____ Age (yrs) Sex Male=1 Female=2

Place of residence: Shehia _____ District _____

Do you know about Schistosomiasis Yes= 1 No= 0

Have you ever been treated for schistosomiasis Yes= 1 No= 0 I don't know= 99

Have you ever passed blood in urine Yes= 1 No= 0 I don't know= 99

How much time do you spend in water on daily basis (time in minutes)

Which water source do you normally use Pond Stream Tap well others

Why do you go to the pond/stream? wash clothes fetch water swim Fish bath Others

How was your health before taking the drug? Good Poor

Did you have any of these diseases? Schistosomiasis: Yes= 1 No= 0 STH Yes= 1 No= 0 LF Yes= 1 No= 0 I don't know= 99

How many tablets were you given Number of ALB tablet given Number of PZQ tablet given I don't know= 99

How many tablets did you take Number of ALB tablet taken Number of PZQ tablet taken

Did everyone in your family take the drugs Yes= 1 No= 0 I don't know= 99

Were you happy to take them Yes= 1 No= 0

Were there any side effect Yes= 1 No= 0

Page 1 of 3

POST MDA SURVEY FORM NOV 2012

Have you suffered from any of the following symptom following taking the drugs last week?

Dizzness	Yes = 1 No = 0	☐	Headache	Yes = 1 No = 0	☐
Sleepy	Yes = 1 No = 0	☐	Tiredness	Yes = 1 No = 0	☐
Abdominal pain	Yes = 1 No = 0	☐	Cramp	Yes = 1 No = 0	☐
Nausea	Yes = 1 No = 0	☐	Vomitting	Yes = 1 No = 0	☐
Diarrhoea	Yes = 1 No = 0	☐	Blood in faeces	Yes = 1 No = 0	☐
Sweating	Yes = 1 No = 0	☐	Night fever	Yes = 1 No = 0	☐
Lower back pain	Yes = 1 No = 0	☐			

When did any of this occured ____ dd mon yy

How long after drugs were taken ☐ Hours OR ☐ Days

Do you think this adverse experience was life-threatening Yes = 1 No = 0 ☐ I don't know = 99 ☐☐

Page 2 of 3

POST MDA SURVEY FORM NOV 2012

Did any of the adverse reaction led to seek health care (to see doctor or nurse) Yes = 1 No = 0 ☐

Were you admitted ? Yes = 1 No = 0 ☐

Date of admission ____ dd ____ mon ____ yy

Reason for admisssion _____

Date of discharge ____ dd ____ mon ____ yy

Did you fully recovered Yes = 1 No = 0 ☐

I don't know = 99 ☐☐

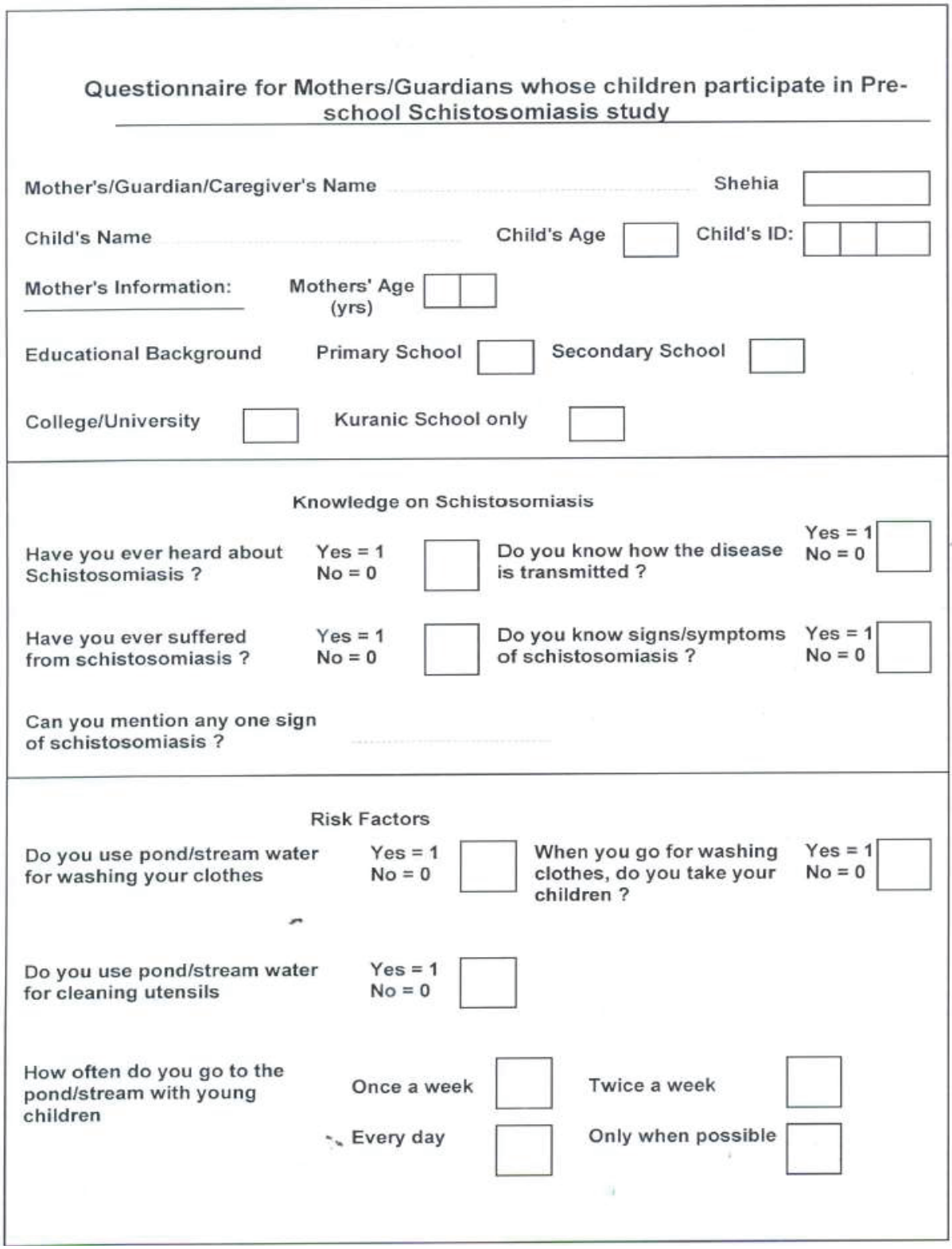
Is the problem still ongoing Yes = 1 No = 0 ☐

Any persistence or significant disability/ incapacity Yes = 1 No = 0 ☐

Death Yes = 1 No = 0 ☐

Date of death occured ____ dd ____ mon ____ yy

Appendix 6.1 Questionnaire administered to mothers/guardians whose children enrolled in the pre-school schistosomiasis study



Questionnaire for Mothers/Guardians whose children participate in Pre-school Schistosomiasis study

Mother's/Guardian/Caregiver's Name ..... Shehia ☐

Child's Name ..... Child's Age ☐ Child's ID: ☐☐☐

Mother's Information: Mothers' Age (yrs) ☐☐

Educational Background Primary School ☐ Secondary School ☐

College/University ☐ Kuranic School only ☐

Knowledge on Schistosomiasis

Have you ever heard about Schistosomiasis ? Yes = 1 No = 0 ☐ Do you know how the disease is transmitted ? Yes = 1 No = 0 ☐

Have you ever suffered from schistosomiasis ? Yes = 1 No = 0 ☐ Do you know signs/symptoms of schistosomiasis ? Yes = 1 No = 0 ☐

Can you mention any one sign of schistosomiasis ? .....

Risk Factors

Do you use pond/stream water for washing your clothes Yes = 1 No = 0 ☐ When you go for washing clothes, do you take your children ? Yes = 1 No = 0 ☐

Do you use pond/stream water for cleaning utensils Yes = 1 No = 0 ☐

How often do you go to the pond/stream with young children Once a week ☐ Twice a week ☐ Every day ☐ Only when possible ☐

What other activities which lead you to use pond/stream water .....

Do you use pond/stream for bathing? Yes = 1
No = 0 ☐

Appendix 7.1 List of snails that were tested for cercarial shedding, Dra-1 gene and characterized by sequencing,

Snail number	Shed cercariae	Dra-1 (*S.haem*)+ve	*B nasutus/globosus*	Site collected
3	-	+ve	SND	Kangagani
4	-	+ve	SND	Kangagani
5	-	uncertain	SND	Piki
6	-	+ve	SND	Piki
7	-	+ve	SND	Mavungwa
8	-	-ve	SND	Mavungwa
9	-	+ve	SND	Vitongoji
10	-	-ve	SND	Vitongoji
11	-	+ve	SND	KImbuni
12	-	-ve	SND	Kimbuni
13	-	+ve	B.g	Mavungwa
14	-	-ve	B.g	Mavungwa
15	-	+ve	SND	Mavungwa
16	-	+ve	SND	Mavungwa
17	-	+ve	SND	Mavungwa
18	-	-ve	SND	Mavungwa
19	-	+ve	SND	Mavungwa
20	-	-ve	SND	Mavungwa
21	-	+ve	SND	Mavungwa
22	-	-ve	SND	Mavungwa
23	-	+ve	B.g	KImbuni
24	-	+ve	B.g	KImbuni
25	-	+ve	SND	KImbuni
26	-	+ve	SND	KImbuni
27	-	+ve	SND	KImbuni
28	-	+ve	SND	KImbuni
29	-	-ve	SND	KImbuni
30	-	-ve	SND	KImbuni
31	-	-ve	SND	KImbuni
32	-	-ve	SND	KImbuni
33	-	-ve	B.n	Vitongoji
34	-	-ve	B.n	Vitongoji
35	-	-ve	B.n	Vitongoji
36	-	-ve	SND	Vitongoji
37	-	-ve	SND	Vitongoji
38	-	-ve	SND	Vitongoji
39	-	+ve	SND	Vitongoji
40	-	-ve	SND	Vitongoji
41	-	+ve	B.g	Piki
42	-	+ve	B.g	Piki
43	-	+ve	SND	Piki
44	-	uncertain	SND	Piki
45	-	+ve	SND	Piki
46	-	-ve	SND	Piki
47	-	uncertain	SND	Piki
48	-	+ve	SND	Piki

49	-	uncertain	SND	Piki
50	-	+ve	SND	Piki
51	-	-ve	SND	Piki
52	-	-ve	SND	Piki
53	-	-ve	B.n	Kangagani
54	-	+ve	SRU	Kangagani
55	-	-ve	SND	Kangagani
56	-	+ve	SND	Kangagani
57	-	+ve	SND	Kangagani
58	-	+ve	SND	Kangagani
59	-	-ve	SND	Kangagani
60	-	+ve	SND	Kangagani
61	-	-ve	SND	Kangagani
62	-	+ve	SND	Kangagani
63	-	+ve	SND	Kangagani
64	-	+ve	SND	Kangagani
65	-	+ve	B.g	Mkungu
66	-	+ve	B.g	Mkungu
67	-	+ve	SND	Mkungu
68	-	uncertain	SND	Mkungu
69	-	uncertain	SND	Mkungu
70	-	+ve	SND	Mkungu
71	-	-ve	SND	Mkungu
72	-	uncertain	SND	Mkungu
73	-	+ve	SND	Mkungu
74	-	uncertain	SND	Mkungu
75	-	+ve	SND	Mkungu
76	-	+ve	SND	Mkungu
77	+	-ve	SRU	Kangagani
78	+	+ve	B.n	Kangagani
79	+	uncertain	B.g	Mavungwa
80	+	+ve	B.n	Kangagani
81	+	+ve	B.n	Kangagani
82	+	+ve	SRU	Kangagani
83	+	+ve	SRU	Kangagani
84	+	+ve	B.g	Kangagani
85	+	+ve	B.n	Kangagani
86	+	uncertain	B.n	Kangagani
87	+	uncertain	B.n	Kangagani
88	+	+ve	B.n	Kangagani
89	+	uncertain	B.n	Kangagani
90	+	+ve	B.n	Kangagani
91	+	+ve	SRU	Kangagani
92	+	+ve	SRU	Kangagani
93	+	uncertain	B.n	Kangagani
94	+	+ve	B.n	Kangagani
95	-	+ve	B.n	Kangagani
96	-	-ve	SND	Kangagani

SND= sequencing not done;SRU = sequencing results unclear

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Parasites & Vectors

SHORT REPORT Open Access

Development of novel multiplex microsatellite polymerase chain reactions to enable high-throughput population genetic studies of *Schistosoma haematobium*

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Abstract

Background: Human urogenital schistosomiasis caused by *Schistosoma haematobium* is widely distributed across Africa and is increasingly targeted for control and regional elimination. The development of new high-throughput, cost-effective molecular tools and approaches are needed to monitor and evaluate the impact of control programs on the parasite populations. Microsatellite loci are genetic markers that can be used to investigate how parasite populations change over time and in relation to external influences such as control interventions.

Findings: Here, 18 existing *S. haematobium* microsatellite loci were optimised to enable simultaneous amplification across two novel multiplex microsatellite PCR's, each containing nine loci. Methods were developed for the cost effective and rapid processing and microsatellite analysis of *S. haematobium* larval stages stored on Whatman-FTA cards and proved robust on miracidia and cercariae collected from Zanzibar and Niger.

Conclusion: The development of these novel and robust multiplex microsatellite assays, in combination with an improved protocol to elute gDNA from Whatman-FTA fixed schistosome larval stages, enables the high-throughput population genetic analysis of *S. haematobium*. The molecular resources and protocols described here advance the way researchers can perform multi locus-based population genetic analyses of *S. haematobium* as part of the evaluation and monitoring of schistosomiasis control programmes.

Keywords: Cercariae, High-throughput, Microsatellites, Miracidia, Multiplex, Population genetics, *Schistosoma haematobium*

Findings

Introduction

Infection with the blood fluke *Schistosoma haematobium* causes human urogenital schistosomiasis throughout Africa, parts of the Middle East, Madagascar and the Indian Ocean Islands, with an estimated 110 million people infected [1]. Several efforts are underway to control morbidity and ultimately to eliminate *S. haematobium* infection predominantly through the large-scale administration of the drug praziquantel (PZQ) [1]. The development of new high-throughput, low cost, molecular tools and approaches are now imperative, not only to elucidate the epidemiology and evolution of schistosomiasis but also to monitor and evaluate the impact of progressing control programs [2]. Here we present an enhanced method enabling the high-throughput and cost effective preparation of gDNA from individual schistosome larval stages facilitating multi-loci genetic analysis together with two novel *S. haematobium* multiplex microsatellite PCRs. Microsatellite loci are highly variable DNA markers in widespread use within the schistosomiasis research community as they enable population-level analysis [3]. The principal drawback of microsatellite markers has been the cost and labour associated with the

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need to genotype multiple loci. Significant cost and timesaving can be achieved by developing multiplex PCR systems that amplify multiple microsatellite loci in single reactions. The methods outlined here facilitate the high-throughput microsatellite-based population genetic analyses of *S. haematobium*.

Microsatellite multiplex design and optimisation

S. haematobium microsatellite loci were available from [4] and [3]. Loci that were di, tri or tetra-mer repeats, non-compound, robust and had multiplexing potential were selected for further optimisation. Eighteen loci were chosen in total (15 from [3] and three from [4], Table 1). Initially the functionality and specificity of all the primer pairs were confirmed by amplifying all the loci in singleplex 12.5 μl reactions using 10 ng of *S. haematobium* reference gDNA obtained from the Schistosomiasis Collection at the Natural History Museum (SCAN [5]) and the Type-it Microsatellite PCR Kit (Qiagen) according to the manufacturer's protocol.

The loci were successfully divided into two multiplex panels each incorporating nine loci that gave the maximum size difference between each locus and a maximum of four overlapping loci at any size range, together with minimal variance of the annealing temperature of all the primers (T_m) (Table 1). Within each panel the forward primer for each locus was 5' labelled with a fluorescent reporter dye according to the 5-dye detection system. Overlapping fragments were assigned a different dye and the maximum distance was maintained between fragments labelled with the same dye to enable accurate identification. The multiplex microsatellite PCRs for each panel were carried out in 12.5 μl reactions using 10 ng of *S. haematobium* reference gDNA and the Type-it Microsatellite PCR Kit (Qiagen) according to the manufacturer's protocol. Different T_m values were tested with the optimal T_m that gave uniform and specific amplification for all loci in each panel determined at 54 °C. Singleplex and multiplex amplicons were visualised on 3 % gel red agarose gels before 2 μl of 1: 50 dilutions were mixed with 0.35 μl of GS500Liz size standard (Applied Biosystems) before being denatured for 5 mins at 95 °C and injected at a 10 s injection speed into an Applied Biosystems 3130xl DNA Analyser. Allele peaks were visualised in Geneious version 6.1.4 (www.geneious.com [6]) using the microsatellite plugin. The multiplex PCRs proved robust giving identical peak scores in repeated reactions, in singleplex *versus* multiplex

Table 1 Details of the 18 selected microsatellite loci and the characteristics of the two multiplex microsatellite PCR assays. Loci Sh1-15 are from Travis *et al.*, 2013 and Loci C102, C111 and C131 are from Gower *et al.*, 2011. For Niger $Ho = 0.596$, $He = 0.609$, for Pemba $Ho = 0.599$, $He = 0.638$. The overall $Ho = 0.597$, $He = 0.623$

Panel	Marker	Forward Primer 5'- 3'	Reverse Primer 5'- 3'	Dye	Size Range (bp)	Repeat	A	Niger		Zanzibar	
								H_o	H_e	H_o	H_e
Panel 1	C102	TGTCTCTGTGAATGACCGAAT	TTAGATGAATAATAATGTTGAAACCAC	VIC	184–199	ATT	6	0.42	0.37	0.02	0.02
	Sh1	GCATCCAATTTCGTACAC	CCACATTAGGCCAACAAG	VIC	245–284	AAT	13	0.76	0.72	0.84	0.80
	Sh14	GTCCTCCTTCCCTCTTTG	CACATTCGTCCTAGATATCG	NED	184–240	ACTC	15	0.94	0.85	0.86	0.88
	C131	CTTGTCATTTGGGCATTGTG	CATGGTGAGGTTCAAACGTG	NED	253–265	AAT	4	0.00	0.00	0.00	0.00
	Sh6	GGGATGTATGCAGACTTG	TTGTTTGGCTGCAGTAAC	NED	309–321	AAT	7	0.48	0.44	0.84	0.76
	Sh9	GCTGAGCTTGAGATTG	CTTCTGTCCCATCGATACC	6-FAM	197–227	AAT	11	0.46	0.76	0.46	0.86
	Sh3	GCTGAGCTTGAGATTG	CTTCTGTCCCATCGATACC	6-FAM	270–366	AAT	30	0.76	0.86	0.94	0.86
	C111	CCCTTGTCTTCAATGCGTTA	GAACGTCTAACTGGCGATCA	PET	201–225	ATT	9	0.74	0.67	0.76	0.68
	Sh7	TCCAAGCACCATTATCAAG	ACGGAAACTTGTTGAAATG	PET	293–311	AAT	7	0.46	0.62	0.42	0.48
Panel 2	Sh2	TTAGTGTGTTTGGCTTCAAC	CCTCGAATGAAATCCTGAC	NED	155–218	AAT	21	0.84	0.90	0.56	0.89
	Sh5	TGTGCACAAGAAAGATTAAATG	ACGACAATGTTGCAAGTTC	NED	263–314	AAT	16	0.78	0.81	0.36	0.48
	Sh13	GAGCAGCTATTTCGTATCG	ACCGTGGACAGTTCATCAG	6-FAM	163–211	AAT	17	0.78	0.72	0.68	0.64
	Sh4	CCCATCGCTGATATTAAAG	TCTAGTCGTCTTGGGATCC	6-FAM	268–313	AAT	13	0.84	0.78	0.72	0.79
	Sh10	CGCATGTCATACCTATCTCC	GCTTATCAGGCCTATCTCC	PET	183–207	AAT	9	0.18	0.34	0.74	0.70
	Sh12	CGTCTTAGTGAGCCAGATG	CTCGTGGACATCATCAG	PET	245–278	AAC	11	0.06	0.06	0.56	0.65
	Sh8	CTAAACTGGCAAGATTTC	CAACGTGCCTTTATTTC	PET	282–321	AAT	14	0.76	0.81	0.84	0.83
	Sh11	TTGGTTTAGAAATTACATCACC	CCAACAATATTAATGGACAGC	VIC	183–213	ATC	9	0.68	0.58	0.68	0.69
	Sh15	CTTTCAGTAGGATTTGTTG	CGACGTCAAGCACTGTAC	VIC	274–301	ATC	10	0.78	0.65	0.50	0.466

Panel = single multiplex PCR. A = observed number of alleles. Dye = the fluorescent dye label of the forward primer (VIC = green, NED = yellow, 6-FAM = Blue, PET = red). *Ho* = observed heterozygosity, *He* = expected heterozygosity

reactions, and significant stutter peaks, n-1 products and allelic drop-out were not observed.

Multiplex PCR optimisation and application on field-collected S. haematobium miracidia and cercaria

A novel, high-throughput and cost effective non-wash Whatman-FTA alkaline DNA elution protocol has been developed which provides ~38 μl of eluted DNA from a single schistosome larval stage which has been fixed on a classic indicating Whatman-FTA card. This three-step protocol is very simple, quick and is suitable for multi-well processing. Individual larval DNA is alkaline eluted from a single 2.0 mm Whatman-FTA punch and subsequently neutralised, providing usable DNA for many downstream applications including microsatellite and fragment analysis, mitochondrial and nuclear DNA/gene amplification (http://www.gelifesciences.com). The solutions (1 and 2) needed for the DNA elution steps can be easily made with standard laboratory chemicals at an insignificant cost, especially compared to alternative DNA preparation methods.

Individual *S. haematobium* miracidia were collected directly from individual urine samples of infected children in Niger and Pemba Island (Zanzibar, United Republic of Tanzania [7]). *S. haematobium* cercariae were also obtained from naturally infected *Bulinus globosus* snails from Niger. All samples were collected and individually preserved on Whatman-FTA cards [8, 9].

DNA elutions were carried out in low profile 1.2 ml 96 square well storage microplates with 96 square well sealing cap mats which facilitates DNA elution. The 2.0 mm Whatman-FTA punch containing the DNA from a single larval stage was incubated at room temperature in 14 μl of Solution 1 (0.1 M NaOH, 0.3 mM EDTA, pH13.0) for 5 mins. Subsequently, 26 μl of Solution 2 (0.1 M Tris–HCl, pH7.0) was added, the mixture was pulse vortexed three times, incubated for a further ten minutes at room temperature and then pulse vortexed ten times. The eluted DNA was then transferred to a 96 well storage plate and either used immediately or stored at -20 °C for future use.

The two multiplex microsatellite PCRs were performed on each available sample in 12.5 μl reactions using 2 μl of the eluted DNA and the Type-it Microsatellite PCR Kit (Qiagen) according to the manufacturer's protocol with the addition of 1.25 μl of the Type-it Microsatellite PCR Kit Q-Solution. Optimal cycling parameters were, an initial denaturing step of 95 °C for 5 mins followed by 32 cycles of 95 °C for 30 s, 54 °C for 90 s, 72 °C for 3 mins and followed by a final elongation step of 60 °C for 30 mins. Reactions were checked by 3 % agarose gel electrophoresis and then diluted 1 in 10 before being denatured and injected at an optimal speed of 12 s into the Applied Biosystems 3130xl DNA analyser for analysis.

Allele peaks were checked and edited using Geneious 6.1.4 (www.geneious.com [6]) before being placed into amplicon size "bins" and exported for analysis. Panel 1 and 2 allele data were compiled for each sample for analysis (Additional file 1: Table S1). Data were analysed from ten miracidia, from five children from Koutoukale Zeno (Lat. 13.680, Long. 1.738) in Niger, five children from Chambani school (Lat. 5.33457 Long. 39.77256) on Pemba Island, Zanzibar, United Republic of Tanzania and also from 16 cercariae from two infected *Bulinus* snails from Niger.

All loci amplified successfully with no significant stutter peaks or n-1 products. Whilst low peak height was often observed in the loci Sh7 (Panel 1) compared to the other loci and was lower in samples from Niger compared to Pemba, the data were still scorable. Genetic diversity indices were calculated using the program GenAlEx 6.5 [10] and the presence of null alleles and allele dropout was evaluated using Micro-Checker [11]. The numbers of alleles observed across the loci ranged from 2 to 33 with loci C131 being the least diverse. Higher genetic diversity was observed in the Pembamiracidial population compared to that from Niger (Table 1). Cercariae obtained from each individual snail had identical genotypes, showing they were clonal, derived from a single miracidium.

Inter-species specificity

The cross-reactivity of the multiplex microsatellite PCRs was also assessed on *S. mansoni*, which causes intestinal

Table 2 Cross reactivity of the two multiplex microsatellite PCR assays on *S. mansoni*

	Marker	Size Range (bp)
Panel 1	C102	allelic drop-out
	Sh1	245–284
	Sh14	low amplification
	C131	low amplification
	Sh6	309–321
	Sh9	low amplification
	Sh3	allelic drop-out
	C111	allelic drop-out
	Sh7	allelic drop-out
Panel 2	Sh2	allelic drop-out
	Sh5	low amplification
	Sh13	allelic drop-out
	Sh4	254
	Sh10	168
	Sh12	242–272
	Sh8	allelic drop-out
	Sh11	allelic drop-out
	Sh15	allelic drop-out

schistosomiasis and is very common throughout Africa and can sometimes be found ectopically excreted in urine samples in endemic co-infection foci [12]. Singleplex and multiplex reactions were performed on 10 ng of reference gDNA from individual *S. mansoni* male worms obtained from the Schistosomiasis Collection at the Natural History Museum (SCAN [5]). Cross-reactivity was found to be low: seven loci failed to amplify, six gave low and/or non-specific amplification, two exhibited a size shift and only three among the total of 18 loci amplified well and were within the size range expected (Table 2).

In conclusion, this study describes two novel robust and informative multiplex microsatellite assays enabling the simultaneous amplification of 18 individual loci; facilitating population genetic analysis of all *S. haematobium* life-cycle stages. Protocols are presented that facilitate high-throughput, and cost effective processing and robust genetic analysis of *S. haematobium* larval stages. Such tools can greatly assist large-scale population genetic analysis of human schistosome populations such as that now underway within the SCORE programme (http://score.uga.edu). The alkaline elution of larval schistosome DNA from Whatman-FTA stored samples is simple, quick, high-throughput and low cost, providing adequate amounts of gDNA preparations for multiple molecular analyses and repeats, significantly overcoming the limitations encountered from the standard Whatman-FTA preparations [2]. Additionally, the multiplexing of the microsatellite loci significantly reduces the resources associated with genotyping multiple microsatellite loci for analysis.

Ethics statement

For the Niger sample collection, ethical approval was obtained from the St Mary's Hospital Local Ethics Research Committee (part of the Imperial College London Research Ethics Committee (ICREC; (EC NO: 03.36. R&D No: 03/SB/033E)) in London, United Kingdom. For the Zanzibar sample collection, ethical approval was obtained from the Zanzibar Medical Research and Ethics Committee (ZAMREC, reference no. ZAMREC 0003/Sept/011) in Zanzibar, United Republic of Tanzania, the "Ethikkomission beider Basel" (EKBB, reference no. 236/11) in Basel, Switzerland, and the Institutional Review Board of the University of Georgia (project no. 2012-10138-0). Within both Niger and Zanzibar, all aspects of sample collections were carried out in the framework of the disease control activities implemented and approved by the local Ministry of Health (MoH) and adopted by regional and local administrative and health authorities. The study participants were informed about the study objectives and procedures. Written consent was obtained from parents prior to sample collection from children. Participation was voluntary and children could withdraw or be withdrawn from the study at any time without obligation. All children were offered PZQ (40 mg/kg single oral dose) treatment in the frame of the following school-based or community-wide treatment carried out by the MoH.

Additional file

Additional file 1: Table S1. Allele sizes for all 18 loci for 50 miracidia from both Niger and Pemba (Zanzibar). (CSV 15 kb)

Abbreviations

SCORE: Schistosomiasis Consortium for Operational Research and Evaluation; PCR: Polymerase chain reaction; gDNA: Genomic deoxyribonucleic acid; PZQ: Praziquantel.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

BW, FA and AE refined the FTA elution protocol. BW designed the experiments and carried out all the laboratory work. BW, MR and TP carried out the data analysis. BW, JW and DR wrote the manuscript with comments and editing from all of the co-authors. All other authors were involved in the fieldwork and/or sample collection/storage. All authors read and approved the final version of the manuscript.

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References

1. World Health Organization. Accelerating work to overcome the global impact of neglected tropical diseases: a roadmap for implementation. 2012. Available: http://www.who.int/neglected_diseases/NTD_RoadMap_2012_Fullversion.pdf
2. Webster JP, Gower CM, Norton AJ. Evolutionary concepts in predicting and evaluating the impact of mass chemotherapy schistosomiasis control programmes on parasites and their hosts. Evol Appl. 2008;1:66–83.
3. Glenn TC, Lance SL, McKee AM, McKee AM, Webster BL, Emery AM, et al. Significant variance in genetic diversity among populations of Schistosoma

haematobium detected using microsatellite DNA loci from a genome-wide database. Parasites & Vectors. 2013;6:300.
4. Gower CM, Gabrielli AF, Sacko M, Dembele R, Golan R, Emery AM, et al. Population genetics of *Schistosoma haematobium*: development of novel microsatellite markers and their application to schistosomiasis control in Mali. Parasitology. 2011;138:978–94.
5. Emery AM, Allan FE, Rabone ME, Rollinson D. Schistosomiasis collection at NHM (SCAN). Parasites & Vectors. 2012;5:185.
6. Kearse M, Moir R, Wilson A, Stones-Havas S, Cheung M, Sturrock S, et al. Geneious Basic: an integrated and extendable desktop software platform for the organization and analysis of sequence data. Bioinformatics. 2012;28:1647–9.
7. Knopp S, Mohammed KA, Ali SM, Khamis IS, Ame SM, et al. Study and implementation of urogenital schistosomiasis elimination in Zanzibar (Unguja and Pemba islands) using an integrated multidisciplinary approach. BMC Public Health. 2012;12:930.
8. Webster BL, Emery A, Webster JP, Gouvras A, Garba A, Diaw O, et al. Genetic diversity within *Schistosoma haematobium*: DNA barcoding reveals two distinct groups. PLoS Neg Trop Dis. 2012;6:e1882.
9. Gower CM, Shrivastava J, Lamberton PHL, Rollinson D, Webster BL, Emery A, et al. Development and application of an ethical and epidemiologically appropriate assay for the multi-locus microsatellite analysis of *Schistosoma mansoni*. Parasitology. 2007;134:523–36.
10. Peakall R, Smouse PE. GenAlEx 6.5: genetic analysis in Excel. Population genetic software for teaching and research—an update. Bioinformatics. 2012;28:2537–9.
11. Van Oosterhout C, Hutchinson WF, Wills DPM, Shipley P. MICRO-CHECKER: software fro identifying and correcting genotyping errors in microsatellite data. Mol Ecol Notes. 2004;4:535–8.
12. Meurs L, Mbow M, Vereecken K, Menten J, Mboup S, Polman K. Epidemiology of mixed *Schistosoma mansoni* and *Schistosoma haematobium* in Northern Senegal. Int J for Parasitology. 2012;42:305–11.

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PLOS | NEGLECTED TROPICAL DISEASES

Elimination of Schistosomiasis Transmission in Zanzibar: Baseline Findings before the Onset of a Randomized Intervention Trial

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Abstract

Background: Gaining and sustaining control of schistosomiasis and, whenever feasible, achieving local elimination are the year 2020 targets set by the World Health Organization. In Zanzibar, various institutions and stakeholders have joined forces to eliminate urogenital schistosomiasis within 5 years. We report baseline findings before the onset of a randomized intervention trial designed to assess the differential impact of community-based praziquantel administration, snail control, and behavior change interventions.

Methodology: In early 2012, a baseline parasitological survey was conducted in ~20,000 people from 90 communities in Unguja and Pemba. Risk factors for schistosomiasis were assessed by administering a questionnaire to adults. In selected communities, local knowledge about schistosomiasis transmission and prevention was determined in focus group discussions and in-depths interviews. Intermediate host snails were collected and examined for shedding of cercariae.

Principal Findings: The baseline *Schistosoma haematobium* prevalence in school children and adults was 4.3% (range: 0–19.7%) and 2.7% (range: 0–26.5%) in Unguja, and 8.9% (range: 0–31.8%) and 5.5% (range: 0–23.4%) in Pemba, respectively. Heavy infections were detected in 15.1% and 35.6% of the positive school children in Unguja and Pemba, respectively. Males were at higher risk than females (odds ratio (OR): 1.45; 95% confidence interval (CI): 1.03–2.03). Decreasing adult age (OR: 1.04; CI: 1.02–1.06), being born in Pemba (OR: 1.48; CI: 1.02–2.13) or Tanzania (OR: 2.36; CI: 1.16–4.78), and use of freshwater (OR: 2.15; CI: 1.53–3.03) showed higher odds of infection. Community knowledge about schistosomiasis was low. Only few infected *Bulinus* snails were found.

Conclusions/Significance: The relatively low *S. haematobium* prevalence in Zanzibar is a promising starting point for elimination. However, there is a need to improve community knowledge about disease transmission and prevention. Control measures tailored to the local context, placing particular attention to hot-spot areas, high-risk groups, and individuals, will be necessary if elimination is to be achieved.

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Competing Interests: The molluscicide niclosamide was donated by Bayer for the control of intermediate host snails in Zanzibar. This does not alter our adherence to all PLOS NTDs policies on sharing data and materials.

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Introduction

Schistosomiasis ranks third after soil-transmitted helminthiasis and leishmaniasis regarding disease burden estimates of neglected tropical diseases (NTDs), and causes an estimated 3.3 million disability-adjusted life years (DALYs) [1]. In Africa alone, it is estimated that some 200 million people are infected with the blood fluke of the genus *Schistosoma* [2].

Encouragingly, over the past decade, efforts to control NTDs have been scaled up [3]. In early 2012, the World Health Organization (WHO) issued an ambitious goal to control schistosomiasis globally by the year 2020 and put forward a roadmap as to how this could be achieved [4]. A number of influential public and private organizations now support this goal and contributed to the London Declaration [5]. In May 2012, the

Author Summary

Schistosomiasis is a chronic and debilitating disease caused by parasitic worms. It negatively impacts on the health and wellbeing of mainly rural dwellers in tropical and sub-tropical countries. The World Health Organization recently put forward an ambitious goal for the year 2020: to control schistosomiasis globally. Interruption of transmission and elimination of schistosomiasis are encouraged whenever resources allow. After careful consideration, the Schistosomiasis Consortium for Operational Research and Evaluation (SCORE) selected the Zanzibar archipelago to learn how best to eliminate schistosomiasis. We report the baseline findings of a 5-year program. Parasitological examination of about 20,000 people on Unguja and Pemba islands revealed a low overall prevalence of *Schistosoma haematobium* (7%). Nevertheless, hot-spots with high prevalence (>20%) and high-risk groups (males, young adults, people born in Pemba or mainland Tanzania, and people using natural freshwater) were identified. The community knowledge about schistosomiasis transmission and prevention was poor. Few of the collected intermediate host snails shed *S. haematobium* cercariae. A multi-arm randomized trial is now being implemented to determine the differential impact of mass deworming, snail control, and behavior change interventions. Lessons learned from this schistosomiasis elimination program will be important for other settings.

World Health Assembly (WHA) resolution 65.21 was adopted, which encourages member states and the international community not only to make available the necessary and sufficient means and resources in terms of medicines, but also in terms of water, sanitation, and hygiene interventions [6].

While preventive chemotherapy is considered as the mainstay of schistosomiasis control [7–9], there is considerable evidence that control packages integrating anti-schistosomal treatment, the provision of clean water and improved sanitation, snail control, and behavior change, readily adapted to the local settings and fine-tuned over time, are necessary to sustain control achievements and to reach elimination of schistosomiasis [10–13]. Political will and support from national governments, institutions, and the local population coupled with inter-sectoral collaboration between the health, water and sanitation, and education sectors are key features to achieve sustainable control of schistosomiasis [14–17]. Examples of where schistosomiasis has been successfully controlled or even eliminated using integrated measures include, besides others, Japan and the People's Republic of China (*S. japonicum*), Martinique and Saudi Arabia (*S. mansoni*), and Tunisia and Mauritius (*S. haematobium*) [12]. The Zanzibar archipelago, part of the United Republic of Tanzania, has been identified as a candidate area, where schistosomiasis elimination might be achieved [4,18–20]. Indeed, after careful consideration, the Schistosomiasis Consortium for Operational Research and Evaluation (SCORE), selected the Zanzibar archipelago to learn how best to eliminate schistosomiasis and to evaluate different intervention combinations. Selection criteria included (i) the strong political commitment from the Zanzibar President and the government; (ii) the restriction to only urogenital schistosomiasis caused by *S. haematobium*; (iii) the relatively low *S. haematobium* prevalence and infection intensity on both islands; and (iv) the creation of an alliance determined to achieve schistosomiasis elimination in Zanzibar. This alliance – Zanzibar Elimination of Schistosomiasis Transmission (ZEST) – was formed in 2011 and consists of the Zanzibar government, particularly the Ministries of Health and Education, the Public Health Laboratory – Ivo de Carneri (PHL–IdC) Pemba, and a growing number of partners, including SCORE, Natural History Museum (NHM) in London, WHO, Schistosomiasis Control Initiative (SCI), Swiss Tropical and Public Health Institute (Swiss TPH), and other institutions and individuals. ZEST aims at (i) eliminating schistosomiasis as a public health problem on Unguja island in 3 years and to interrupt transmission in 5 years; (ii) controlling schistosomiasis throughout Pemba island (prevalence <10%) in 3 years and eliminating it as a public health problem in 5 years; and (iii) gaining experiences and drawing lessons for successful and durable schistosomiasis control, including costs and barriers associated with three different control interventions. Elimination of schistosomiasis as a public health problem is defined as the reduction of the prevalence of *S. haematobium* to <1% heavy infections based upon direct egg-detection methods in the school-aged population [12].

Here, we describe the baseline characteristics of local communities in Zanzibar, prior to the implementation of a randomized intervention trial, that consists of biannual mass drug administration (MDA) of praziquantel to the whole at-risk population (arm 1), compared to MDA plus snail control interventions (arm 2), and to MDA plus behavior change interventions (arm 3) [19]. Challenges and opportunities are discussed.

Methods

Ethics Statement

The study protocol received ethical approval from the Zanzibar Medical Research Ethics Committee (ZAMREC, reference no. ZAMREC 0003/Sept/011), the "Ethikkomission beider Basel" (EKBB) in Switzerland (reference no. 236/11), and the Institutional Review Board of the University of Georgia (project no. 2012-10138-0). Formative research on behavior change interventions was approved by the National Center for Emerging Zoonotic Diseases (NCEZID) of the Centers for Disease Control and Prevention (NCEZID tracking no. 103111BP). The study is designed as randomized intervention trial and is registered at the International Standard Randomised Controlled Trial Number Register (ISRCTN48837681).

The purpose and procedures of the study were verbally explained to village and school authorities and to study participants. Participants received an information sheet and were asked to submit a written informed consent. All minors (e.g., children below the age of 16 years) included into the study had written informed consent given by their parents/guardians and all participating adults signed and provided their own consent.

All participants were offered praziquantel (40 mg/kg) against schistosomiasis and albendazole (400 mg) against soil-transmitted helminthiasis free of charge in the frame of the island-wide MDA campaign conducted in late April 2012.

Study Area and Population

The Zanzibar archipelago includes the two large islands of Unguja and Pemba. Unguja is divided into six and Pemba into four districts, which are further subdivided into smaller administrative units, known as shehias. The local administration in the shehias is governed by the community leader (sheha). According to the 2002 census, Unguja consists of 176 and Pemba of 73 shehias with a total population of 979,637 inhabitants. The mean annual growth rate is 3.1%, and hence, the estimated population in 2012 was 1,330,000. The majority of the population is Muslim.

For inclusion into our intervention trial, we randomly selected 45 shehias in both Unguja and Pemba [19]. The three intervention arms (i.e., MDA alone, MDA plus snail control,

and MDA plus behavior change) will be monitored longitudinally in 15 shehias each, on both islands, by means of annual cross-sectional parasitological surveys together with snail surveys, and qualitative behavioral assessments [19].

Field Procedures

Details of the study surveys are provided elsewhere [19]. In brief, before the onset of regular biannual MDA in April 2012, we conducted a baseline parasitological survey assessing *S. haematobium* infection in adults and school children. The adult survey was conducted in November and December 2011. In each study shehia, the sheha was invited to answer a set of standard questions about the demographics, sanitary infrastructure, and water availability and use in the shehia. Moreover, 50 randomly selected households in each shehia were visited by a member of a 4–12 headed trained interviewer team. In each household, one present adult, aged 20–55 years, was randomly selected, informed about the ZEST program, and asked for consent to participate. Participation included answering standard questions about demographics such as age, occupation, and risk factors potentially associated with *S. haematobium* transmission. Moreover, all participants were invited to submit a urine sample right after the questionnaire interview (between 09:00 and 18:00 hours).

The baseline survey in 45 primary schools was conducted from January to March 2012. A school was visited on two subsequent days by two field teams consisting of 2–4 fieldworkers for registration and 4–6 fieldworkers for sample collection, respectively. On the first visit, approximately 130 children attending standard 1 and 130 children from standards 3 and 4 were stratified by sex and randomly selected for participation. Children's names and demographic details were registered. After explaining the study purpose and procedures, children were provided with a consent sheet to be signed by the parents/guardians and to be returned the next day. Upon submission of the signed informed consent sheet, a urine sample was collected from children attending standards 1, 3, and 4 (between 09:00 and 14:00 hours).

Urine samples from adults and school children were transferred to the laboratory in Zanzibar Town (Helminth Control Laboratory Unguja, HCLU) or Chake (PHL–IdC) immediately after collection.

Laboratory Procedures

Urine samples were processed either the same day (HCLU) or stored in the fridge until the next morning (PHL–IdC). Urine samples of sufficient quantity (≥10 ml) were visually inspected for blood (macrohematuria) using a color chart, for microhematuria using reagent strips (Hemastix; Siemens Healthcare Diagnostics GmbH, Eschborn, Germany), and for *S. haematobium* eggs by filtering 10 ml of urine through a polycarbonate filter (Sterlitech, Kent, United States of America) that was quantitatively examined under a microscope by experienced laboratory technicians.

Snail Survey

In Zanzibar, four species of *Bulinus* spp. snails are recognized [21,22]. While *B. globosus* and *B. nasutus* are allopatric, *B. forskalii* may be found in association with both species. *Bulinus* sp., another *B. forskalii* group species, has a very limited distribution [22]. In Unguja, *B. nasutus* only occurs in the south of the island in areas that are not part of our survey. We are hence only dealing with *B. globosus* and *B. forskalii* in our surveyed shehias in Unguja. In Pemba, however, the distribution lines of *B. globosus* and *B. nasutus* are not as clear cut. Since it is difficult to differentiate the taxa of *B. globosus* and *B. nasutus* using shell characteristics, we refer in the following to *B. globosus/nasutus* for snails collected in Unguja or Pemba. It must be noted, however, that in Zanzibar only *B. globosus* seems susceptible for *S. haematobium* miracidia infection [23]. The snail control arm in our study aims to minimize schistosomiasis transmission by reducing intermediate host snail populations of *B. globosus* by the use of the molluscicide niclosamide [19]. Before the onset of niclosamide application, between November 2011 and December 2012, freshwater bodies in the 15 randomly selected shehias on each island were identified with the help of local people and mapped using a hand-held global positioning system (Garmin GPSMap 62, Garmin Ltd., Southampton, United Kingdom). Water characteristics such as conductivity, dissolved oxygen, pH, salinity, temperature, total dissolved solids (TDS), and velocity were measured and recorded with a hand-held water meter (PcTest 35K, Thermo Fisher Scientific Inc., Loughborough, United Kingdom). To assess snail densities, a sample area of 15 m was measured and subsequently surveyed for snails for 15 min by four trained staff using scoops, sieves, and hands. The collected snails per site were placed into one collecting tray, identified at genus level, and counted on the spot. Once the species and number of snails were recorded, all organisms, except *Bulinus* spp., were returned and evenly distributed to their original habitats. All *B. globosus/nasutus* were transferred to HCLU or PHL–IdC to observe shedding of cercariae and all *B. forskalii* for more detailed species investigations.

In the laboratory, *B. globosus/nasutus* snails were assessed the following day for *S. haematobium* infection by placing snails individually into a flat-bottom vial, in clean water, exposing the snails to sun light for 2 hours and subsequently observing cercariae shed using a dissection microscope [24,25]. *S. haematobium* cercariae were identified microscopically by experienced technicians of the "snail team" and captured on Whatman FTA classic cards (Whatman, Part of GE Healthcare, Florham Park, United States of America) to identify molecular characteristics at a later point in time [26].

Formative Research for Behavior Change

The behavior change study arm aims to interrupt the transmission cycle of *S. haematobium* by modifying people's behavior [19]. To identify and implement community-owned behavior change interventions, we follow a 'Human-centered Design Process' [27]. This process creates solutions to problems in joint collaboration with the community. The first step was to conduct qualitative formative research with study community members, in five shehias on Unguja (Chaani, Dole, Kilombero, Mwera, and Uzini) and two shehias on Pemba (Chambani and Kizimbani), among the 15 selected study shehias on each island. We explored community perceptions and practices associated with transmitting, having, treating and preventing schistosomiasis, locally known as *kichocho*. Community leaders, religious leaders, teachers, parents and school children were asked for their opinion in focus group discussions (FGDs) and in-depth interviews (IDIs) conducted in Kiswahili by trained members of the "behavioral team" of the HCLU and PHL–IdC in 2011.

Statistical Analysis

Registration details and quantitative data from laboratory examinations were entered in Microsoft Excel version 10.0 (2002 Microsoft Corporation) and snail records and questionnaire data into EpiInfo version 3.5.1 (Centers for Disease Control and Prevention, Atlanta, United States of America) by local staff in Zanzibar. Statistical analyses were carried out with STATA version 10 (StataCorp., College Station, United States of America). FGDs and IDIs were transcribed verbatim, translated from Kiswahili into English, and entered into Atlas-ti version 6.0

(Software Development GmbH, Berlin, Germany) [19]. Sections of narrative data were open-coded to create specific categories and axial-coding was employed to relate the categories to each other. A thematic analysis was conducted through a grounded theory framework. Sets of codes were integrated and emergent themes identified.

Into the analyses of parasitological and questionnaire data only adults aged 20–55 years, first-year students and children aged 9–12 years were included, adhering to SCORE guidelines [19]. Macrohematuria was graded with numbers from 1 to 6 from transparent to dark red urine using a pretested color chart [28,29]. Microhematuria in urine was coded semi-quantitatively according to the Hemastix manufacturer's instructions (0, negative; 1, +; 2, ++; 3, +++; and 4, trace). *S. haematobium* infection intensity was determined according to the number of eggs found in a 10 ml filtrate with 1–49 eggs/10 ml considered as light and ≥50 eggs/10 ml as heavy infections [30]. Association between *S. haematobium* infection (binary variable) or egg counts per 10 ml urine (continuous variable) and macro- or microhematuria (categorical variables) was assessed by univariable logistic regression.

Stratified by island, univariable and stepwise backward multivariable regression analyses were employed to identify significant associations between *S. haematobium* infection and risk factors, as assessed in the questionnaire interviews with adults. Candidate explanatory variables for the multivariable logistic regression were sex (binary variable) and age (continuous variable) and those which were significantly associated with *S. haematobium* infection in the univariable analyses. In the backward stepwise multivariable logistic regression, we removed non-predicting covariates up to a significance level of 0.2 and allowed for possible clustering by using the sandwich estimator robust cluster option in STATA. Similarly, the association between *Bulinus* spp. snails and water chemistry parameters (conductivity, pH, temperature, and TDS; continuous variables; velocity: ordinary variable) were analyzed using univariable and multivariable regression, including only covariables that were significant in univariable analysis.

Results

Study Compliance

The shehas of all 90 study shehias agreed to answer the questions about shehia characteristics. Both on Unguja and Pemba, 2,250 adult individuals were invited to participate in the study and provided written informed consent. In Unguja, 2,196 and in Pemba 1,867 adults were in the 20–55 years age range, and hence included in the analyses. On both islands, the adults' mean age was 34 years. In Unguja 1,670 (76.1%) and in Pemba 1,242 (66.6%) participants were female. All participants replied at least partially to the questionnaire survey. The number of individuals who provided a urine sample of sufficient quantity for hematuria assessment and urine filtration is shown in Table 1. The compliance of the 8,912 and 10,593 school children, who were invited to participate in the study in Unguja and Pemba, respectively, is detailed in Figure 1.

Shehia Characteristics

On average, a shehia on Unguja and Pemba has a size of 9.5 km^2 and 13.5 km^2, respectively. In Unguja, the population in the 45 study shehias ranged from 880 in the rural shehia Donge Mnyimbi to 15,000 in the urban shehia Melinne. In 2011, on average, 19 (range: 0–186) people immigrated and 10 (range: 0–50) emigrated per shehia. Rice farming (62.9%), vegetable farming (17.1%), and banana farming (11.4%) were the activities that most shehas attributed as primary occupation related to being exposed to open freshwater bodies in their shehia. Natural freshwater was used as optional drinking, washing, or bathing water in 24.4%, 28.9%, and 28.9% of shehias, respectively. A number of shehas also reported the implementation of new wells (11 shehias), new taps (eight shehias), new polytanks (five shehias), new rainwater tanks (three shehias), new household toilets (40 shehias), and new public toilets (17 shehias) in their shehia in 2011.

In Pemba, between 2,043 (Makombeni) and 12,781 (Msuka) people inhabited a shehia. On average, 16 (range: 0–60) immigrants and nine (range: 0–30) emigrants per shehia were counted by the shehas in 2011. Shehas mentioned rice farming as the predominant agricultural activity involving freshwater contact (88.9%). Natural freshwater was not mentioned as drinking water source in Pemba, but 77.8% and 75.6% of shehas reported the use of natural freshwater for bathing or washing, respectively. In some shehias, new wells (four shehias), new taps (one shehia), new household toilets (42 shehias), and new public toilets (six shehias) were implemented in 2011.

S. haematobium Infection Characteristics, Stratified by Sentinel Group

Microhematuria was detected in the urine of 10.4% and 14.3% of surveyed adults in Unguja and Pemba, respectively (Table 1). The respective *S. haematobium* infection prevalence in this age group was 2.7% and 5.5%. The highest *S. haematobium* prevalence in adults was found in the shehia Koani (26.5%) in Unguja, and in the shehia Uwandani (23.4%) in Pemba (Figure 2).

In Unguja, 3.4% of the children from standard 1 had microhematuria and 5.2% had *S. haematobium* eggs diagnosed in their urine. Microhematuria was detected in 7.3% and *S. haematobium* infections in 3.8% of the school children aged 9–12 years. The highest *S. haematobium* prevalence of 26.8% in standard 1 children was found in the shehia Upenja (Figure 3) and of 20.0% in 9- to 12-year-old children from the shehia Kinyasini (Figure 4).

In Pemba, microhematuria and *S. haematobium* eggs in urine were diagnosed in 14.9% and 12.2% of children attending standard 1 and in 11.1% and 8.1% of school children aged 9–12 years, respectively. The highest prevalence in standard 1 and among 9- to 12-year-old children was observed in the shehias Uwandani (37.0%) and Kizimbani (29.0%), respectively.

As detailed in Table 2, macro- and microhematuria were strongly associated with *S. haematobium* infection in adults and school children, both in Unguja and Pemba. Children and adults with eggs identified in their urine had significantly higher odds of a trace, +, ++, or +++ result indicated by the reagent strip. Combining all data from adults and children and both islands, we found a significant correlation between the number of eggs detected in 10 ml urine and the color grading for macrohematuria (odds ratio (OR): 1.24, 95% confidence interval (CI): 1.07–1.21) and microhematuria (OR: 3.32, 95% CI: 3.17–3.48).

Risk Factors for *S. haematobium* Infection

In Unguja, males (OR: 2.56, 95% CI: 1.50–4.35), people of young adult age (OR: 1.05; 95% CI: 1.02–1.08), Christians (OR: 3.67, 95% CI: 1.41–9.56), those born in mainland Tanzania (OR: 2.51; 95% CI: 1.24–5.08), and those using natural freshwater (OR: 2.09, 95% CI: 1.11–3.94) had an elevated risk of *S. haematobium* infection according to univariable regression analysis. With the exception of religion, these explanatories remained significant also in the adjusted multivariable regression analyses (n = 2,131).

In Pemba, young adult age (OR: 1.03; 95% CI: 1.01–1.05) and the use of natural freshwater (OR: 2.18; 95% CI: 1.46–3.27) were significant risk factors for *S. haematobium* infection in univariable

Table 1. *S. haematobium* infection characteristics stratified by survey group.

Study participants	Infection characteristics	Unguja			Pemba		
		n	n pos	%	n	n pos	%
Children (1st year)	Hematix hematuria	3,430			3,543		
	0		3,313	96.6		3,014	85.1
	Trace		3	0.1		157	4.4
	+		23	0.7		78	2.2
	++		38	1.1		167	4.7
	+++		53	1.5		127	3.6
	S. haematobium eggs	3,364	174	5.2	3,533	432	12.2
	Low infection intensity		142	81.6		258	59.7
	High infection intensity		32	18.4		163	37.7
Children (9–12 years)	Hematix hematuria	4,327			4,017		
	0		4,011	92.7		3,572	88.9
	Trace		11	0.3		134	3.3
	+		98	2.3		105	2.6
	++		94	2.2		110	2.7
	+++		113	2.6		96	2.4
	S. haematobium eggs	4,262	164	3.8	4,004	326	8.1
	Low infection intensity		145	88.4		219	67.2
	High infection intensity		19	11.6		107	32.8
Adults (20–55 years)	Hematix hematuria	2,155			1,864		
	0		1,931	89.6		1,598	85.7
	Trace		50	2.3		84	4.5
	+		55	2.6		86	4.6
	++		52	2.4		67	3.6
	+++		67	3.1		29	1.6
	S. haematobium eggs	2,134	57	2.7	1,861	102	5.5
	Low infection intensity		53	93.0		95	93.1
	High infection intensity		4	7.0		7	6.9

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analyses and remained significant in the adjusted multivariable analyses (n = 1,858).

Intermediate Host Snail Occurrence and Infection

Between November 2011 and December 2012, a total of 46 freshwater bodies were identified in the 15 selected shehias where MDA plus snail control interventions will be conducted in Unguja. The predominant freshwater bodies were ponds (n = 27), followed by streams (n = 19), mostly of permanent nature. In these water bodies, the average conductivity was 364.6 ms (range: 32–1,657 ms), pH was 8.4 (range: 7.2–12.9), temperature was 29.0°C (range: 24.2–37.9°C), and TDS was 154.3 (range: 15–456). *Bulinus* spp. snails were found in 11 streams (57.9%) and 16 ponds (59.3%). None of the water characteristics was associated with the presence of *Bulinus* spp. None of the *B. globosus/nasutus* shed *S. haematobium* cercariae.

In Pemba, between November 2011 and October 2012, there were a large number of waterbodies (n = 146) identified in the 15 study shehias. The average values of the water chemistry were as follows: conductivity: 298.5 ms (range: 24.4–1,064 ms), pH: 7.1 (range: 4.7–9.4), temperature: 29.6°C (range: 23.0–36.7°C), and TDS: 150.9 (range: 11.2–810). *Bulinus* spp. snails were found in 49 water bodies (33.6%) and their presence was significantly associated with temperature (OR: 1.15; 95% CI: 1.03–1.29) in univariable regression analysis. In total, four *B. globosus/nasutus* from three different sites shed *S. haematobium* cercariae.

Community Perceptions of Schistosomiasis

FGDs were conducted with 16 groups of primary and 13 groups of secondary school children (n = 150), and with five groups of community members (n = 47). Additionally, IDIs were conducted with 21 teachers, 16 parents, and 12 community leaders.

Most people we talked with associated *kichocho* with urinating blood because of spending time in a dirty river. Despite this association, only a few students and teachers could describe the transmission cycle associated with a parasite and snail. Standing in someone else's urine when in a latrine, stepping in someone's urine in the bush, witchcraft and hexes, walking in dirt infected with *kichocho* organisms, eating chilies, and sexual intercourse between a man and woman were all described as ways to get *kichocho*.

Symptoms of *kichocho* were most often described as abdominal pain, itching of one's private parts with severe pain during urination and bloody urine. Importantly, most people characterized *kichocho* as a boy's disease rather than a girl's disease. Even women and girls believed it to be a disease of boys. One female community member reported, "*I don't know which symptoms are for*

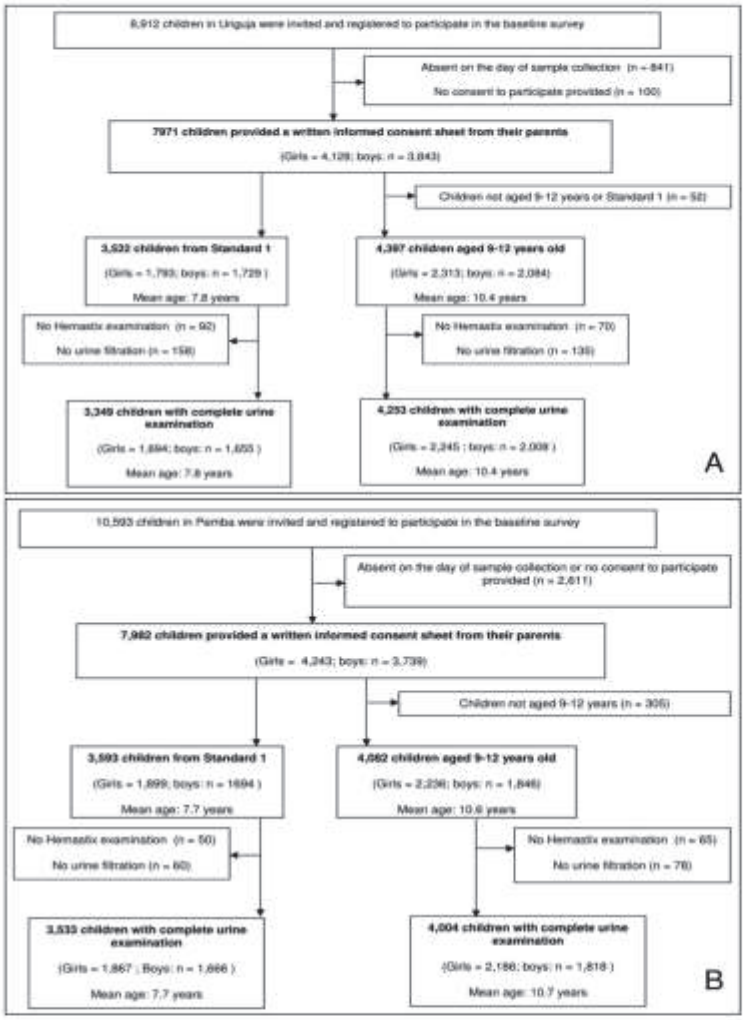

Figure 1. Flowchart detailing study participation in the schools surveyed in Unguja (A) and Pemba (B) in January till March, 2012.
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male and which symptoms are for female. I think it is only a disease of boys." Parents knew the least about the transmission of *kichocho*.

People reported that some people self-treated with plant-based teas (often the root of a plant), by drinking lots of water to flush the system, or fail to seek treatment because of anticipated costs. A teacher told us, *"There are some kind of roots made into teas which are used by people (…).* Kichocho *can be treated by these roots."* Some people told us *kichocho* treatment was free and some described paying for treatment. Even if treatment was free, the cost of transportation was reported as a barrier to seeking care. People also described negative interactions with hospital staff and the lack of available drugs when arriving for treatment at their local health care facility as barriers to seeking care.

People reported that children urinating in the river was a practice contributing to the risk of *kichocho* transmission. Boys were identified as engaging in the riskiest behaviors for acquiring

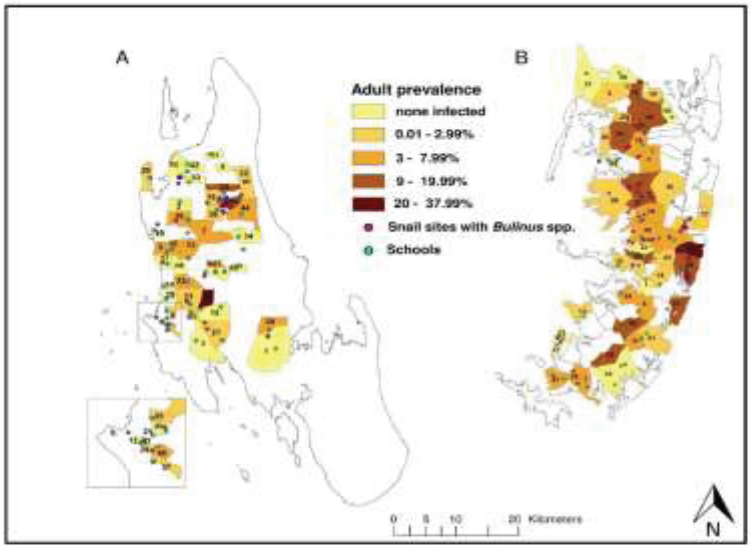

Figure 2. *S. haematobium* prevalence in adults in 45 study shehias in Unguja (A) and Pemba (B). Map indicating the *S. haematobium* prevalence in the adult population of the 45 study shehias in Unguja (A) and Pemba (B) at the baseline survey conducted in November/December 2011, surveyed schools in all 45 study shehias per island and sites in the 15 snail control shehias per island, where *Bulinus* spp. snails were found. Shehias in Unguja and their assigned intervention: Biannual mass drug administration with praziquantel: 1 = Cheju, 2 = Donge Mnyimbi, 3 = Fuoni Kibondeni, 4 = Gamba, 5 = Kama, 6 = Kiboje Mkwajuni, 7 = Kitope, 8 = Mbuzini, 9 = Mchangani, 10 = Mfenesini, 11 = Mkwajuni, 12 = Muungano, 13 = Mwakaje, 14 = Mwanyanya, 15 = Ubago; biannual mass drug administration with praziquantel plus snail control: 16 = Bandamaji, 17 = Chuini, 18 = Donge Mchangani, 19 = Fujoni, 20 = Jendele, 21 = Jumbi, 22 = Kandwi, 23 = Kianga, 24 = Kilimahewa Juu, 25 = Kinyasini, 26 = Mafufuni, 27 = Miwani, 28 = Mtopepo, 29 = Nyerere, 30 = Welezo; biannual mass drug administration with praziquantel plus behaviour change interventions: 31 = Chaani Kubwa, 32 = Dole, 33 = Donge Mtambile, 34 = Kilombero, 35 = Koani, 36 = Mahonda, 37 = Melinne, 38 = Mgambo, 39 = Mtoni, 40 = Mwanakwerekwe, 41 = Mwera, 42 = Pale, 43 = Sebleni, 44 = Upenja, 45 = Uzini. Shehias in Pemba and their assigned intervention: Biannual mass drug administration with praziquantel: 1 = Chanjaani, 2 = Kangani, 3 = Kiwani, 4 = Konde, 5 = Matale, 6 = Ole, 7 = Pandani, 8 = Selemu, 9 = Tibirinzi, 10 = Tumbe, 11 = Ukutini, 12 = Uwandani, 13 = Wambaa, 14 = Wawi, 15 = Wesha; Biannual mass drug administration with praziquantel plus snail control: 16 = Finya,17 = Kangagani, 18 = Kinowe, 19 = Kisiwani, 20 = Kwale, 21 = Makangale, 22 = Makombeni, 23 = Mbuzini, 24 = Mgogoni, 25 = Mkanyageni, 26 = Msuka, 27 = Piki, 28 = Shumba Viamboni, 29 = Vitongoji, 30 = Ziwani; biannual mass drug administration with praziquantel plus behaviour change interventions: 31 = Chambani, 32 = Jadida, 33 = Kengeja, 34 = Kinyasini, 35 = Kizimbani, 36 = Mchangamdogo, 37 = Michenzani, 38 = Mtambile, 39 = Mtambwe Kusini, 40 = Ng'ambwa, 41 = Ng'ombeni, 42 = Ngwachani, 43 = Pujini, 44 = Shungi, 45 = Sizini.
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kichocho – playing in the river, fishing, and swimming. Young girls were considered at greater risk than older girls for getting *kichocho* because they often played in the river as they had not reached the stage of modesty associated with their culture. People also identified washing clothes in the river as a major risk behavior for both, boys and girls.

When asking the participants for their ideas for preventing children from urinating in the river and spreading schistosomiasis, many people suggested fear and punishment to change urination behaviors and prevent children from going to the river, while at the same time admitting that these methods rarely work. Some people also expressed the need for the community to work together against the disease. A few people reported that it was the role of the government to handle the problem. We were told, "*The best option is give education to our children. But we have nothing in the schools to teach. We need teaching materials and curricula.*" Most people described some ideas for educational, behavioral, or structural interventions to prevent *kichocho* in children.

Discussion

Elimination of schistosomiasis is now being considered in different parts of the world, including Brazil, the WHO Western Pacific Region, and several countries of the African Region [4]. After careful consideration, SCORE has selected the Zanzibar archipelago to evaluate what intervention combinations are needed to eliminate urogenital schistosomiasis. Lessons from this program will be documented so that elimination programs elsewhere in Africa can benefit. We presented the results from baseline surveys carried out at the onset of a 5-year randomized multi-faceted intervention trial. Hence, the data presented here can serve as a benchmark for monitoring progress as the program unfolds.

In Unguja, we found an overall *S. haematobium* prevalence below 3% in adults and below 5% in school children. Heavy infection intensities in egg-positive school children were rare (15%). In Pemba, the overall prevalence of *S. haematobium* was considerably higher than in Unguja: 6% in adults and 10% in school children,

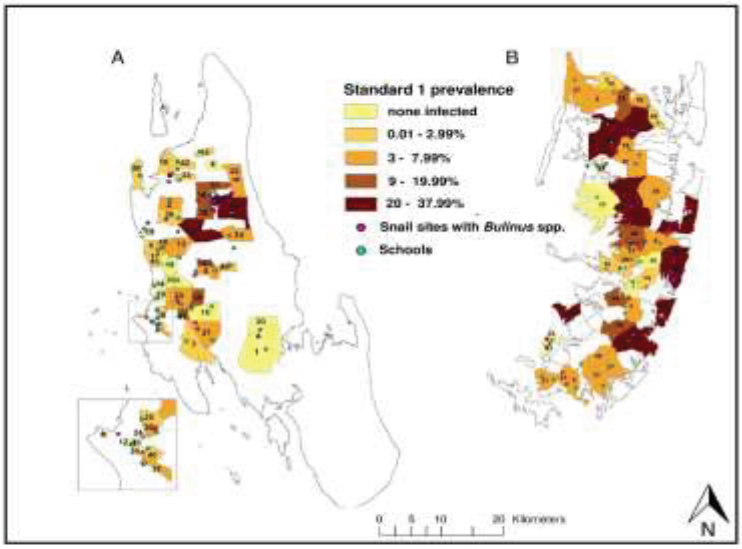

Figure 3. *S. haematobium* prevalence in first-year school children in 45 study shehias in Unguja (A) and Pemba (B). Map indicating the *S. haematobium* prevalence in first-year school children in the 45 study shehias in Unguja (A) and Pemba (B) at the baseline survey conducted in January-March 2012, surveyed schools in all 45 study shehias per island and sites in the 15 snail control shehias per island, where *Bulinus* spp. snails were found. Shehias in Unguja and their assigned intervention: Biannual mass drug administration with praziquantel: 1 = Cheju, 2 = Donge Mnyimbi, 3 = Fuoni Kibondeni, 4 = Gamba, 5 = Kama, 6 = Kiboje Mkwajuni, 7 = Kitope, 8 = Mbuzini, 9 = Mchangani, 10 = Mfenesini, 11 = Mkwajuni, 12 = Muungano, 13 = Mwakaje, 14 = Mwanyanya, 15 = Ubago; biannual mass drug administration with praziquantel plus snail control: 16 = Bandamaji, 17 = Chuini, 18 = Donge Mchangani, 19 = Fujoni, 20 = Jendele, 21 = Jumbi, 22 = Kandwi, 23 = Kianga, 24 = Kilimahewa Juu, 25 = Kinyasini, 26 = Mafufuni, 27 = Miwani, 28 = Mtopepo, 29 = Nyerere, 30 = Welezo; biannual mass drug administration with praziquantel plus behaviour change interventions: 31 = Chaani Kubwa, 32 = Dole, 33 = Donge Mtambile, 34 = Kilombero, 35 = Koani, 36 = Mahonda, 37 = Melinne, 38 = Mgambo, 39 = Mtoni, 40 = Mwanakwerekwe, 41 = Mwera, 42 = Pale, 43 = Sebleni, 44 = Uperja, 45 = Uzini. Shehias in Pemba and their assigned intervention: Biannual mass drug administration with praziquantel: 1 = Chanjaani, 2 = Kangani, 3 = Kiwani, 4 = Konde, 5 = Matale, 6 = Ole, 7 = Pandani, 8 = Selemu, 9 = Tibirinzi, 10 = Tumbe, 11 = Ukutini, 12 = Uwandani, 13 = Wambaa, 14 = Wawi, 15 = Wesha; Biannual mass drug administration with praziquantel plus snail control: 16 = Finya,17 = Kangagani, 18 = Kinowe, 19 = Kisiwani, 20 = Kwale, 21 = Makangale, 22 = Makombeni, 23 = Mbuzini, 24 = Mgogoni, 25 = Mkanyageni, 26 = Msuka, 27 = Piki, 28 = Shumba Viamboni, 29 = Vitongoji, 30 = Ziwani; biannual mass drug administration with praziquantel plus behaviour change interventions: 31 = Chambani, 32 = Jadida, 33 = Kengeja, 34 = Kinyasini, 35 = Kizimbani, 36 = Mchangamdogo, 37 = Michenzani, 38 = Mtambile, 39 = Mtambwe Kusini, 40 = Ng'ambwa, 41 = Ng'ombeni, 42 = Ngwachani, 43 = Pujini, 44 = Shungi, 45 = Sizini.
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with heavy infections detected in 36% of egg-positive school children. Our data mean that the study objective of "controlling schistosomiasis throughout Pemba island (prevalence <10%) in 3 years" is achieved at this point in time. However, we found considerable heterogeneity. Indeed, we identified a number of "hot-spot" communities on both islands, where the prevalence of *S. haematobium* was above 20% in adults and school children. Population groups with higher odds for *S. haematobium* infections were, besides school children, adult males, younger adults, people born in Pemba or mainland Tanzania, and individuals using natural freshwater.

The existence of some of the hot-spots for schistosomiasis transmission on both, Unguja and Pemba islands is known from previous studies [31,32] and it seems they have been resilient to the preventive chemotherapy campaigns over the past years, maintaining high prevalences and infection intensities. It will therefore be important to intensify control interventions particularly in these communities in future years to reduce significantly and to interrupt transmission. Future surveillance of the recrudescence of the disease will need to pay special attention to ex-hotspots. It is also widely acknowledged that school boys or adolescents, people using natural freshwater, and those pursuing specific occupations that expose them to open freshwater bodies (e.g., rice farmers) are at an elevated risk of *S. haematobium* infection [16,33–36]. A new and interesting finding of our study is that adult immigrants from mainland Tanzania showed higher odds of *S. haematobium* infection than their counterparts from Zanzibar. Reasons might be arriving from a highly endemic area of schistosomiasis, a different behavior, or a lack of acquired immunity and increased susceptibility to *S. haematobium* infection. For example, immigrants might not have the same exposure history as the local population and could therefore not develop the same level of resistance. A similar observation was made in lifelong residents and residential newcomers in Kenya [37]. It might also be that as *S. haematobium* species are more genetically diverse in Zanzibar than in mainland Africa [38], immigrants might be exposed to *S. haematobium* genotypes to which they are more susceptible. Noteworthy, a study on the neighboring Mafia Island

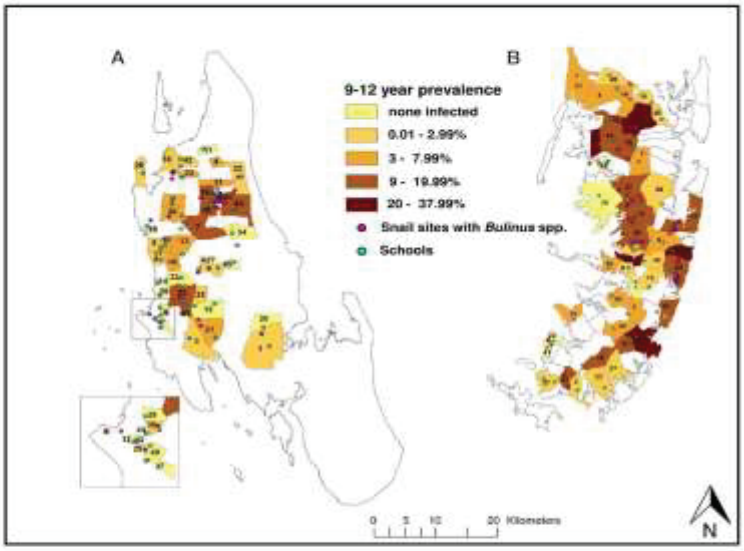

Figure 4. *S. haematobium* prevalence in 9–12-year-old school children in 45 study shehias in Unguja (A) and Pemba (B). Map indicating the *S. haematobium* prevalence in 9- to 12-year school children in the 45 study shehias in Unguja (A) and Pemba (B) at the baseline survey conducted in January-March 2012, surveyed schools in all 45 study shehias per island and sites in the 15 snail control shehias per island, where *Bulinus* spp. snails were found. Shehias in Unguja and their assigned intervention: Biannual mass drug administration with praziquantel: 1 = Cheju, 2 = Donge Mnyimbi, 3 = Fuoni Kibondeni, 4 = Gamba, 5 = Kama, 6 = Kiboje Mkwajuni, 7 = Kitope, 8 = Mbuzini, 9 = Mchangani, 10 = Mfenesini, 11 = Mkwajuni, 12 = Muungano, 13 = Mwakaje, 14 = Mwanyanya, 15 = Ubago; biannual mass drug administration with praziquantel plus snail control: 16 = Bandamaji, 17 = Chuini, 18 = Donge Mchanganí, 19 = Fujoni, 20 = Jendele, 21 = Jumbi, 22 = Kandwi, 23 = Kianga, 24 = Kilimahewa Juu, 25 = Kinyasini, 26 = Mafufuni, 27 = Miwani, 28 = Mtopepo, 29 = Nyerere, 30 = Welezo; biannual mass drug administration with praziquantel plus behaviour change interventions: 31 = Chaani Kubwa, 32 = Dole, 33 = Donge Mtambile, 34 = Kilombero, 35 = Koani, 36 = Mahonda, 37 = Melinne, 38 = Mgambo, 39 = Mtoni, 40 = Mwanakwerekwe, 41 = Mwera, 42 = Pale, 43 = Sebleni, 44 = Upenja, 45 = Uzini. Shehias in Pemba and their assigned intervention: Biannual mass drug administration with praziquantel: 1 = Chanjaani, 2 = Kangani, 3 = Kiwani, 4 = Konde, 5 = Matale, 6 = Ole, 7 = Pandani, 8 = Selemu, 9 = Tibirinzi, 10 = Tumbe, 11 = Ukutini, 12 = Uwandani, 13 = Wambaa, 14 = Wawi, 15 = Wesha; Biannual mass drug administration with praziquantel plus snail control: 16 = Finya,17 = Kangagani, 18 = Kinowe, 19 = Kisiwani, 20 = Kwale, 21 = Makangale, 22 = Makombeni, 23 = Mbuzini, 24 = Mgogoni, 25 = Mkanyageni, 26 = Msuka, 27 = Piki, 28 = Shumba Viamboni, 29 = Vitongoji, 30 = Ziwani; biannual mass drug administration with praziquantel plus behaviour change interventions: 31 = Chambani, 32 = Jadida, 33 = Kengeja, 34 = Kinyasini, 35 = Kizimbani, 36 = Mchangamdogo, 37 = Michenzani, 38 = Mtambile, 39 = Mtambwe Kusini, 40 = Ng'ambwa, 41 = Ng'ombeni, 42 = Ngwachani, 43 = Pujini, 44 = Shungi, 45 = Sizini.
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found that all children with a *S. haematobium* infection reported a travel history to mainland Tanzania [39]. Since there is no evidence of active *S. haematobium* transmission on Mafia Island, these cases were almost certainly imported. Therefore, in future surveys, it will be important to determine the influence of migration on the study outcomes, and special attention will be needed on the monitoring of immigrants and potentially imported *S. haematobium* infections from mainland Africa, as well as between Pemba and Unguja.

Prevalence of *S. haematobium* in Pemba has consistently been reported higher than in Unguja [40,41], most likely due to social-ecological contexts. From our questionnaire surveys, we found that less shehas in Pemba than in Unguja reported the establishment of new clean water sources and latrines in the respective shehia in 2011. In Pemba more people (>75%) than in Unguja (<30%) reported the use of natural freshwater for washing or bathing. This might be due to the fact that Pemba has an undulating landscape with many creeks and streams available in close proximity to houses, while Unguja has a relatively flat terrain with a comparatively low number of streams [42], but also due to the lower availability of artificial clean water sources in Pemba due to its lower economic development [43,44]. The monitoring of improvements in the water and sanitation infrastructure and the use of clean water and latrines by the people will be essential to adjust correctly our future analyses on the impact of our interventions on *S. haematobium* prevalence and intensity for confounders.

Encouraging for our aim to interrupt schistosomiasis transmission in Unguja over the next 5 years is that none of the snails in Unguja and only very few of the collected *B. globosus/nasutus* snails in Pemba shed *S. haematobium* cercariae. This observation is in contrast to results obtained in a previous study, where patent *S. haematobium* infections were detected, on average, in 4% of the *B. globosus/nasutus* snails collected in Unguja [25]. However, the season and areas where snails were collected differed, and hence data cannot be readily compared. Whether the prepatent infection level in the snails collected in the present survey between November 2011 and December 2012 is higher

Table 2. Association between *S. haematobium* infection and macro- and microhematuria.

Study participants	Infection characteristics	Variable	Unguja			Pemba		
			n	OR	95% CI	n	OR	95% CI
Adults (20–55 years)	Visible hematuria	continuous	2,133	1.8	1.3–2.5	1,860	1.3	1.01–1.6
	Hemastix hematuria	Trace	2,133	8.4	3.1–22.9	1,860	10.8	5.7–20.5
		+		2.8	0.7–12.3		10.5	5.5–19.9
		++		15.8	7.0–35.8		21.0	11.3–38.9
		+++		25.6	11.9–47.0		49.2	22.0–109.8
Children (9–12 years)	Visible hematuria	continuous	4,253	1.8	1.5–2.3	4,004	2.0	1.7–2.3
	Hemastix hematuria	Trace	4,242	NA	NA	4,004	126.9	77.4–208.1
		+		6.8	3.7–12.4		245.8	142.6–423.6
		++		7.6	4.2–13.6		223	130.9–379.8
		+++		18.8	12.1–29.4		898.8	435.8–1853.8
Children (1st year)	Visible haematuria	continuous	3349	1.1	0.111	3533	2.2	<0.001
	Hemastix haematuria	trace	3349	12.7	0.039	3533	438.9	<0.001
		1		5.6	<0.001		624.5	<0.001
		2		21.5	<0.001		1247.7	<0.001
		3		29.5	<0.001		3016.6	<0.001

CI: confidence interval.
OR = odds ratio.
NA = not applicable.
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than the patent observations, remains to be elucidated with molecular methods using a *Dra*I repeat polymerase chain reaction (PCR) approach [25,45,46]. The association of *Bulinus* spp. snail presence with temperature in our study in Pemba and with velocity in a previous study [25], highlights the preferences of the snails for specific climatic conditions and their fluctuation in dependence of the rainy season [47,48]. Given a higher velocity and colder temperature of freshwater in or shortly after the rainy season, we suggest that molluscicides should be applied after the rains have ceased, but before the non-permanent water bodies start to dry out. Snail control will be best harmonized with MDA and ideally be implemented before the population is treated with praziquantel to minimize the risk of rapid re-infection [49].

The qualitative research using FGDs and IDIs showed that, despite Unguja and Pemba having a history of more than 20 years of schistosomiasis control using preventive chemotherapy and, to some extent, health education booklets [50–52], the communities' knowledge of disease transmission is only rudimentary. This finding is in line with studies conducted elsewhere in Africa, where poor knowledge on the causes of schistosomiasis was observed [53–55]. The behaviors that emerged from our formative research as most important to change in order to reduce schistosomiasis transmission in Zanzibar can be summarized as follows: (i) children urinating in streams and ponds and (ii) children playing, swimming, and washing laundry in the same streams and ponds. Involving community members in the design and implementation of behavioural change interventions will result in a human-centered design intervention tailored to the cultural and social norms of the community with an increase in the likelihood of adoption of the desired protective and preventive behaviors. Participatory hygiene and sanitation transformation (PHAST) interventions already succeeded in increasing knowledge of communities, and transformed into active prevention of schistosomiasis transmission elsewhere in Tanzania [56].

Our finding that macro- and microhematuria were strongly associated with *S. haematobium* infections in adults and school children on both islands is in line with many previous reports from Zanzibar and elsewhere, where the urine examination with reagent strips is suggested as rapid diagnostic tool to identify high-risk areas and to monitor the impact of MDA with praziquantel [57–59]. Despite low prevalence and infection intensities found in our baseline survey, the number of egg counts correlated with the color grading of macrohematuria and microhematuria charts. Hence, both macrohematuria and microhematuria are still valid indicators for the detection of true *S. haematobium* cases, even in settings with a long-term history of consistent preventive chemotherapy as found with Zanzibar, and might be used not only for monitoring the impact of our interventions in Zanzibar, but also for future surveillance and response to avoid the recrudescence or reintroduction of the disease. The overall prevalence (9%) of microhematuria particularly in urines from adults implies that there is still considerable morbidity due to schistosomiasis. Hence, when approaching elimination of schistosomiasis transmission, we must not forget that urogenital schistosomiasis is a chronic debilitating disease that affects the urinary and genital tracts of many people and it may continue to impact on public health after the interruption of transmission [35,60–63].

The observation of a higher *S. haematobium* prevalence in first years students compared to 9- to 12-year-old children that attended standards 3 and 4 most likely reflects the impact of praziquantel treatment administered over the past years to school children, but not to pre-school aged children, in school-based treatment programs. Preventive chemotherapy campaigns, and likely also improvements in the sanitary infrastructure, have reduced schistosomiasis prevalences from very high levels (>50%) in the 1980s to today's low level [18,40,52,64].

The low prevalence and intensities of *S. haematobium* infection detected on the Zanzibar islands support our aim to achieve elimination of urogenital schistosomiasis. We must be aware, however, that in addition to the application of MDA, snail control,

and behavior change interventions to communities, there will be a need for specifically tailored and sustained control measures to target hot-spot areas, high-risk groups, and individuals with acute and chronic schistosomiasis to achieve and sustain elimination [10–12,65]. Regular assessment of the efficacy of praziquantel and niclosamide will be essential to detect potential resistance development in humans and snails. Finally, to enhance an effective surveillance-response platform, rigorous monitoring, reporting and management of new cases in all health facilities will be essential to avoid a reintroduction of the disease by immigrants and travelers. It is evident, that the human and financial resources needed to eliminate a disease, including schistosomiasis, are considerable. Funds to provide multiple integrated intervention techniques to seriously address schistosomiasis are yet out of reach for most endemic countries. Therefore, it will be important that the governments, institutions, organizations and people from endemic and wealthy countries take responsibility and combine their forces and resources to jointly tackle the last mile towards elimination.

Supporting Information

Supporting Information S1 Translation of abstract into language German by author Stefanie Knopp.
(DOC)

Supporting Information S2 Study protocol published in BMC Public Health.
(PDF)

Supporting Information S3 STROBE table checklist.
(DOC)

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Author Contributions

Conceived and designed the experiments: SK BP SMAm KAM SMAl ISK FA JU AF DR. Performed the experiments: SK BP SMAm KAM SMAl ISK MR FA AG LB DR. Analyzed the data: SK MR. Contributed reagents/materials/analysis tools: SK BP SMAm KAM SMAl ISK LB AF DR. Wrote the paper: SK BP SMAm KAM SMAl ISK MR FA AG LB AF JU DR.

References

1. Murray CJL, Vos T, Lozano R, Naghavi M, Flaxman AD, et al. (2012) Disability-adjusted life years (DALYs) for 291 diseases and injuries in 21 regions, 1990–2010: a systematic analysis for the Global Burden of Disease Study 2010. Lancet 380: 2197–2223.
2. Steinmann P, Keiser J, Bos R, Tanner M, Utzinger J (2006) Schistosomiasis and water resources development: systematic review, meta-analysis, and estimates of people at risk. Lancet Infect Dis 6: 411–425.
3. Utzinger J, Becker SL, Knopp S, Blum J, Neumayr AL, et al. (2012) Neglected tropical diseases: diagnosis, clinical management, treatment and control. Swiss Med Wkly 142: w13727.
4. WHO (2012) Accelerating work to overcome the global impact of neglected tropical diseases – a roadmap for implementation. Geneva: World Health Organization. 42 p.
5. WHO (2012) The London Declaration. Uniting to combat neglected tropical diseases. Ending the Neglect & Reaching 2020 Goals. Table of commitments. Available: http://www.who.int/neglected_diseases/NTD_London_Event_Table_Commitments.pdf. Accessed 17 July 2013.
6. WHO (2012) WHA65.21. Elimination of schistosomiasis. Sixty-fifth World Health Assembly Geneva 21–26 May 2012 Resolutions, decisions and annexes. Geneva: World Health Organization. pp. 36–37.
7. Fenwick A (2006) New initiatives against Africa's worms. Trans R Soc Trop Med Hyg 100: 200–207.
8. Hotez PJ, Engels D, Fenwick A, Savioli L (2010) Africa is desperate for praziquantel. Lancet 376: 496–498.
9. Fenwick A, Webster JP, Bosque-Oliva E, Blair L, Fleming FM, et al. (2009) The Schistosomiasis Control Initiative (SCI): rationale, development and implementation from 2002–2008. Parasitology 136: 1719–1730.
10. Utzinger J, Raso G, Brooker S, de Savigny D, Tanner M, et al. (2009) Schistosomiasis and neglected tropical diseases: towards integrated and sustainable control and a word of caution. Parasitology 136: 1859–1874.
11. Utzinger J, Bergquist R, Xiao SH, Singer BH, Tanner M (2003) Sustainable schistosomiasis control–the way forward. Lancet 362: 1932–1934.
12. Rollinson D, Knopp S, Levitz S, Stothard JR, Tchuente LA, et al. (2013) Time to set the agenda for schistosomiasis elimination. Acta Trop doi:10.1016/j.actatropica.2012.04.013.
13. Gray DJ, McManus DP, Li Y, Williams GM, Bergquist R, et al. (2010) Schistosomiasis elimination: lessons from the past guide the future. Lancet Infect Dis 10: 733–736.
14. Singer BH, Castro MC (2007) Bridges to sustainable tropical health. Proc Natl Acad Sci USA 104: 16038–16043.
15. Spiegel JM, Dharamsi S, Waaan KM, Yassi A, Singer B, et al. (2010) Which new approaches to tackling neglected tropical diseases show promise? PLoS Med 7: e1000255.
16. Gryseels B, Polman K, Clerinx J, Kestens L (2006) Human schistosomiasis. Lancet 368: 1106–1118.
17. Freeman MC, Ogden S, Jacobson J, Abbott D, Addiss D, et al. (2013) Integration of water, sanitation, and hygiene for the prevention and control of neglected tropical diseases: a rationale for inter-sectoral collaboration. PLoS Negl Trop Dis 7: e2439.
18. Knopp S, Stothard JR, Rollinson D, Mohammed KA, Khamis IS, et al. (2013) From morbidity control to transmission control: time to change tactics against helminths on Unguja Island, Zanzibar. Acta Trop doi:10.1016/j.actatropica.2011.04.010.
19. Knopp S, Mohammed KA, Ali SM, Khamis IS, Amr SM, et al. (2012) Study and implementation of urogenital schistosomiasis elimination in Zanzibar (Unguja and Pemba islands) using an integrated multidisciplinary approach. BMC Public Health 12: 930.
20. Lustigman S, Prichard RK, Gazzinelli A, Grant WN, Boatin BA, et al. (2012) A research agenda for helminth diseases of humans: the problem of helminthiases. PLoS Negl Trop Dis 6: e1582.
21. Rollinson D, Stothard JR, Southgate VR (2001) Interactions between intermediate snail hosts of the genus *Bulinus* and schistosomes of the *Schistosoma haematobium* group. Parasitology 123 Suppl: S245–260.
22. Stothard JR, Loxton NJ, Rollinson D (2002) Freshwater snails on Mafia Island, Tanzania with special emphasis on the genus *Bulinus* (Gastropoda: Planorbidae). J Zool Lond 257: 353–364.
23. Stothard JR, Rollinson D (1997) Molecular characterization of *Bulinus globosus* and *B. senutus* on Zanzibar, and an investigation of their roles in the epidemiology of *Schistosoma haematobium*. Trans R Soc Trop Med Hyg 91: 353–357.
24. Jordan P, Webbe G, Sturrock RF (1993) Human schistosomiasis. Wallingford, Oxfordshire: CAB International. 212 p.
25. Allan F, Dunn AM, Emery AM, Stothard JR, Johnston DA, et al. (2013) Use of sentinel snails for the detection of *Schistosoma haematobium* transmission on Zanzibar and observations on transmission patterns. Acta Trop doi:10.1016/j.actatropica.2013.03.003.
26. Gower CM, Shrivastava J, Lamberton PH, Rollinson D, Webster BL, et al. (2007) Development and application of an ethically and epidemiologically advantageous assay for the multi-locus microsatellite analysis of *Schistosoma mansoni*. Parasitology 134: 523–536.
27. IDEO (2008) IDEO human centered design toolkit fieldguide. Available: http://www.hcdconnect.org/methods. Accessed 17 July 2013.
28. Rollinson D, Klinger EV, Mgeni AF, Khamis IS, Stothard JR (2005) Urinary schistosomiasis on Zanzibar: application of two novel assays for the detection of excreted albumin and haemoglobin in urine. J Helminthol 79: 199–206.
29. Stothard JR, Sousa-Figueiredo JC, Standley C, Van Dam GJ, Knopp S, et al. (2009) An evaluation of urine-CCA strip test and fingerprick blood SEA-ELISA for detection of urinary schistosomiasis in schoolchildren in Zanzibar. Acta Trop 111: 64–70.
30. Montresor A, Crompton DWT, Hall A, Bundy DAP, Savioli L (1998) Guidelines for the evaluation of soil-transmitted helminthiasis and schistosomiasis at community level. Geneva/Switzerland: World Health Organization. 48 p.
31. Savioli L, Mott KE (1989) Urinary schistosomiasis on Pemba island: low-cost diagnosis for control in a primary health care setting. Parasitol Today 5: 333–337.

32. Stothard JR, Mgeni AF, Khamis S, Seto E, Rollinson D, et al. (2002) Urinary schistosomiasis in schoolchildren on Zanzibar island (Unguja), Tanzania: a parasitological survey supplemented with questionnaires. Trans R Soc Trop Med Hyg 96: 507–514.
33. Rudge JW, Stothard JR, Basáñez MG, Mgeni AF, Khamis IS, et al. (2008) Micro-epidemiology of urinary schistosomiasis in Zanzibar: local risk factors associated with distribution of infections among schoolchildren and relevance for control. Acta Trop 105: 45–54.
34. Kosinski KC, Adjei MN, Boscoppein KM, Crocker JJ, Durant JL, et al. (2012) Effective control of *Schistosoma haematobium* infection in a Ghanaian community following installation of a water recreation area. PLoS Negl Trop Dis 6: e1709.
35. Mazigo HD, Nuwaha F, Kinung'hi SM, Morona D, Pinot de Moira A, et al. (2012) Epidemiology and control of human schistosomiasis in Tanzania. Parasit Vectors 5: 274.
36. WHO (2012) Meeting of the International Task Force for Disease Eradication, April 2012. Wkly Epidemiol Rec 33: 305–316.
37. Black CL, Muchiri PN, Muok EM, Abudho B, Fitzpatrick CM, et al. (2010) Influence of exposure history on the immunology and development of resistance to human schistosomiasis mansoni. PLoS Negl Trop Dis 4: e637.
38. Webster BL, Emery AM, Webster JP, Gouvras A, Garba A, et al. (2012) Genetic diversity within *Schistosoma haematobium*: DNA barcoding reveals two distinct groups. PLoS Negl Trop Dis 6: e1852.
39. Stothard JR, Ameri H, Khamis IS, Blair L, Nyandindi US, et al. (2012) Parasitological and malacological surveys reveal urogenital schistosomiasis on Mafia Island, Tanzania to be an imported infection. Acta Trop doi: 10.1016/j.actatropica.2012.09.006.
40. Mgeni AF, Kinunka UM, McCullough FS, Dixon H, Yoon SS, et al. (1990) Metrifonate in the control of urinary schistosomiasis in Zanzibar. Bull World Health Organ 68: 721–730.
41. Savioli L, Hatz C, Dixon H, Kinunka UM, Mott KE (1990) Control of morbidity due to *Schistosoma haematobium* on Pemba Island: egg excretion and haematuria as indicators of infection. Am J Trop Med Hyg 43: 289–295.
42. WCS (2011) Protected area spatial planning for Unguja and Pemba islands, Zanzibar. New York: Wildlife Conservation Society. 41 p.
43. MFEA (2009) Zanzibar human development report 2009. Zanzibar Town: Ministry of Finance and Economic Affairs. 156 p.
44. OCGS (2010) Zanzibar statistical abstract 2010. Zanzibar Town: Office of Chief Government Statistician. 166 p.
45. Hamburger J, Hoffman O, Kariuki HC, Muchiri EM, Ouma JH, et al. (2004) Large-scale, polymerase chain reaction-based surveillance of *Schistosoma haematobium* DNA in snails from transmission sites in coastal Kenya: a new tool for studying the dynamics of snail infection. Am J Trop Med Hyg 71: 765–773.
46. Abbasi I, King CH, Muchiri EM, Hamburger J (2010) Detection of *Schistosoma mansoni* and *Schistosoma haematobium* DNA by loop-mediated isothermal amplification: identification of infected snails from early prepatency. Am J Trop Med Hyg 83: 427–432.
47. Sturrock RF (1993) The intermediate host and host-parasite relationships. In: Jordan P, Webbe G, Sturrock R., editors. Human schistosomiasis. Wallingford: CAB International. pp. 33–85.
48. Appleton CC (1978) Review of literature on biotic factors that influence the distribution and life cycles of bilharziasis intermediate host snails. Malacol Rev 11: 1–25.
49. Sturrock RF (1993) Current concepts of snail control. Mem Inst Oswaldo Cruz 88: 241–248.
50. Savioli L, Dixon H, Kinunka UM, Mott KE (1989) Control of morbidity due to *Schistosoma haematobium* on Pemba Island: programme organization and management. Trop Med Parasitol 40: 189–194.
51. Stothard JR, Mook P, Mgeni AF, Khamis IS, Khamis AN, et al. (2006) Control of urinary schistosomiasis on Zanzibar (Unguja island): a pilot evaluation of the educational impact of the *Jaza na Kichacha* health booklet within primary schools. Mem Inst Oswaldo Cruz 101 (Suppl. 1): 119–124.
52. Stothard JR, Fenwick MD, Khamis IS, Basáñez MG, Rollinson D (2009) The epidemiology and control of urinary schistosomiasis and soil-transmitted helminthiasis in schoolchildren on Unguja island, Zanzibar. Trans R Soc Trop Med Hyg 103: 1031–1044.
53. Mwanga JR, Magnussen P, Mugashe CL, Gabone RM, Aagaard-Hansen J (2004) Schistosomiasis-related perceptions, attitudes and treatment-seeking practices in Magu district, Tanzania: public health implications. J Biosoc Sci 36: 63–81.
54. Mazigo HD, Waihenya R, Mhoji GM, Zinga M, Ambrose EE, et al. (2010) Intestinal schistosomiasis: prevalence, knowledge, attitude and practices among school children in an endemic area of north western Tanzania. J Rural Trop Public Health 9: 53–60.
55. Acka CA, Raso G, N'Goran EK, Tschannen AB, Bogoch I, et al. (2010) Parasitic worms: knowledge, attitudes, and practices in western Côte d'Ivoire with implications for integrated control. PLoS Negl Trop Dis 4: e910.
56. Mwanga JR, Lwambo NJS (2013) Pre- and post-intervention perceptions and water contact behaviour related to schistosomiasis in north-western Tanzania. Acta Trop doi:10.1016/j.actatropica.2012.09.017.
57. Savioli L, Dixon H, Kinunka UM, Mott KE (1989) Control of morbidity due to *Schistosoma haematobium* on Pemba island: selective population chemotherapy of schoolchildren with haematuria to identify high-risk localities. Trans R Soc Trop Med Hyg 83: 803–810.
58. Taylor P, Chandiwana SK, Matanhire D (1990) Evaluation of the reagent strip test for haematuria in the control of *Schistosoma haematobium* infection in schoolchildren. Acta Trop 47: 91–100.
59. Emukah E, Gutman J, Eganjie J, Miri ES, Yinkore P, et al. (2012) Urine heme dipsticks are useful in monitoring the impact of praziquantel treatment on *Schistosoma haematobium* in sentinel communities of Delta state, Nigeria. Acta Trop 122: 126–131.
60. Kjetland EF, Ndhlovu PD, Mduluza T, Gomo E, Gwanzura L, et al. (2005) Simple clinical manifestations of genital *Schistosoma haematobium* infection in rural Zimbabwean women. Am J Trop Med Hyg 72: 311–319.
61. Lyons B, Stothard R, Rollinson D, Khamis S, Simai KA, et al. (2009) A comparison of urinary tract pathology and morbidity in adult populations from endemic and non-endemic zones for urinary schistosomiasis on Unguja Island, Zanzibar. BMC Infect Dis 9: 189.
62. Mbabazi PS, Andan O, Fitzgerald DW, Chitsulo L, Engels D, et al. (2012) Examining the relationship between urogenital schistosomiasis and HIV infection. PLoS Negl Trop Dis 5: e1196.
63. Gilbody M, Bergquist NR (2008) Post-transmission schistosomiasis: a new agenda. Acta Trop 77: 3–7.
64. McCullough FS, Krafft JG (1976) Schistosomiasis in Zanzibar and Pemba. Report on a mission 1 April–7 June 1975. Geneva: World Health Organization.
65. King CH (2009) Toward the elimination of schistosomiasis. N Engl J Med 360: 106–109.

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Study and implementation of urogenital schistosomiasis elimination in Zanzibar (Unguja and Pemba islands) using an integrated multidisciplinary approach

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Abstract

Background: Schistosomiasis is a parasitic infection that continues to be a major public health problem in many developing countries being responsible for an estimated burden of at least 1.4 million disability-adjusted life years (DALYs) in Africa alone. Importantly, morbidity due to schistosomiasis has been greatly reduced in some parts of the world, including Zanzibar. The Zanzibar government is now committed to eliminate urogenital schistosomiasis. Over the next 3–5 years, the whole at-risk population will be administered praziquantel (40 mg/kg) biannually. Additionally, snail control and behaviour change interventions will be implemented in selected communities and the outcomes and impact measured in a randomized intervention trial.

Methods/Design: In this 5-year research study, on both Unguja and Pemba islands, urogenital schistosomiasis will be assessed in 45 communities with urine filtration and reagent strips in 4,500 schoolchildren aged 9–12 years annually, and in 4,500 first-year schoolchildren and 2,250 adults in years 1 and 5. Additionally, from first-year schoolchildren, a finger-prick blood sample will be collected and examined for *Schistosoma haematobium* infection biomarkers. Changes in prevalence and infection intensity will be assessed annually. Among the 45 communities, 15 were randomized for biannual snail control with niclosamide, in concordance with preventive chemotherapy campaigns. The reduction of *Bulinus globosus* snail populations and *S. haematobium*-infected snails will be investigated. In 15 other communities, interventions triggering behaviour change have been designed and will be implemented in collaboration with the community. A change in knowledge, attitudes and practices will be assessed annually through focus group discussions and in-depth interviews with schoolchildren, teachers, parents and community leaders. In all 45 communities, changes in the health system, water and sanitation infrastructure will be annually tracked by standardized questionnaire-interviews with community leaders. Additional issues potentially impacting on study outcomes and all incurring costs will be recordedand monitored longitudinally.

Discussion: Elimination of schistosomiasis has become a priority on the agenda of the Zanzibar government and the international community. Our study will contribute to identifying what, in addition to preventive chemotherapy, needs to be done to prevent, control, and ultimately eliminate schistosomiasis, and to draw lessons for current and future schistosomiasis elimination programmes in Africa and elsewhere.

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Background

Burden and transmission of schistosomiasis, with focus on *Schistosoma haematobium* infection in Zanzibar

Schistosomiasis is a group of diseases caused by parasitic worms of the genus *Schistosoma*. These blood-dwelling flukes have a complicated life cycle involving freshwater snail intermediate hosts and transmission of the parasite is governed by social-ecological systems and intimately linked with conditions of poverty [1,2]. More than 200 million people are infected with more than 95% of the infections concentrated in Africa [3-5]. The global burden due to schistosomiasis is currently estimated at 1.7-4.5 million disability-adjusted life years (DALYs) [2,6,7]. Schistosomiasis predominantly occurs in tropical and sub-tropical areas, but the disease is also seen in specialized travel clinics in Europe and North America among migrants and returning travellers [8,9]. Schistosomiasis can cause acute illness, but its main public health impact is due to chronic infection, including increased risk for anaemia, growth stunting and under-nutrition in affected populations, as well as exacerbation of co-infections and impairment of cognitive development and work capacity [10]. Depending on the species of *Schistosoma*, the liver, intestine, spleen, lungs and urogenital system are affected, which can cause serious health problems in later life [10]. For example, chronic infection with *Schistosoma haematobium* is associated with bladder cancer, and might account for up to 30% of all cancer cases in some endemic regions [11,12].

Humans become infected with *S. haematobium* when they wade, swim or bathe in water, inhabited by compatible intermediate snail hosts, previously contaminated by human urine containing parasite eggs. The adult schistosomes live as paired male and female worms in the perivesical venous plexus of their human host. Eggs produced by female worms get trapped in the tissues and can lead to inflammatory and obstructive disease in the urogenital system [3]. Eggs that pass through the bladder wall are voided in urine. If urination occurs in freshwater, the parasite eggs will hatch in the water and release miracidia (a free swimming larval stage), which subsequently infect the specific intermediate host snails. In Zanzibar, the only intermediate host snail for *S. haematobium* is *Bulinus globosus* [13]. In the snail, asexual reproduction takes place and 4–6 weeks after infection, the snail starts to shed cercariae, the human infective larval stage, into the water. Importantly, a single snail can shed thousands of cercariae over its life-time. On locating a human host, cercariae penetrate the skin and, after migrating via the lungs to the liver develop, within 9–10 weeks, into adult separate sex worms. These worms pair up, and on average, live and produce eggs for 3–5 years. Many eggs leave the body via the excreta, but many more become trapped in the tissues causing an inflammatory reaction that leads to morbidity [3,14,15].

In Zanzibar, the population is at risk of infection with only *S. haematobium* [13,16], the schistosome species that affects the bladder, genital tract and urethras [17-20], causing urogenital schistosomiasis. The highest prevalence of *S. haematobium* infection is found among school-aged children on both islands, Unguja and Pemba [21-25]. Previous research has shown that visible haematuria, microhaematuria and urinary and genital tract pathology are associated with infection, particularly heavy intensity infections [24-27].

Control of schistosomiasis with focus on efforts in Zanzibar

To interrupt the life cycle of *S. haematobium*, there are four main strategies: (i) kill the worms in humans, by anthelminthic drugs; (ii) kill the intermediate host snails, including those carrying intramolluscan parasites, by chemical (i.e. molluscicides) or biological control agents (e.g. competitor snails and fish); (iii) stop people infecting snails, by convincing them not to urinate into open freshwater bodies; and (iv) stop cercariae infecting man, by keeping people out of infested water bodies [28,29].

The current global strategy to control morbidity due to schistosomiasis is preventive chemotherapy that is the regular administration of the anthelminthic drug praziquantel to at-risk populations (e.g. school-aged children) without prior diagnosis [30]. In the frame of preventive chemotherapy programmes in Zanzibar, praziquantel has been administered to school-aged children on both islands since 1994 by multiple rounds of treatment depending on the availability of external funds [23,24,31-33]. As a result, the prevalence and intensity of *S. haematobium* infection in school-aged children in Unguja and Pemba decreased considerably. For example, in Unguja, while a prevalence of >50% was recorded in the early 1980s, it has dropped to <10% in 2006 [23,34,35]. Hence, morbidity

control has largely been achieved in Zanzibar. Of note, in a survey carried out in March 2011 in 24 schools on each island, the overall prevalence of *S. haematobium* was 8% in Unguja and 15% in Pemba.

Current initiatives for schistosomiasis control in Zanzibar

In mid-2010, the Zanzibar government expressed commitment to eliminate urogenital schistosomiasis on the islands of Unguja and Pemba. As backbone for this commitment the Zanzibar Neglected Tropical Disease (NTD) Programme will be implementing its "3-year comprehensive strategic plan to combat neglected tropical diseases in Zanzibar 2009/2011" [36], referred to as the "National Plan". It calls for preventive chemotherapy using praziquantel, accompanied by health education and community mobilization activities to consolidate and enhance the impact of preventive chemotherapy. The National Plan is focusing on the districts, and zones within districts, where urogenital schistosomiasis is known to be endemic.

In support of the National Plan, the World Health Organization (WHO) has agreed to supply sufficient quantities of praziquantel to ensure repeated rounds of preventive chemotherapy and the Schistosomiasis Control Initiative (SCI) will assist with the drug intervention at a large scale. WHO and SCI have long histories of working with the Zanzibar Ministry of Health (MoH) in their fight against schistosomiasis and soil-transmitted helminthiasis [18,37].

An international consortium, called Zanzibar Elimination of Schistosomiasis Transmission (ZEST), is committed to assisting the government of Zanzibar in its efforts to eliminate urogenital schistosomiasis. This consortium includes the Zanzibar MoH, including the Zanzibar NTD Control Programme, the Public Health Laboratory - Ivo de Carneri (PHL-IdC) Pemba, Zanzibar government agencies, WHO, SCI, the Natural History Museum (NHM) in London, the Swiss Tropical and Public Health Institute (Swiss TPH) in Basel, the London School of Hygiene and Tropical Medicine (LSHTM), the University of New Mexico and the Schistosomiasis Consortium for Operational Research and Evaluation (SCORE) based at the University of Georgia, with additional groups expected to join as the efforts progress. Within the ZEST project, the emphasis will shift from morbidity control to comparative methods of transmission control and local elimination of urogenital schistosomiasis. In addition to measuring the outcomes of this project, thorough documentation of the process, including lessons learned, will be crucial so that schistosomiasis control and elimination programmes implemented elsewhere can benefit from the ZEST experiences.

Research for schistosomiasis elimination in Zanzibar

SCORE was established in December 2008 to address operational research questions pertaining to gaining and sustaining control of schistosomiasis, and to establish the proof-of-concept that local elimination is feasible. SCORE is funded through a 5-year grant by the Bill & Melinda Gates Foundation awarded to the University of Georgia Research Foundation (UGARF). The goal of SCORE is to provide pragmatic answers that will help current and future schistosomiasis control programme managers to more efficiently control the disease more efficiently. This includes learning which approaches need to be pursued for controlling and eliminating schistosomiasis, as well as developing and validating new tools and strategies. SCORE's vision is that the work will inform efforts to gain control of schistosomiasis in high-prevalence areas, sustain control and move towards elimination in areas of moderate prevalence, and ultimately eliminate schistosomiasis in areas of low endemicity.

Increased use of praziquantel through preventive chemotherapy and availability of treatments for those in need will be central to the success of the National Plan to control and eliminate schistosomiasis. At the same time, increased efforts are required to reduce transmission of the disease. Layered onto the implementation of the National Plan by the Zanzibar government, SCORE, under the umbrella of the ZEST partners, will simultaneously study and implement additional measures of schistosomiasis control/elimination.

Three promising interventions were discussed at ZEST meetings in Zanzibar in 2011 as possible add-ons to preventive chemotherapy to be implemented as part of the National Plan. These are: (i) water and sanitation changes; (ii) snail control; and (iii) human behaviour change interventions that could interrupt the transmission cycle of *S. haematobium*. The Zanzibar Water Authority (ZAWA) has funding to provide piped water and potentially improved sanitation, but such interventions will take considerable time and effort and are unlikely to make widespread progress within the timeframe of ZEST. Therefore, the SCORE study presented here will focus on evaluating the impact of snail control using the molluscicide niclosamide and environmental management and on a community-informed behaviour change intervention on rates and success of efforts to eliminate schistosomiasis in Zanzibar, in parallel with the National Plan.

Goal, aims and objectives

The goal of this project is to provide an evidence-base for programme decisions about schistosomiasis elimination, not only for the Zanzibar NTD Control Programme, but also for other settings in Africa and elsewhere that aim to eliminate schistosomiasis. The study will be implemented on Unguja and Pemba islands and will compare snail control and behaviour

change strategies as complementary measures to preventive chemotherapy in three study arms, each consisting of 15 communities (shehias). Hence the study involves a total of 45 study communities on each island. The following aims and specific objectives are related to this goal.

Aims

1. Eliminate schistosomiasis as a public health problem* on Unguja in 3 years and interrupt transmission in 5 years.
2. Control schistosomiasis throughout Pemba (prevalence <10% in school-aged children) in 3 years and eliminate it as a public health problem* in 5 years.
3. To identify effective behaviour change strategies with an understanding of the associated costs, motivators, triggers and barriers associated with behaviour change interventions.
4. To identify effective snail control strategies with an understanding of the associated costs, motivators, triggers and barriers associated with snail control interventions.

* Eliminate schistosomiasis as a public health problem is defined: "Reduction of *Schistosoma* prevalence to <1% heavy infections based upon direct egg-detection methods in the school-aged population; continued intervention measures are required to prevent resurgence of transmission" as in Rollinson et al. 2012 [29].

Specific objectives, activities and milestones

1. To assess annually the reduction in prevalence and intensity of *S. haematobium* infection according to standardized, quality-controlled methods (i.e. urine filtration and reagent strip testing) in schoolchildren aged 9–12 years within and between each study arm from year 1 to year 5.
2. To assess the reduction in the prevalence and intensity of *S. haematobium* infection as measured by urine filtration and reagent strip testing in adults in year 1 and in year 5 within and between each study arm.
3. To assess the reduction in the prevalence and intensity of *S. haematobium* infection as measured by urine filtration and reagent strip testing in first-year schoolchildren in year 1 and in year 5 within and between each study arm.
4. To assess the difference in the sero-prevalence of *S. haematobium* infection in first-year schoolchildren after 4 years of control within and between each study arm.
5. To assess annually changes in knowledge on *S. haematobium* transmission and the impact of change of behaviour on transmission.
6. To assess the impact of niclosamide on the presence of *B. globosus* in freshwater ponds and rivers.
7. To test and evaluate recently developed methods for the diagnosis of *S. haematobium* infections (such as: enzyme linked immuno-sorbent assay (ELISA) for assessment of antibody levels in blood; polymerase chain reaction (PCR) for DNA detection in urine) for their sensitivity, specificity and feasibility for elimination programmes.
8. To create, test and validate mathematical models for the prediction of *S. haematobium* prevalence after control interventions (to be developed with external partners).

Methods/Design

Study area and population

The zanzibar archipelago is part of the United Republic of Tanzania and consists of the two main islands, Unguja and Pemba [38], as well as a few much smaller islands. There are two annual wet seasons: the Masika rains from the south lasting usually from March to June, and the Vuli rains from north-east occurring from October to November. The average annual temperature ranges between 23°C and 32°C. The estimated total resident population for Unguja and Pemba was 773,234 and 511,576 inhabitants, respectively, in 2010 [39]. Islam is the predominant religion [40]. The main economic activities include seawater fishing and cash crop production (e.g. coconuts, cloves, chillies, copra and seaweed). Tourism is of growing importance, since the islands offer a host of historical and natural attractions [39].

Both islands are divided into large administrative areas (i.e. districts). The districts are sub-divided into smaller administrative units (i.e. shehias). Unguja consists of six districts (North A, North B, Central, West, South and Urban) and a total of 203 shehias. Pemba consists of four districts (Micheweni, Wete, Chake and Mkoani), subdivided into 88 shehias. In Unguja, schistosomiasis is endemic in districts North A, North B, Central, West and a part of the Urban district (entitled "Zone C" for the operational ease of preventive chemotherapy distribution), whereas all four districts of Pemba are endemic. The schistosome-endemic areas will be targeted for preventive chemotherapy within the National Plan.

Interventions in the frame of SCORE and the National Plan

The SCORE research study is designed as a randomized trial to be layered on the planned preventive chemotherapy campaigns to be conducted by the Zanzibar MoH. The trial will have three study arms, each comprising 15

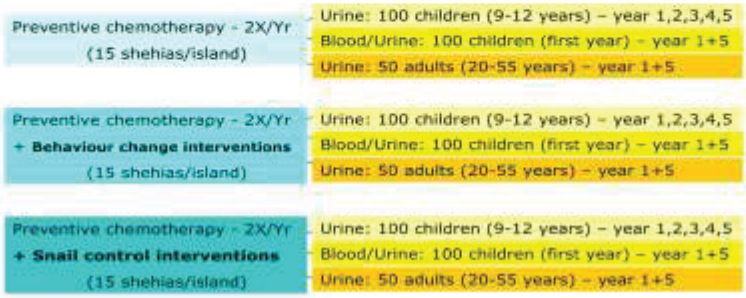

Figure 1 Study arms to assess the impact of different *S. haematobium* control interventions in Zanzibar. In a randomized intervention trial with three study arms we will compare the impact of (i) praziquantel administration as implemented according to the National Plan of the Zanzibar government, (ii) behaviour change interventions in addition to praziquantel administration and (iii) snail control in addition to praziquantel administration on *S. haematobium* infection prevalences and intensities in Zanzibar (Unguja and Pemba islands). Each study arm comprises 15 communities; hence, a total of 45 communities per island is surveyed. The number of study participants per study arm and community is given in Figure 1.

shehias. Hence, on each island, a total of 45 shehias will be included into the study. The three study arms are designed as follows (Figure 1):

i) Implementation of the National Plan of the Zanzibar government (preventive chemotherapy, health education and community mobilization focused on preventive chemotherapy).
ii) Implementation of the National Plan plus targeted snail control.
iii) Implementation of the National Plan plus an intensive behavioural intervention aimed at reducing *S. haematobium* transmission.

Preventive chemotherapy in the frame of the National Plan

In the frame of the National Plan of the Zanzibar MoH, preventive chemotherapy with praziquantel (40 mg/kg using a dose pole) [41] against schistosomiasis and albendazole (400 mg) against soil-transmitted helminthiasis will be conducted twice each year, at 6-month intervals to all children aged >2 years and all adults throughout the islands. Severely sick people and pregnant women will be excluded from preventive chemotherapy interventions. However, presumptive treatment of pregnant women at any stage of pregnancy will be implemented at mother and child health (MCH) facilities, once in pregnancy, in all the areas where schistosomiasis is transmitted.

The anthelminthic drugs for the preventive chemotherapy campaigns will be donated by WHO, whereas SCI will financially support the implementation of drug delivery. In addition to preventive chemotherapy, the National Plan will promote health education and community mobilization campaigns.

Snail control

B. globosus is the only intermediate host snail for *S. haematobium* in Zanzibar and occurs commonly in a variety of temporary and permanent habitats on Pemba and Unguja [13]. Snail populations can be reduced either indirectly by the destruction of their natural habitat, for example by the removal of vegetation and debris from riverbanks (environmental management), or directly by the use of a molluscicide (chemical control). Environmental management and molluscicide represent important, well-tried and effective tools to supplement preventive chemotherapy against schistosomiasis [29,42,43]. The molluscicide niclosamide has been recommended by WHO to control human schistosomiasis [44-46]. It has been applied earlier on Unguja in a study for the control of schistosomiasis in the early 1980s [35], and has been widely and effectively used for snail control in schistosomiasis control programmes in Morocco, Egypt and the People's Republic of China [47-50]. Moreover, niclosamide 70% wettable powder (WP) was tested in a pilot study in Zanzibar in June-August 2011, preceding the study proposed here.

In the randomly selected shehias of the current study, niclosamide 70% WP will be applied to open water bodies (slow flowing rivers, ponds and lakes) in concordance with preventive chemotherapy. Since, the optimum time for administering praziquantel is when the snail populations are absent and there is no risk of re-infection for the treated population, molluscicide is most effective if applied immediately before a pre-planned chemotherapy campaign to avoid immediate re-infection [28]. Areas where humans are in regular contact with open freshwater bodies (e.g. for washing or bathing) in the selected

shehias will be identified and treated with niclosamide 70% WP in the months preceding preventive chemotherapy in Zanzibar, aiming for two applications per year. Before each intervention, all involved shehas (community leaders) and community members will be informed about the purpose of the studies and their concerns and suggestions will be taken into consideration. Since snails often lay eggs on rubbish in the rivers and ponds, the community members will be encouraged to engage in the clearance of vegetation and rubbish from open water bodies; especially from potential transmission sites where humans have regular water contact. Full safety briefings will be required.

Behaviour change interventions

School-aged children who live in *S. haematobium*-endemic areas are usually at the highest risk of becoming infected and are most likely to be involved in transmitting the disease, because they tend to spend time swimming or playing in open water bodies that may contain infected snails and because children have no or limited protective immunity [51-54]. Adults are also at risk of infection through bathing or washing clothes in contaminated water or through occupational exposures (e.g. fishing, rice farming and car washing) [40,55]. Health communication taking into account local knowledge, attitudes and practices and the integration of communities in priority settings, decision making and planning of schistosomiasis control interventions, will be essential to achieve a change in behaviour [56].

The behaviours we believe most important to modify in order to reduce schistosomiasis transmission in Zanzibar are (i) children urinating in streams and ponds and (ii) children playing, swimming and washing laundry in the same streams and ponds. The urination behaviour is particularly difficult to target because it is not observable, and children with schistosomiasis may have increased urgency and difficulty in restraining urination because of bladder irritation from the parasite. Additionally, with little else to do, rivers and ponds are a source of recreation for children.

The behavioural intervention is being guided by human-centred design (HCD) processes and techniques [57]. This process starts with a specific challenge such as the prevention of children urinating in rivers and ponds. In partnership with the community we explore community knowledge, beliefs, current practices and social norms related to the challenge through formative qualitative methods. The resulting findings are shared with the community and together through a participatory process a behavioural intervention is designed and implemented as concrete and tangible solutions. This process helped to identify cultural norms around urination in freshwater bodies and messages, activities and approaches that might be useful to change children's behaviour in Zanzibar. In addition we identified alternative structural solutions and replacement behaviours to urinating in the water that might be acceptable in Zanzibar, as well as best channels and approaches for communications [58]. Behavioural interventions based on formative findings and the participatory work of the community are being designed to raise awareness of schistosomiasis transmission, diagnosis, treatment and prevention and to influence the adoption of new behaviours by: (i) training teachers, coaches and students in local primary schools; (ii) training teachers and students in religious schools; (iii) designing and installing locally produced male and female urinals at targeted water hotspots where children congregate; (iv) providing alternative play activities and play structures for children; and (v) providing washing platforms at designated tap water sources and areas a short distance from local washing water sources.

Justification of the number of participants

With regard to the goal and aims of the SCORE study, the comparison of outcomes between the three intervention arms will document benefits of interventions added to preventive chemotherapy. However, due to the overall low *S. haematobium* prevalence in Unguja (8% in school-aged children, as determined in 24 schools surveyed in March 2011) and the estimated further decrease in prevalence due to biannual treatment in the coming 3 years, the difference attributed to additional interventions could be very small. Hence, to reach a desired power of 80% in our randomized trial, we would need a sample size of clusters (i.e. shehias) that exceeds the total number of shehias in schistosomiasis-endemic settings in Unguja and Pemba and a sample size of participants that is not logistically feasible. The choice of 15 shehias per intervention arm per island, and the number of people to be tested (per island: 4,500 children aged 9–12 years annually, 4,500 first-year schoolchildren in years 1 and 5 and 2,250 adults in years 1 and 5) is a compromise between what is optimal and what is practically achievable.

Selection and randomization of study shehias and participants

Eligibility and randomization of shehias

Unguja and Pemba are divided into 203 and 88 shehias, respectively. The following exclusion criteria were applied. First, in Unguja, all shehias with no endemic schistosomiasis according to expert opinion (n=104) and in Pemba all shehias without a stream indicated on an aerial photography (n=13) were excluded. Second, all shehias without a primary school were excluded (Unguja: n=43; Pemba: n=23). Third, if a shehia had

more than one primary school, the school with lower pupil numbers was excluded. Fourth, all shehias with schools that were attended by fewer than 200 children in 2008 (most recent available data) were excluded (Pemba: n=1). Primary schools in Zanzibar include school grades 1–7, consisting of children mainly aged between 7 and 13 years. Hence, we anticipate that, in a school attended by at least 200 children in 2008, there are at least 100 children aged 9–12 years that can be enrolled for the current study. Fifth, one shehia in Unguja and one shehia in Pemba were excluded as the indicated school was not located in the same shehia. Adhering to these inclusion and exclusion criteria resulted in 45 eligible shehias in Unguja and 50 eligible shehias in Pemba.

For the random selection and allocation of 45 shehias both in Unguja and Pemba to one of the three intervention arms, in a first step, all eligible shehias having participated in the annual 24-school surveys formerly conducted by the *Piga vita Kichocho* programme [23] (Unguja: n=13; Pemba: n=17) were included in a computer-based randomization to one of the three study arms. In a second step, out of the remaining 33 shehias in Pemba, 28 were randomly selected to be part of the study. In a third step, the 32 shehias in Unguja and the 28 shehias in Pemba were randomized to one of the three study arms. Although the randomization may have resulted in differences in the starting prevalence of *S. haematobium* infection or other factors among study arms, we did not re-randomize. Figure 2 shows the distribution of shehias to each of the three study arms in Unguja and Pemba.

Eligibility and randomization of schoolchildren

In years 1 to 5 of the study, all schoolchildren aged 9–12 years and in years 1 and 5 additionally all first-year schoolchildren are eligible to participate in the study. For the respective surveys, (i) 130 children aged 9–12 years and (ii) 130 first-year schoolchildren will be randomly selected for urine collection and additional finger-prick blood collection for first-year schoolchildren. For this purpose, all eligible children will line up, in separate lines for boys and girls and school class. Subsequently, we will systematically select each third child in the lines to be included in the study. This procedure will be continued until we reach a total of 130 selected children, accounting for a 20% drop-out with the final aim to sample 100 children aged 9–12 years and 100 first-year schoolchildren. We anticipate to collect each year 9,000 urine samples from 9-12-year-old children and 9,000 urine and finger-prick blood samples from first-year schoolchildren in years 1 and 5 on each of the two islands.

Eligibility and randomization of adults from communities

All adults aged 20–55 years are eligible to participate in the study. In years 1 and 5, we will select a quota sample of 50 houses per shehia according to a method suggested by Winkler et al. [59]. For this purpose, a gyro with a marked arrow-star (equal to the number of field interviewers in a team) pointing into different directions will be spun on a central point in the shehia. Subsequently, each interviewer will count the households to the edge of the shehia following a path in direction of the arrow. On return to the centre point, the number of households in that direction will be reported and the interviewer will state a number within the range of counted households. This number will be compared to a list with computer-generated random numbers created for each shehia. The random number corresponding to the number stated by the interviewer will assign where to start the first questionnaire interview. Proximity sampling will then be pursued with interviewers moving from one household to the next nearest household until 50 households are covered. People sharing the same kitchen or pot will define a household. If more than one of the present household members is eligible to participate, we will use a "drawing cards" approach for randomly selecting only one of the members to participate in the study. We plan to collect 4,500 urine samples and questionnaire data from adults aged 20–55 years in years 1 and 5 on each of the two islands.

Data collection

Recruitment of participants and collection of specimen and questionnaire data

The community leaders and headmasters of the selected shehias and primary schools will be informed about the aims and procedures of the study. Each year, in the selected schools, the teachers will be informed about the forthcoming activities. The study will be explained in lay terms to the children and they will be asked for their oral assent to participate. Children will be selected according to the eligibility criteria and randomization as described above. Name, sex, age, school grade and village and shehia of residency of the selected children will be recorded and the children will be provided with an information and consent form, the latter to be signed by their parents or legal guardians and to be returned the following day. On that day, children who submitted a signed consent form will be provided with a plastic container (120 ml) labelled with the individual's ID, for subsequent urine collection on the spot between 10 AM and 12 AM. From the selected children from the first-year at school, in addition to a urine sample, a finger-prick blood sample will be collected in small tubes (BD Microtainer, Ref.: 365967; BD, Oxford, UK) labelled with the individual's ID and stored on ice after clotting.

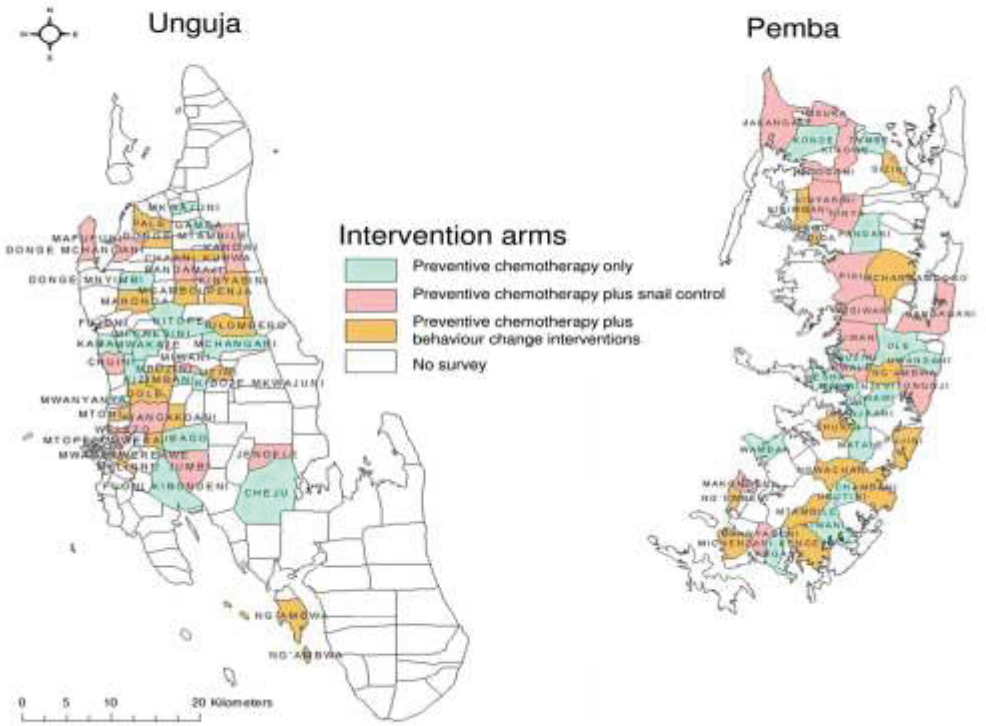

Figure 2 Assignment of communities (shehias) to control interventions aiming to eliminate urogenital schistosomiasis in Zanzibar. On both, Unguja and Pemba islands, 45 shehias were randomly assigned to one of three intervention arms: 15 shehias on each island will receive praziquantel administration as implemented according to the National Plan of the Zanzibar government, 15 shehias will be targeted by behaviour change interventions in addition to praziquantel administration and 15 shehias will receive snail control in addition to praziquantel administration.

In the study communities, the shehas will be informed about the purpose and procedures of the study and invited to inform the community about the forthcoming visits of households. The sheha will be interviewed in Kiswahili with a "village inventory form" and asked a set of questions on the demographic characteristics of his shehia. In addition to general information, data on occupation of the population closely related to water contact, on health facilities and available drugs, on water contact sites, on availability and use of water sources and sanitary facilities will be collected.

While visiting the households selected as described above, the study procedure will be explained to the present household members in Kiswahili and they will be asked for their oral assent to participate. The selected household member will be invited to sign a written informed consent form, given a urine collection container labelled with a unique ID and asked to provide a urine sample on the spot, ideally produced between 10 AM and 2 PM of the same day. Additionally, the participant's ID, sex, age, occupation, place of birth, time of residency in the respective shehia and sanitary behaviour will be recorded using a pre-tested questionnaire administered in Kiswahili.

Laboratory procedures to assess S. haematobium *infection in humans*

In the laboratory, each urine sample will be screened for visual blood (macrohaematuria) and for microhaematuria using reagent strips (Haemastix; Siemens Healthcare Diagnostics GmbH, Eschborn, Germany). When tested visually, the colour of the urine will be coded

semi-quantitatively according to a pretested colour chart (1, 2, 3, 4, 5, 6); the colorimetric test of the reagent strip for microhaematuria will be recorded semi-quantitatively (0 = negative, 1 = +, 2 = ++, 3 = +++, 4 = trace). Additionally, urine samples will be vigorously shaken and 10 ml of each sample will be filtered using a plastic syringe with a filter-holder containing a 13 mm polycarbonate filter (Sterlitech, Kent, WA, USA) [60]. All *S. haematobium* eggs present on the filter will be counted under a microscope by experienced laboratory technicians and exact egg counts will be recorded for each individual.

From a subset of fresh urine samples with microhaematuria according to positive reagent strip tests, *S. haematobium* eggs will be hatched and miracidia collected and stored on Whatman FTA indicator cards (Whatman plc, Maidstone, UK) [61] for population genetic studies layered onto the current study to evaluate the impact of the interventions on the genetic diversity and population structure of the schistosomes in the shehias.

Additionally, on each survey day in years 1 and 5, from every 10^{th} of the collected urines of sufficient amount after the urine filtration has been performed, 10 ml will be transferred into 15 ml Falcon tubes, labelled with the ID of the respective participant and stored for future use at −20°C at HCLU/PHL-IdC. The frozen samples will be examined for *S. haematobium* infections using novel sub-microscopic diagnostic approaches (i.e. PCR or ELISA) evaluated either within SCORE studies or by external partners who have yet to be identified.

The clotted finger-prick blood samples will be centrifuged at 6,000 *g* for 10 min immediately after arrival at HCLU/PHL-IdC. The sera will be transferred into semi-skirted 96 well plates (maximum 250 μl), labelled and stored at −80°C at PHL-IdC pending further analyses. Antibody levels against *S. haematobium* antigen will be tested using ELISA, following the manufacturer's instructions or using newly developed multiplexing assays.

For quality control, microscope slides with the filter containing potential *S. haematobium* eggs will be covered with cellophane soaked in glycerol and stored in slide storing boxes that are kept at room temperature. After each survey, 10% of the slides of each technician will be re-read by external senior laboratory technicians by adding a drop of Lugol's solution on the hydrophilic cellophane. The number of *S. haematobium* eggs will be recorded and compared with the original egg counts. In the case of significant discrepancies between the original and re-read slides (false negatives, false positives, egg counts resulting in a different infection intensity category) that exceed a defined threshold, all stored slides of the respective technician will be re-examined.

Monitoring of snail populations and infection level

Preceding each round of preventive chemotherapy, transmission sites at open water bodies where humans have regular contact with water (identified on maps and by consulting community members and children) in the 15 selected shehias on both islands will be treated with niclosamide 70% WP. The number of freshwater bodies treated, their kind, size and exact location will be recorded. The amount of niclosamide 70% WP used will be recorded after each intervention. Before treating the water bodies, snail densities will be assessed at each site. For this purpose, a sample area of 15 m^2 will be measured and surveyed for any snails for 15 min by two trained staff. All snails will be deposited into a basin and all organisms alive and dead in the collection tube will be counted and recorded. Snails will be classified to species level. Once the number and species of snails and the genus of the other organisms have been recorded, all organisms, except *B. globosus*, will be returned and evenly distributed to their original collection sites. Temperature, pH, salinity and conductivity of the water will be measured and recorded at each of the sites, on all survey and sample days using standard protocols and forms. All collection sites will be located using a hand-held global positioning system (GPS) device (Garmin GPSMap 62, Garmin (Europe) Ltd; Southhampton, UK).

B. globosus snails will be transferred to HCLU in Unguja and PHL-IdC in Pemba to determine whether they are infected with *S. haematobium*. Snails will be investigated for parasitic infection using the shedding method. For this purpose the snails will placed individually in flat-bottomed glass vials (height: 7.5 cm, diameter: 2.5 cm) containing dechlorinated water, and exposed to indirect sunlight for a maximum duration of 4 hours [62]. Cercariae shedding will be observed using a dissection microscope. Snails that do not shed cercariae on the first sunlight exposure will be re-exposed on the second day. Based on their morphology, cercariae will be categorized either as those of *S. haematobium* or those of other trematodes (non-*S. haematobium* cercariae) [63]. *S. haematobium* cercariae will be collected using a pipette and placed on Whatman FTA cards for future molecular analysis. All collected snails will be preserved in small glass tubes containing 100% ethanol, which will be deposited in the schistosomiasis repository (SCAN; http://www.nhm.ac.uk/research-curation/collections/curation-groups/scan/index.html) held at NHM in London, and made available for future investigations as needed.

Assessment of behaviour change

The impact of interventions on the behaviour of people living in the targeted 15 shehias in the behaviour change study arm on each island will be assessed in qualitative baseline and annual follow-up studies. In-depth

interviews (IDIs) and focus group discussions (FGDs) with young and older schoolchildren, teachers, parents and community leaders will be conducted in Kiswahili by trained members of the Zanzibar NTD Programme. The content of each FGD and IDI will be recorded and transcribed into English and analyzed through a modified grounded theory approach. Additionally, pre-tested structured observation checklists will be used to capture critical observations of the same behaviours in households and public venues. Brief questionnaires, based upon behavioural theory constructs, will be administered to measure behavioural determinants associated with the intervention implementation process and to assess its impact on *S. haematobium* infections in schoolchildren and communities.

Collection of additional data relevant for the study outcomes

Baseline data on the 90 selected shehias will be collected from the existing health management information system (HMIS), the Office of the Chief Government Statistician Zanzibar, available aerial photographs, and by consulting the local authorities (shehas and school headmasters) and key informants. This baseline information will include data on the area of the shehias (in km^2), number of inhabitants, number and size of primary and secondary schools, number of public and private health centres, number of MCH facilities, water sources for private and domestic use (e.g. piped water, wells, rivers and ponds), sanitation coverage (e.g. number of pit latrines, ventilated improved pit latrines, flush toilets), immigration and main income source of the population, mainly covered within the village inventory form. To track multiple factors that could affect study outcomes, shehas will be interviewed on an annual basis. In addition to direct village inventories, we will gather and store reports from non-governmental organisations (NGOs) involved in water and sanitation as well as other health-related projects and be in regular contact and exchange with ZAWA to track their activities.

Praziquantel treatments in community health centres will be recorded and the data transferred to the HMIS of the MoH. In relation to preventive chemotherapy administration according to the National Plan, the Zanzibar MoH will collect data on treatment coverage, use of praziquantel from primary health care units (PHCUs) and treatment of pregnant women in MCH facilities. Costs incurred for all activities will be recorded and used to estimate the cost-effectiveness of the different intervention arms.

Data management and analysis

Quantitative data from laboratory examinations, questionnaires and snail records will be entered in Microsoft Excel version 10.0 (2002 Microsoft Corporation) or EpiInfo version 3.5.1 (Centers for Disease Control and Prevention; Atlanta, GA, USA) by local staff in Zanzibar. Statistical analyses will be carried out with STATA version 10 (StataCorp.; College Station, TX, USA). Descriptive and regression analyses will be conducted to evaluate the effectiveness of the interventions under study, including evaluation of cost and cost-effectiveness. In addition, spatial analysis will be conducted to identify clusters and spatially defined factors that may be modifying outcomes. For each year, *S. haematobium* prevalence and infection intensity data will be calculated. The results from the different study arms will be compared on an annual basis, after 2 years of intervention and at the end of the 4-year intervention period.

For the assessment of effectiveness of behaviour change interventions, FGDs and IDIs will be transcribed verbatim, translated into English by bilingual research assistants and entered as Microsoft Word documents into Atlas-ti version 6.0 (Software Development GmbH; Berlin, Germany) to facilitate text searching, data coding and analysis. Open, axial and selective coding will be used to analyse the transcribed narratives. Open coding and a word-by-word analysis will be used to identify, name and categorize explanations and descriptions of the day-to-day reality of participants as related to schistosomiasis and other water-related issues. Structured observations will be summarised and descriptive open-ended questions analysed in the same manner for the FGDs and IDIs narratives.

Data storage and handling

All original data records will remain within the office of the HCLU or PHL-IdC of the MoH Zanzibar. Electronic data files will be transferred to the NHM in London and Swiss TPH in Basel and original and cleaned data will be stored in a password-protected folder accessible for all investigators. Name-linked information on participants will remain confidential and be shared only by the study team. Unique identifiers will be used for data records, transcripts and computer-based software data management. When discussing or showing the results of analyses in public venues, the information will always be reported at an aggregate level so that individual participants cannot be identified.

Protocol review and ethical clearance

The study protocol summarised here was reviewed by the SCORE secretariat consisting of several scientists with long-term experience in the epidemiology and control of schistosomiasis before it was submitted to and accepted by (i) the Zanzibar Medical Research Ethical Committee (ZAMREC), (ii) the "Ethikkomission beider Basel" (EKBB) in Switzerland, and (iii) the institutional review board of the University of Georgia (IRB UGA). The trial has been registered at the International Standard Randomised Controlled Trial Number Register (ISRCTN48837681; http://www.controlled-trials.com/ISRCTN48837681).

At the onset of each study segment, all participants will be informed about the purpose and procedures of the study and the respective study segment and asked to submit a written informed consent prior to collection of urine or finger-prick blood samples, or the collection of questionnaire, FGD or IDI data. The obtained information will be treated with strict confidentiality and data made anonymous before analysis.

Discussion

In November 2011, the Secretariat of the WHO considered that elimination of schistosomiasis "is feasible in all epidemiological settings, provided that there is strong political commitment to the goal, supplies of anthelminthic medicines for preventive chemotherapy are adequate, and support is provided by the international community" [64]. Further, the Executive Board called "on all countries endemic for schistosomiasis to intensify control interventions and strengthen surveillance, with the aim of eliminating the disease" [64]. A few months later, in May 2012, the 65th World Health Assembly announced that schistosomiasis elimination campaigns should be initiated where appropriate [65].

The islands of Zanzibar are meeting the requirements specified by the secretariat, rendering the archipelago a suitable candidate for schistosomiasis elimination. Control of schistosomiasis has a long-term history on Unguja and Pemba [18,19,23,35,66,67]. Elimination of urogenital schistosomiasis on the islands of Zanzibar is a health priority, endorsed by the President, and the Zanzibar MoH is committed to fulfil the National Plan [36]. The MoH and the Zanzibar NTD Programme have backing from important and influential partners such as WHO and SCI, which will strongly support the control and elimination efforts on both islands by donating praziquantel and funding treatment implementation costs, respectively. The rigid boundaries of the islands, the strong public health system and a number of successful previous campaigns against malaria [68], cholera [38], lymphatic filariasis [69], soil-transmitted helminthiasis [70,71] and schistosomiasis [18,37] have sensitized the population [72]. Huge steps towards elimination of lymphatic filariasis and malaria in Zanzibar have been made [68,69] and the elimination of urogenital schistosomiasis in Unguja seems now an achievable goal [29,73].

However, a broad consensus has been reached in the international community that long-term commitment by influential partners and efforts going beyond preventive chemotherapy are needed to achieve elimination [29,74,75]. Indeed, examples of countries having achieved schistosomiasis elimination show that preventive chemotherapy must be fully integrated with other tools of transmission control such as molluscicides against snail intermediate hosts, changing behaviour to avoid the contamination of water bodies and prevent (re-)infection and improving water and sanitation [29,75–77].

The study on urogenital schistosomiasis elimination in Zanzibar that we describe here will contribute to identifying what, in addition to preventive chemotherapy, needs to be done to prevent, control and ultimately eliminate schistosomiasis, and to draw lessons for current and future schistosomiasis elimination programmes. It will provide important information for the Zanzibar NTD Programme and for other endemic areas in Africa and elsewhere that aim to eliminate schistosomiasis, and particularly urogenital schistosomiasis.

Based on the findings and outcomes of the proposed study, and in close collaboration with WHO and other partners, new guidelines for schistosomiasis control programmes progressing from morbidity control to transmission control/local elimination may be developed.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

SK drafted the original protocol and manuscript. KAM, SaMA and DR initiated the study. ISK and ShMA advised on local conditions and distinctions. SK, ISK, ShMA and JU developed the parasitological part. SK, ISK, ShMA and DR focussed on the snail control design. BP, KAM and SaMA developed the behaviour change component of the study. SK, KAM, SaMA, ISK, ShMA, MA, AG, JU and DR are substantially involved in the study preparation and conduction. MA, AG, AF, LS, DGC, JU, KAM and DR provided expert knowledge for the study design and implementation and support the study on all levels. All authors contributed to the full conception and design of the study, revised the manuscript and approved its final version.

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References

1. King CH: **Parasites and poverty: the case of schistosomiasis.** *Acta Trop* 2010, **113**(2):95–104.
2. Utzinger J, N'Goran EK, Caffrey CR, Keiser J: **From innovation to application: social-ecological context, diagnostics, drugs and integrated control of schistosomiasis.** *Acta Trop* 2011, **120**(Suppl 1):S121–S137.
3. Gryseels B, Polman K, Clerinx J, Kestens L: **Human schistosomiasis.** *Lancet* 2006, **368**(9541):1106–1118.
4. Steinmann P, Keiser J, Bos R, Tanner M, Utzinger J: **Schistosomiasis and water resources development: systematic review, meta-analysis, and estimates of people at risk.** *Lancet Infect Dis* 2006, **6**(7):411–425.
5. Utzinger J, Raso G, Brooker S, de Savigny D, Tanner M, Ørnbjerg N, Singer BH, N'Goran EK: **Schistosomiasis and neglected tropical diseases: towards integrated and sustainable control and a word of caution.** *Parasitology* 2009, **136**(13):1859–1874.
6. Chan MS: **The global burden of intestinal nematode infections–fifty years on.** *Parasitol Today* 1997, **13**(11):438–443.
7. WHO: **Prevention and control of schistosomiasis and soil-transmitted helminthiasis: report of a WHO expert committee.** *WHO Tech Rep Ser* 2002, **912**:1–57.
8. Ross AGP, Bartley PB, Sleigh AC, Olds GR, Li Y, Williams GM, McManus DP: **Schistosomiasis.** *N Engl J Med* 2002, **346**(16):1212–1220.
9. Clerinx J, Van Gompel A: **Schistosomiasis in travellers and migrants.** *Travel Med Infect Dis* 2011, **9**(1):6–24.
10. King CH, Dangerfield-Cha M: **The unacknowledged impact of chronic schistosomiasis.** *Chronic Illn* 2008, **4**(1):65–79.
11. Mostafa MH, Sheweita SA, O'Connor PJ: **Relationship between schistosomiasis and bladder cancer.** *Clin Microbiol Rev* 1999, **12**(1):97–111.
12. Botelho MC, Machado JC, da Costa JM: ***Schistosoma haematobium* and bladder cancer: what lies beneath?** *Virulence* 2011, **1**(2):84–87.
13. Stothard JR, Rollinson D: **Molecular characterization of *Bulinus globosus* and *B. nasutus* on Zanzibar, and an investigation of their roles in the epidemiology of *Schistosoma haematobium*.** *Trans R Soc Trop Med Hyg* 1997, **91**(3):353–357.
14. King CL: **Initiation and regulation of disease in schistosomiasis.** In *Schistosomiasis*. Edited by Mahmoud AAF, James S. London: Imperial College Press; 2001:213–264.
15. Gryseels B: **Schistosomiasis.** *Infect Dis Clin North Am* 2012, **26**(2):383–397.
16. Savioli L, Mott KE: **Urinary schistosomiasis on Pemba island: low-cost diagnosis for control in a primary health care setting.** *Parasitol Today* 1989, **5**(10):333–337.
17. Rollinson D: **A wake up call for urinary schistosomiasis: reconciling research effort with public health importance.** *Parasitology* 2009, **136**(12):1593–1610.
18. Savioli L, Dixon H, Kisumku UM, Mott KE: **Control of morbidity due to *Schistosoma haematobium* on Pemba Island: programme organization and management.** *Trop Med Parasitol* 1989, **40**(2):189–194.
19. Stothard JR, Loxton N, Rollinson D, Mgeni AF, Khamis S, Ameri H, Ramsan M, Savioli L: **The transmission status of *Bulinus* on Zanzibar island (Unguja), with implications for control of urinary schistosomiasis.** *Ann Trop Med Parasitol* 2000, **94**(1):87–94.
20. Mbabazi PS, Andan O, Fitzgerald DW, Chitsulo L, Engels D, Downs JA: **Examining the relationship between urogenital schistosomiasis and HIV infection.** *PLoS Negl Trop Dis* 2012, **5**(12):e1396.
21. Stothard JR, Mgeni AF, Khamis S, Seto E, Ramsan M, Rollinson D: **Urinary schistosomiasis in schoolchildren on Zanzibar island (Unguja), Tanzania: a parasitological survey supplemented with questionnaires.** *Trans R Soc Trop Med Hyg* 2002, **96**(5):507–514.
22. French MD, Rollinson D, Basanez MG, Mgeni AF, Khamis IS, Stothard JR: **School-based control of urinary schistosomiasis on Zanzibar, Tanzania: monitoring micro-haematuria with reagent strips as a rapid urological assessment.** *J Pediatr Urol* 2007, **3**:364–368.
23. Stothard JR, French MD, Khamis IS, Basáñez MG, Rollinson D: **The epidemiology and control of urinary schistosomiasis and soil-transmitted helminthiasis in schoolchildren on Unguja island, Zanzibar.** *Trans R Soc Trop Med Hyg* 2009, **103**(10):1031–1044.
24. Savioli L, Dixon H, Kisumku UM, Mott KE: **Control of morbidity due to *Schistosoma haematobium* on Pemba island: selective population chemotherapy of schoolchildren with haematuria to identify high-risk localities.** *Trans R Soc Trop Med Hyg* 1989, **83**(6):805–810.
25. Lwambo NJS, Savioli L, Kisumku UM, Alawi KS, Bundy DAP: **The relationship between prevalence of *Schistosoma haematobium* infection and different morbidity indicators during the course of a control programme on Pemba Island.** *Trans R Soc Trop Med Hyg* 1997, **91**(6):643–646.
26. Lyons B, Stothard R, Rollinson D, Khamis S, Simai KA, Hunter PR: **A comparison of urinary tract pathology and morbidity in adult populations from endemic and non-endemic zones for urinary schistosomiasis on Unguja Island, Zanzibar.** *BMC Infect Dis* 2009, **9**:189.
27. Rollinson D, Klinger EV, Mgeni AF, Khamis IS, Stothard JR: **Urinary schistosomiasis on Zanzibar: application of two novel assays for the detection of excreted albumin and haemoglobin in urine.** *J Helminthol* 2005, **79**(3):199–206.
28. Sturrock RF: **Current concepts of snail control.** *Mem Inst Oswaldo Cruz* 1995, **90**(2):241–248.
29. Rollinson D, Knopp S, Levitz S, Stothard JR, Tchuente LA, Garba A, Mohammed KA, Schur N, Person B, Colley DG, *et al*: **Time to set the agenda for schistosomiasis elimination.** *Acta Trop* 2012, [Epub ahead of print]. http://dx.doi.org/10.1016/j.actatropica.2012.04.013.
30. WHO: *Preventive chemotherapy in human helminthiasis: coordinated use of anthelminthic drugs in control interventions: a manual for health professionals and programme managers*. Geneva: World Health Organization; 2006:1–62.
31. Mohammed KA, Haji HJ, Gabrielli A, Mubila L, Biswas G, Chitsulo L, Bradley MH, Engels D, Savioli L, Molyneux DH: **Triple co-administration of ivermectin, albendazole and praziquantel in Zanzibar: a safety study.** *PLoS Negl Trop Dis* 2008, **2**(1):e171.
32. Stoltzfus RJ, Albonico M, Tielsch JM, Chwaya HM, Savioli L: **School-based deworming program yields small improvement in growth of Zanzibari school children after one year.** *J Nutr* 1997, **127**(11):2187–2193.
33. Renganathan E, Ercole E, Albonico M, De Gregorio G, Alawi KS, Kisumku UM, Savioli L: **Evolution of operational research studies and development of a national control strategy against intestinal helminths in Pemba Island, 1988–92.** *Bull World Health Organ* 1995, **73**(2):183–190.
34. McCullough FS, Gayral P, Duncan J, Christie JD: **Molluscicides in schistosomiasis control.** *Bull World Health Organ* 1980, **58**(5):681–689.
35. Mgeni AF, Kisumku UM, McCullough FS, Dixon H, Yoon SS, Mott KE: **Metrifonate in the control of urinary schistosomiasis in Zanzibar.** *Bull World Health Organ* 1990, **68**(6):721–730.
36. MoHSW: *3-year comprehensive strategic plan to combat neglected tropical diseases in Zanzibar 2009/2011*. Zanzibar Town: Ministry of Health and Social Welfare (Revolutionary Government of Zanzibar); 2009:1–25.
37. Stothard JR, Mook P, Mgeni AF, Khamis IS, Khamis AN, Rollinson D: **Control of urinary schistosomiasis on Zanzibar (Unguja island): a pilot evaluation of the educational impact of the Juma na Kichocho health booklet within primary schools.** *Mem Inst Oswaldo Cruz* 2006, **101**(Suppl. 1):119–124.
38. Schaetti C, Hutubessy R, Ali SM, Pach A, Weiss MG, Chaignat CL, Khatib AM: **Oral cholera vaccine use in Zanzibar: socioeconomic and behavioural features affecting demand and acceptance.** *BMC Public Health* 2009, **9**:99.
39. OCGS: *Zanzibar statistical abstract 2010*. Zanzibar Town: Office of Chief Government Statistician (Revolutionary Government of Zanzibar); 2010:1–109.
40. Knopp S, Mohammed KA, Stothard JR, Khamis IS, Rollinson D, Marti H, Utzinger J: **Patterns and risk factors of helminthiasis and anemia in a rural and a peri-urban community in Zanzibar, in the context of helminth control programs.** *PLoS Negl Trop Dis* 2010, **4**(5):e681.
41. Montresor A, Engels D, Ramsan M, Fourn A, Savioli L: **Field test of the 'dose pole' for praziquantel in Zanzibar.** *Trans R Soc Trop Med Hyg* 2002, **96**(3):323–324.
42. Sturrock RF: **Schistosomiasis: complimentary snail control in chemotherapeutic based control programmes.** In *Parasitic diseases: treatment and control*. Edited by Miller MJ, Love EJ. Baton Rouge: CRC Press; 1989:51–60.
43. Utzinger J, Zhou XN, Chen MG, Bergquist R: **Conquering schistosomiasis in China: the long march.** *Acta Trop* 2005, **96**(2–3):69–96.
44. McCullough FS: *The role of mollusciciding in schistosomiasis control. WHO/Q/107*. Geneva: World Health Organization; 1993:1–35.

45. WHO: **The control of schistosomiasis.** *WHO Tech Rep Ser* 1993, **830**:1–86.
46. WHO: *The role of mollusciciding in schistosomiasis control.* Geneva: World Health Organization; 1983:72–73.
47. Yang GJ, Li W, Sun LP, Wu F, Yang K, Huang YX, Zhou XN: **Molluscicidal efficacies of different formulations of niclosamide: result of meta-analysis of Chinese literature.** *Parasit Vectors* 2010, **3**:84.
48. Laamrani H, Khallaayoune K, Madsen H, Mahjour J, Gryseels B: **New challenges in schistosomiasis control in Morocco.** *Acta Trop* 2000, **77**(1):61–67.
49. Laamrani H, Mahjour J, Madsen H, Khallaayoune K, Gryseels B: ***Schistosoma haematobium* in Morocco: moving from control to elimination.** *Parasitol Today* 2000, **16**(6):257–260.
50. World Bank: *Project performance assessment report. Arab Republic of Egypt. National schistosomiasis control project (Credit no. 2403-EGT).* World Bank; 2008:71.
51. Etard JF, Audibert M, Doko A: **Age-acquired resistance and predisposition to reinfection with *Schistosoma haematobium* after treatment with praziquantel in Mali.** *Am J Trop Med Hyg* 1995, **52**(6):549–558.
52. Kapito-Tembo AP, Mwapasa V, Meshnick SR, Samanyika Y, Banda D, Bowie C, Redke S: **Prevalence distribution and risk factors for *Schistosoma haematobium* infection among school children in Blantyre, Malawi.** *PLoS Negl Trop Dis* 2009, **3**(1):e361.
53. Ndyomugyenyi R, Minjas JN: **Urinary schistosomiasis in schoolchildren in Dar-es-Salaam, Tanzania, and the factors influencing its transmission.** *Ann Trop Med Parasitol* 2001, **95**(7):697–706.
54. Rudge JW, Stothard JR, Basáñez MG, Mgeni AF, Khamis IS, Khamis AN, Rollinson D: **Micro-epidemiology of urinary schistosomiasis in Zanzibar: local risk factors associated with distribution of infections among schoolchildren and relevance for control.** *Acta Trop* 2008, **105**(1):45–54.
55. Black CL, Steinauer ML, Mwinzi PN, Secor EW, Karanja DM, Colley DG: **Impact of intense, longitudinal retreatment with praziquantel on cure rates of schistosomiasis mansoni in a cohort of occupationally exposed adults in western Kenya.** *Trop Med Int Health* 2009, **14**(4):450–457.
56. Aagaard-Hansen J, Mwanga JR, Bruun B: **Social science perspectives on schistosomiasis control in Africa: past trends and future directions.** *Parasitology* 2009, **136**(13):1747–1758.
57. **IDEO human centred design toolkit field guide.** [http://www.ideo.com/images/uploads/work/case-studies/pdfs/IDEO_HCD_FieldGuide_for_download.pdf].
58. Person B: *A human centered design project for schistosomiasis elimination in Zanzibar (Unguja and Pemba islands) - formative research findings.* Atlanta: Centers for Disease Control and Prevention; 2012:1–37.
59. Winkler MS, Divall MJ, Krieger GR, Schmidlin S, Magassouba ML, Knoblauch AM, Singer BH, Utzinger J: **Assessing health impacts in complex eco-epidemiological settings in the humid tropics: modular baseline health surveys.** *Environ Impact Asses* 2012, **33**:15–22.
60. Peters PA, Mahmoud AA, Warren KS, Ouma JH, Siongok TK: **Field studies of a rapid, accurate means of quantifying *Schistosoma haematobium* eggs in urine samples.** *Bull World Health Organ* 1976, **54**(2):159–162.
61. Gower CM, Shrivastava J, Lamberton PH, Rollinson D, Webster BL, Emery A, Kabatereine NB, Webster JP: **Development and application of an ethically and epidemiologically advantageous assay for the multi-locus microsatellite analysis of *Schistosoma mansoni*.** *Parasitology* 2007, **134**(4):523–536.
62. Kariuki HC, Clennon JA, Brady MS, Kitron U, Sturrock RF, Ouma JH, Ndzovu ST, Mungai P, Hoffman O, Hamburger J, *et al*: **Distribution patterns and cercarial shedding of *Bulinus nasutus* and other snails in the Msambweni area, Coast province, Kenya.** *Am J Trop Med Hyg* 2004, **70**(4):449–456.
63. Frandsen F, Christensen NO: **An introductory guide to the identification of cercariae from African freshwater snails with special reference to cercariae of trematode species of medical and veterinary importance.** *Acta Trop* 1984, **41**(2):181–202.
64. WHO: *Elimination of schistosomiasis. Provisional agenda item 13.11.* Geneva: World Health Organization; 2011:1–3.
65. WHA: *Elimination of schistosomiasis. Agenda item 13.11.* Geneva: World Health Assembly; 2012:1–2.
66. Goatly KD, Jordan P: **Schistosomiasis in Zanzibar and Pemba.** *East Afr Med J* 1965, **42**:1–9.
67. Guidi A, Andolina C, Makame Ame S, Albonico M, Cioli D, Juma Haji H: **Praziquantel efficacy and long-term appraisal of schistosomiasis control in Pemba island.** *Trop Med Int Health* 2010, **15**(5):614–618.
68. Smith DL, Cohen JM, Moonen B, Tatem AJ, Sabot OJ, Ali A, Mugheiry SM: **Solving the Sisyphean problem of malaria in Zanzibar.** *Science* 2011, **332**(6036):1584–1585.
69. Mohammed KA, Molyneux DH, Albonico M, Rio F: **Progress towards eliminating lymphatic filariasis in Zanzibar: a model programme.** *Trends Parasitol* 2006, **22**(7):340–344.
70. Albonico M, Chwaya HM, Montresor A, Stoltzfus RJ, Tielsch JM, Alawi KS, Savioli L: **Parasitic infections in Pemba Island school children.** *East Afr Med J* 1997, **74**(5):294–298.
71. Knopp S, Mohammed KA, Rollinson D, Stothard JR, Khamis IS, Utzinger J, Marti H: **Changing patterns of soil-transmitted helminth infections in Zanzibar in the context of national control programs.** *Am J Trop Med Hyg* 2009, **81**(6):1071–1078.
72. WHO: *The global elimination of lymphatic filariasis. The story of Zanzibar.* Geneva: World Health Organization; 2002:1–48.
73. Knopp S, Stothard JR, Rollinson D, Mohammed KA, Khamis IS, Marti H, Utzinger J: **From morbidity control to transmission control: time to change tactics against helminths on Unguja Island, Zanzibar.** *Acta Trop* 2012, [Epub ahead of print]. http://dx.doi.org/10.1016/j.actatropica.2011.04.010.
74. Taylo VL, Carabin H, Alday PP, Balolong E Jr, Olveda RM, McGarvey ST: **Is mass treatment the appropriate schistosomiasis elimination strategy?** *Bull World Health Organ* 2008, **86**(10):765–771.
75. Gray DJ, McManus DP, Li Y, Williams GM, Bergquist R, Ross AG: **Schistosomiasis elimination: lessons from the past guide the future.** *Lancet Infect Dis* 2010, **10**(10):733–736.
76. Zhang Z, Jiang Q: **Schistosomiasis elimination.** *Lancet Infect Dis* 2011, **11**(5):345, author reply 346–347.
77. Utzinger J, Bergquist R, Xiao SH, Singer BH, Tanner M: **Sustainable schistosomiasis control–the way forward.** *Lancet* 2003, **362**(9999):1932–1934.

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