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A systematic review and meta-analysis of the effects of treatment and immunization against schistosomiasis

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Declaration

This dissertation is submitted in accordance with the requirements for a Doctorate of Philosophy by the School of Biomedical Sciences at the University of Edinburgh. The work included in this thesis has not been submitted for any other degree or professional qualification. The data described in chapter 2 and 3 were collected by the collaboration of PhD students of our research group, Kate M Mitchell, Clair D Bourke, and me. The data described in chapter 4 and 5 were collected by me. All the data analysis presented in this thesis is my own work.

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Abstract

Schistosomiasis is a water-borne parasitic disease of great public health importance mainly in sub-Saharan African countries. The majority of current control programmes use the antihelminthic drug praziquantel to reduce disease burden in endemic areas. Praziquantel treatment has been reported to accelerate the development of protective immunity against re-infection that otherwise takes years to develop. To date, there is no licensed vaccine for schistosomiasis in humans but an attenuated schistosome parasite vaccine has been tested in animal models.

Employing systematic review and meta-analysis approaches, my PhD research has four main objectives relating to attenuated schistosome vaccine and praziquantel treatment: 1) to identify predictors that determine protection levels after treatment with attenuated *Schistosoma mansoni* vaccines in the mouse model, 2) to quantify the influence of host and schistosome parasite species on attenuated parasite vaccine efficacy, 3) to explore the direction of change (increase/decrease) in schistosome parasite-specific antibody isotypes after praziquantel treatment in humans, 4) to identify predictors of praziquantel efficacy in humans.

My analyses revealed three factors that have an influence on the protection levels provided by attenuated schistosome parasite vaccines: increasing numbers of immunizing parasites had a positive effect on the levels of protection whereas increasing the radiation dose and the

time to challenge infection had negative effects. Analyses showed that the attenuated schistosome vaccine has the potential to achieve protection levels as high as 79% after a single dose in mice. Alongside this, baboon studies consistently reported protective effects of attenuated schistosome vaccines against re-infection. These results show there is a high potential for an attenuated schistosome parasite vaccine to be effective in humans.

A meta-analysis of the influence of praziquantel treatment on the direction of change in schistosome-specific antibody isotypes was conducted. The analysis revealed considerable variability in the antibodies' direction of change among populations. The results also demonstrated an increase of anti-worm IgA and IgE in the majority of studies. These antibodies have been reported to have a protective effect against re-infection. The combination of age and infection intensity, and the number of days after treatment were identified as influential predictors for some antibody isotypes, but there was no single predictor that consistently affected all antibody isotypes in the same way.

Praziquantel efficacy levels in humans were investigated and the analyses revealed that cure rates for schistosomiasis increase with praziquantel dose, and were affected by the identity of the schistosome parasite species (*S. mansoni* vs. *S. haematobium*) and the age of the participants (children: 0-19 years old vs. adults: ≥ 20 years old). There has been no clear efficacy level reduction over the treatment years (1979-2013) suggesting that praziquantel is still effective in the treatment of schistosomiasis despite concerns about possible resistance.

The development of a schistosome vaccine will benefit from a closer investigation into the mechanisms through which protection is acquired in attenuated schistosome parasite vaccine studies showing high potential efficacy in animal models. Nevertheless, it will take time to develop a schistosome vaccine for human use. The uptake of the vaccine will be made even more challenging by the lack of adequate infrastructure in schistosomiasis endemic areas. In the meantime, close monitoring of praziquantel efficacy levels is necessary to confirm the effectiveness of schistosomiasis control in endemic areas.

Lay summary

Schistosomiasis is a water-borne disease caused by a group of flatworm parasites known as schistosomes. In areas with schistosomiasis, schistosome parasites initiate infection by penetrating the skin of people who come into contact with natural fresh water sources such as streams and ponds. We have an effective drug (praziquantel) for the treatment of schistosome infection that has been used for decades in schistosomiasis affected areas. However, re-infection is common following drug treatment, since streams and ponds are often essential water sources for people living in affected areas. Therefore, the development of additional methods for disease control is important. A considerable number of studies have been conducted to develop an effective vaccine for schistosomiasis. However, to date, we do not have a licensed schistosomiasis vaccine for human use. This study aims to answer the key questions that relate to drug treatment and vaccine development for schistosomiasis. Relevant scientific publications were identified by a systematic literature review, and reports from these articles were used for this study.

By analysing 131 scientific articles investigating schistosomiasis vaccines in animals, I found that weakened live-schistosome parasite vaccines are protective against schistosome infection in mice, baboons, and rat hosts. In addition, I found that an increase in vaccination dose could improve protection levels. This protection slowly declines over time but stays high for at least 6 months after vaccination in mice. The results of these animal experiments

confirm that there is potential for a weakened live-schistosome parasite vaccine to be effective against schistosome infection in previously unexposed populations. Further studies are required to estimate the influence of previous persistent schistosome infection and/or treatment on the protection levels.

I identified 106 scientific articles which reported efficacy levels of praziquantel treatment for schistosomiasis. I found that praziquantel treatment is still effective for schistosomiasis despite concerns that the parasites might be acquiring resistance against the drug after decades of use. In addition, my analyses revealed that efficacy levels of praziquantel treatment increased with drug dose, and were affected by the identity of the schistosome parasite species (*S. mansoni* vs. *S. haematobium*), and the age of the participants (child vs. adult).

There were 26 articles that reported whether levels of antibodies against schistosome parasites increased or decreased following praziquantel treatment. I found that there were more reports of an increase in antibodies which have been reported to be associated with protective immunity against re-infection: schistosome worm specific IgA and IgE antibodies. However, I also found that the pattern of changes in antibody levels was not consistent among different populations. These results suggest that the levels of protective immunity which can be stimulated by praziquantel treatment might vary among different populations.

The development of a schistosome vaccine will benefit from a closer investigation of the weakened live-schistosome parasite vaccine studies which provide significant levels of protection in animal models. Nevertheless, it will take time to develop a suitable vaccine for human use in developing countries. In the meantime, close monitoring of praziquantel efficacy is necessary to confirm the effectiveness of treatment programmes in affected areas.

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Contents

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Acronyms and abbreviations

CART	Classification and Regression Tree
DALYs	Disability-adjusted life years
df	Degree of freedom
ELISA	Enzyme-linked immunosorbent assay
Ig	Immunoglobulin
HIV	Human immunodeficiency virus
MDA	Mass Drug Administration
NTD	Neglected Tropical Disease
OD	Optical Density
SEA	Schistosome soluble egg antigen
WWA	Schistosome soluble worm antigen
WHO	World Health Organization
SE	Standard error of the mean
SD	Standard deviation
CI	Confidence interval

Chapter 1: Introduction

1.1. Background

Schistosomiasis, which is caused by blood fluke parasites in the genus *Schistosoma*, is a water-borne disease of great public health importance. Schistosomiasis transmission has been reported from 78 countries, where more than 260 million people are infected (WHO 2015). With this chronic parasitic disease, more than 4.5 million Disability Adjusted Life Years (DALYs) are lost each year worldwide (King *et al.* 2005; King *et al.* 2008; Gray *et al.* 2010). Schistosomiasis mainly affects the poorest populations of remote areas in African countries where people have limited access to safe water supplies and proper sanitary facilities (WHO 2015). Current control strategies for schistosomiasis consist mainly of mass administration of the antihelminthic drug praziquantel, a drug that has been used for more than 30 years in the treatment and control of schistosome infections (Gönnert *et al.* 1977; WHO 2015). Similar to other parasitic diseases, protective immunity against schistosome infection takes years to develop, leading to reductions in both infection prevalence and intensity in older age groups in endemic areas (Woolhouse *et al.* 1999; Mitchell *et al.* 2011). Some studies report that praziquantel treatment can accelerate the development of this protective immunity by exposing the parasite's hidden antigens (Harnett *et al.* 1986; Mutapi *et al.* 2005; Doenhoff *et al.* 2008). There are multiple schistosome parasite antigens that have been considered as vaccine candidates; however, to date there is still no licensed vaccine against schistosomiasis.

In this thesis, a systematic review and meta-analysis methods are used to identify the predictors that influence schistosome parasite vaccine efficacy levels in experimental animal models. The influence of praziquantel treatment on the direction of change (increase or decrease) in schistosome parasite-specific antibody isotypes are also explored. In addition, the predictors that influence the cure rate of praziquantel treatment for schistosome infections are identified. In this introductory chapter, the basic biology, epidemiology, and control methods of schistosomiasis are reviewed along with an overview of current knowledge about protective immunity against schistosomiasis. The methodology upon which the thesis is based, i.e. a systematic review and meta-analysis, is also briefly introduced. Although the main focus of this thesis is on *S. mansoni* and *S. haematobium* infections which cause intestinal and urogenital schistosomiasis respectively (Cline *et al.* 1977; King *et al.* 1988), findings from other schistosome species are mentioned where relevant.

1.2. Schistosome parasites, their lifecycle (Figure 1.1)

Schistosome parasites reproduce themselves by asexual reproduction in intermediate snail hosts and sexual reproduction in mammalian hosts, including humans. Inside the definitive host body, schistosome parasites develop into sexually matured adult worms that start to produce eggs, which can be released into the environment through contaminated urine and faeces. When eggs are released into environmental fresh water, the eggs hatch and release miracidia which infect intermediate snail hosts for asexual reproduction. Miracidia develop

into free-swimming mammalian-infective stage parasites (cercariae) in snails which are capable of penetrating human skin when humans become exposed to contaminated fresh water (Jordan *et al.* 1969). Cercariae transform into schistosomula by shedding their swimming tail. Schistosomulae pass to the hepatic portal system where males and females pair up. Paired adult worms migrate to the portal system (intestinal schistosomiasis caused by *S. mansoni* and *S. japonicum*) or the bladder (urinary schistosomiasis caused by *S. haematobium*). During their reproduction period, schistosome parasites produce large numbers of eggs daily. For example, a single *S. japonicum* worm pair can lay about 1,000-22,000 eggs per day, and in the case of *S. mansoni*, it is about 350 eggs per a day (Cheever *et al.* 1994). These eggs are mainly responsible for the pathogenesis of schistosome infections. While some eggs move from the veins to the lumen of the intestine (*S. mansoni* and *S. japonicum*) or the vesical veins surrounding the bladder (*S. haematobium*), over half of the eggs remain trapped in host tissues and cause chronic immune stimulation, and subsequent granuloma and fibrosis formation (Christie *et al.* 1986; Smith *et al.* 1986; Andrade 2009). Eggs excreted in urine and faeces are, in areas with poor sanitation, released to fresh water sources where schistosome parasites complete their life cycle.

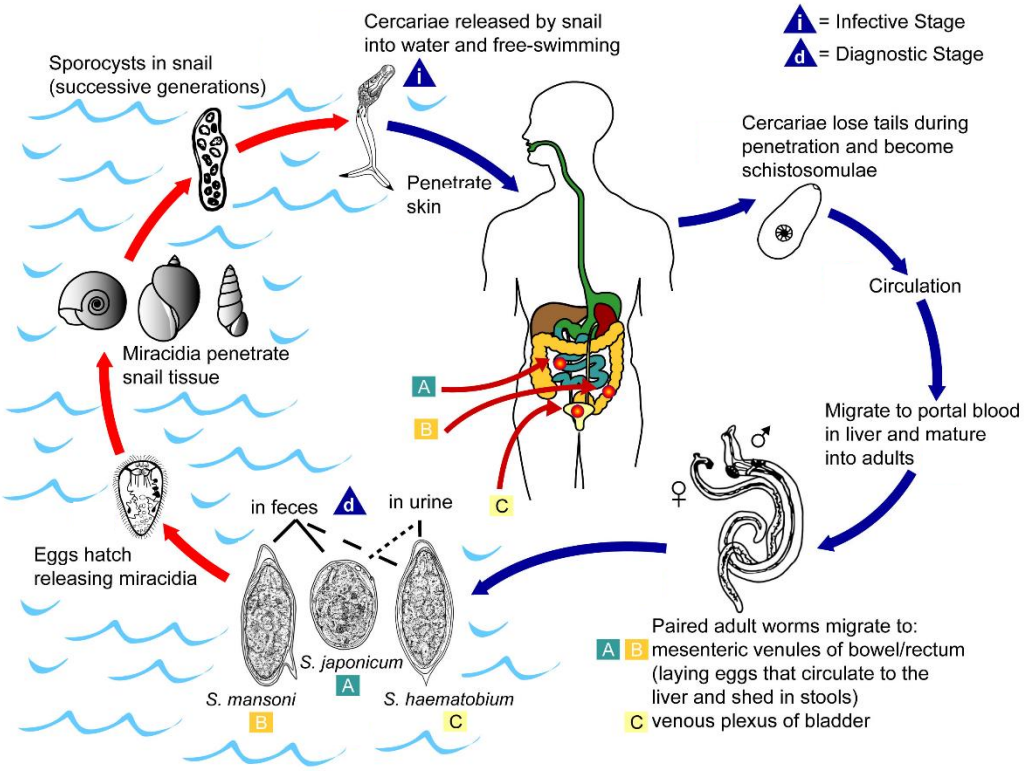


Figure 1.1: The life cycle of main schistosome species affecting humans. Adapted from the Centre for Disease Control and Prevention (CDC) online resources: http://www.cdc.gov/dpdx/schistosomiasis/ (accessed 15/01/2016).

1.3. Epidemiology of schistosomiasis

Humans are natural hosts for *S. mansoni*, *S. haematobium*, *S. japonicum*, *S. intercalatum*, *S. guineensis*, and *S. mekongi* (Ratard *et al.* 1990; Kato-Hayashi *et al.* 2010; Mone *et al.* 2012). Nevertheless, the majority of human schistosomiasis is caused by three major schistosoma species: *S. mansoni*, *S. haematobium*, and *S. japonicum* (Steinmann *et al.* 2006). Of the estimated 260 million people infected with schistosomiasis globally, more than 90% of cases are reported from sub-Saharan Africa where both *S. mansoni* and *S. haematobium* infections

are endemic (WHO 2015). *S. mansoni* is found throughout Africa, the Middle East, and a part of South America, *S. haematobium* is distributed throughout Africa and the Middle East, and *S. japonicum* is mainly found in China and Southeast Asia (WHO 2015). While the major hosts for *S. mansoni* and *S. haematobium* are humans, *S. japonicum* is capable of infecting both humans and other animal hosts including sheep, cattle and water buffalo in natural conditions. There are 10 common species associated with animal schistosomiasis, and among them, *S. mattheei* and *S. bovis* stand out because of their veterinary significance (Vercruysse *et al.* 2005). Several other schistosomiasis species that mainly infect birds have also been reported as being capable of causing human infection (Horak *et al.* 1999; Lichtenbergova *et al.* 2008).

In schistosomiasis endemic areas, the intensity and prevalence of disease infection tends to be highest among children, and lower in older individuals within the same populations (Fulford *et al.* 1992; Mutapi *et al.* 2006; Colley *et al.* 2014). This trend of infection intensity and prevalence has been associated with possible behavioural change by age. There are epidemiological reports that water contact peak among young children and then decrease as they get older (Chan *et al.* 2000). Nevertheless, there are some reports of peak of infection intensity among children despite higher water contact rate among adults than children (Kabatereine *et al.* 1999). Therefore, the change of water contact rate alone cannot explain the relationship between age and infection intensity. In addition, epidemiological studies have reported that the age that harbors the highest intensity of infections depends on the

disease transmission levels of the areas (Woolhouse 1992; Woolhouse 1998). The peak of infection intensity is higher and occurs at a younger age in schistosome-infected populations in highly endemic areas (i.e., the peak shift, as in Figure 1.).

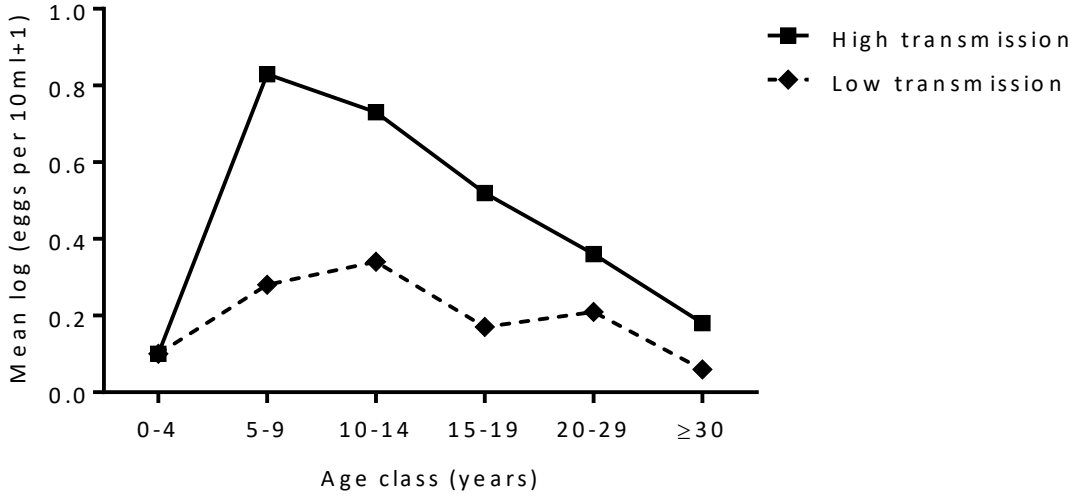


Figure 1.2: Age-infection intensity profiles from different high and low transmission areas indicating the peak shift. The plots show the age-infection intensity profiles of *S. haematobium* infections between populations of high (squares, solid line) and low (diamonds, dashed line) transmission areas. Adapted from Woolhouse (1998).

1.4. Protective immunity against schistosome infections

The age-related changes in infection levels (as in figure 1.3) have been associated with the development of naturally acquired protective immunity against schistosome infections (Woolhouse *et al.* 1999; Mitchell *et al.* 2012). The protective immunity is thought to take years to develop, as in endemic areas infants get infected by schistosome parasites as soon as they are old enough to come into contact with natural water sources (Ruganuza *et al.* 2015). A recent quantitative study has concluded that the naturally acquired protective immunity is

stimulated by the death of adult schistosome worms and consequently reduced fecundity (Mitchell *et al.* 2012).

Schistosome parasite specific antibodies have been reported to be associated with protection against schistosome infections. High levels of schistosome specific IgE have been reported to be associated with low re-infection rates of *S. mansoni* (Dunne *et al.* 1992), *S. haematobium* (Hagan *et al.* 1991) and *S. japonicum* (Zhang *et al.* 1997). Similarly, epidemiological studies have reported the association with high levels of IgA with low re-infection rates of *S. mansoni* (Vereecken *et al.* 2007). On the other hand, high levels of schistosome specific IgG4 have been associated with high susceptibility to both *S. mansoni* and *S. haematobium* infections (Grogan *et al.* 1997; Oliveira *et al.* 2012). In particular, epidemiological studies have reported that a high IgE ratio to IgG4 is associated with resistance to re-infection in both *S. mansoni* and *S. haematobium*, suggesting that IgG4 has a blocking effect on IgE (Grogan *et al.* 1997; Pinot de Moira *et al.* 2010).

1.5. Current control strategies

1.5.1. Mass Drug Administration (MDA)

The majority of schistosomiasis control programmes use the anthelminthic drug praziquantel for mass administrations. Since its discovery in the 1970s, praziquantel has been used as the first drug of choice against schistosomiasis in many endemic areas (Gönnert *et al.* 1977; WHO 2015). Currently, praziquantel is sold at US$ 0.08 per single 600 mg tablet with an average cost of US$ 0.14–0.30 per treatment (Evans *et al.* 2011). This reasonably-priced

efficacious drug has achieved a significant reduction in the disease's prevalence, infectious intensity, and morbidity in many endemic areas (Vennervald *et al.* 2005; Koukounari *et al.* 2007). In mass drug administration (MDA) programmes, praziquantel is given at 40mg/kg body weight regardless of the age or sex of participants (WHO 2006). The recommended frequency of praziquantel treatment in control programmes depends on the prevalence of schistosome infection among primary school children who are the main target of the treatment (WHO 2006). A yearly treatment of school-age children is recommended in areas with more than 50% prevalence of schistosome infection among school-age children (High-risk community), and a biannual treatment is recommended in areas with 10-50% prevalence of schistosome infection (Moderate-risk community). In areas with lower than 10% prevalence of schistosome infection among school-age children is recommended to occur twice during their primary school years. Recently praziquantel has confirmed to be safe and effective for infants and preschool-age children (aged 5 years and below) who were previously excluded from the treatment (Mutapi *et al.* 2011; Coulibaly *et al.* 2012). In 2010, WHO amended praziquantel treatment guidelines by recommending praziquantel treatment for infants and preschool-age children through regular health services (WHO 2011). This change could make it possible to apply MDA more widely in the future.

1.5.2. Intermediate Snail Host Control

The geographical distribution of schistosome parasites and schistosomiasis cases is limited by the distribution of fresh water intermediate host snails (Loker *et al.* 2005). Therefore, the control of intermediate host snail populations is regarded as another important intervention

against schistosomiasis. The snails can shed thousands of cercariae into waters for about a month while they are infected by miracidia larvae (Ward *et al.* 1988). Kariuki *et al.* have reported that molluscicide-based snail control, together with MDA, achieved significantly low levels of re-infection compared with drug administration alone (Kariuki *et al.* 2013). Similarly, King *et al.* have reviewed molluscicide control studies and have reported that molluscicide interventions can reduce the risk of new infections in targeted areas (King *et al.* 2015). However, Kariuki *et al.* have also mentioned the difficulty of maintaining control of snail populations, as the number of snails tends to recover when the interventions are interrupted (Kariuki *et al.* 2013). In addition, the toxic effect of a commonly used molluscicide (niclosamide) on fish and aquatic animals makes it difficult to use the molluscicide constantly (Takougang *et al.* 2006; Takougang *et al.* 2007; Yang *et al.* 2010). Therefore, more environmentally friendly approaches such as re-formulations of niclosamide and alternative types of molluscicide have been investigated to achieve sustainable snail control (Dai *et al.* 2008; Yuan *et al.* 2011; Xia *et al.* 2014).

Besides chemical molluscicide usage, a number of biological snail control programmes have also reported successful reduction of the snail populations. This includes the introduction of snail competitors (Pointier *et al.* 1989; Pointier *et al.* 1992) or snail predators (mainly fish) (Ben-Ami *et al.* 2001; Stauffer *et al.* 2006). The best snail competitors are closely related snail species that are not intermediate hosts for schistosome parasites. Although the competitor snail species are selected due to their resistance against schistosome infections,

there is still a risk of their becoming susceptible to the local schistosome parasites during the intervention (Lardans *et al.* 1998).

1.5.3. The improvement of infrastructure

Similar to several other neglected tropical diseases, proper sanitation and clean water supply systems are also very important factors in the success of control programmes (Asaolu *et al.* 2003; Zhou *et al.* 2013). Without appropriate sanitation systems, even just a single infected person can release a large number of schistosoma eggs into the environment (Cheever *et al.* 1994). Having a schistosome parasite-free water supply is also important, as in many schistosomiasis endemic areas where natural water sources are still essential for daily lives, people cannot avoid coming into contact with water in streams and/or ponds that can be contaminated with schistosome parasites (WHO 2015). However, the improvement of infrastructure is both time and resource consuming, especially because schistosomiasis is endemic mainly in the remote rural areas of developing countries. In addition, there are some reports that agricultural irrigation development is a risk factor for schistosomiasis (Steinmann *et al.* 2006). For example, a schistosomiasis epidemic occurred in the delta of the Senegal River Basin after a dam was constructed in the river, although there was no report of schistosome infection prior to dam construction (Talla *et al.* 1990). This is thought to be because the dam has changed the water flow in the local rivers, which made it suitable for intermediate host snail to inhabit (Talla *et al.* 1990; Southgate 1997).

1.5.4. Education

Health education has been shown to improve understanding of schistosomiasis transmission among people in the endemic areas (Aryeetey *et al.* 1999; Asaolu *et al.* 2003) which could lead the reduction of re-infection rates after chemotherapy (Hu *et al.* 2005). However, there are some people who cannot escape from water contact due to their occupation (e.g. agricultural and fishing populations) or housekeeping activities (e.g. washing, bathing) (Li *et al.* 2003). Added to this, endemic areas' ponds and rivers are often essential for inhabitants not only as water sources but also as places of socialization for adults and as playgrounds for children, making it difficult for them to change their behaviour.

1.5.5. Vaccine development against Schistosomiasis

In spite of the large research effort expended on the development of an effective vaccine against schistosomiasis, no vaccine has yet been approved for human use. Recent technological developments have enabled the production of recombinant vaccines that contain only those molecules of pathogens or molecules secreted by pathogens that are considered to stimulate most effectively host protective immunity (de Veer *et al.* 2011). In the early 1990s the WHO funded evaluations of *S. mansoni* candidate antigens to select the most promising vaccine candidates. Six proteins were tested in their study: the 63 kD parasite myosin, the 97 kD paramyosin, the 28 kD triose phosphate isomerase (TPI), a 23 kD integral membrane protein (Sm23), and the 26 and 28 kD glutathione-S-transferases (GSTs) (Wilson *et al.* 2006). Unfortunately, none of the above vaccine candidates conferred more than partial protection against future infections in experimental mice. The resulting

protections varied between 30% and 50% and was therefore too inconsistent to be fully satisfactory (Bergquist *et al.* 1998; Mountford *et al.* 2005; Wilson *et al.* 2006).

There are two promising recombinant vaccines against schistosomiasis they are currently undergoing clinical trials. The 28 kD *S. haematobium* GST (Sh28GST, named Bilhvax) for schistosomiasis has passed phase one and two clinical trials and now is in phase three clinical trials (Mountford *et al.* 2005; clinicaltrials.gov 2012; Riveau *et al.* 2012). Alongside this, a recombinant 14KDa, fatty acid-binding protein from *S. mansoni* (rSM14) has successfully passed phase one clinical trials has now proceeded into the phase two clinical trials (Santini-Oliveira *et al.* 2016).

As well as subunit vaccine studies, attenuated live schistosome parasite vaccines have been studied extensively in animal models (Wilson *et al.* 1999; Hewitson *et al.* 2005). Immunization with attenuated schistosome cercariae has been reported to be able to achieve as high as 79% protection against *S. mansoni* re-infection in murine studies (Fukushige *et al.* 2015). There are multiple attenuated parasite vaccines that have been used for veterinary purposes (Meeusen *et al.* 2007). For example, the attenuated vaccine for *Dictyocaulus viviparous* infection in cattle has been used for over 30 years (Jarrett *et al.* 1960; McKeand 2000). Nevertheless, it is not easy to move from animal studies into humans due to ethical and safety reasons. In fact, no live parasite vaccine is currently used in humans.

1.6. Systematic literature review and meta-analysis

Recent advances in technology have enabled us to access scientific publications from around the world more easily. Reflecting this, the number of articles that have reported results of the systematic review and meta-analysis approaches has increased dramatically over the last 25 years (figure 1.4). Systematic review and meta-analysis approaches have been commonly used in the fields of medicine, science, social science, business, and ecology (Borenstein 2009).

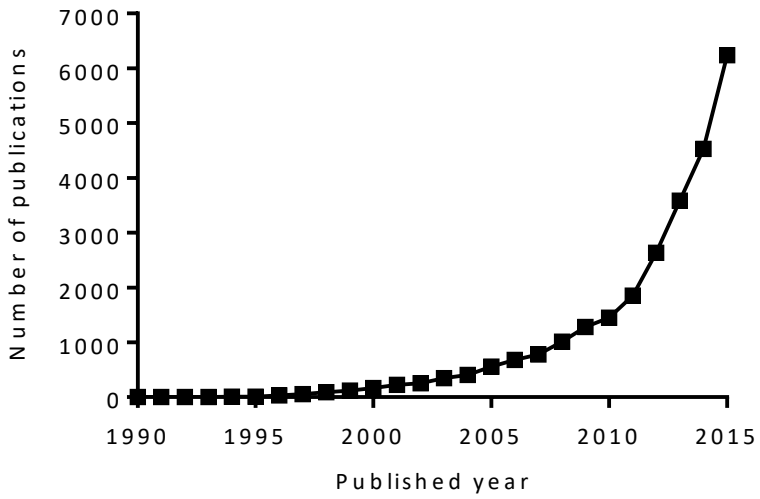


Figure 1.3: The increase in number of articles that use systematic review and meta-analysis methods over the years between 1990 and 2015. The number of publications published each year that contain the terms: "systematic review" AND "meta-analysis" in Pubmed. The search was conducted on 17th Jan. 2016.

1.6.1. The systematic review

The results of systematic review and meta-analysis studies have been regarded as the most reliable evidence in healthcare science (Sinha *et al.* 2006). This is because of the broader applicability of the results, as well as the high precision of pooled summary effect when meta-analysis is performed (Sinha *et al.* 2006). Therefore, the systematic review is currently the standard approach for synthesizing evidence in quantitative and/or qualitative way in health sciences (Shamseer *et al.* 2015). The systematic review approach has methodological advantages over traditional narrative reviews (Shamseer *et al.* 2015). In narrative reviews articles selection procedures are subjective and, therefore, the choice of studies that are included in the review is usually biased. In addition, in narrative reviews normally the methodological approaches used to conduct the review are not reported (Rother 2007). On the other hand, systematic reviews report the review procedure in a detailed and comprehensive manner, which makes study selection procedure clear. In addition, the same review procedure and results can be replicated by any trained reviewer by following a review protocol (Uman 2011).

A systematic review follows the following steps: 1) develop a research question, 2) break a research question down into concepts (e.g., target population, type of intervention, outcome) to develop a set of search terms for the review, 3) define inclusion and exclusion criteria for the article selection, 4) search electronic databases using search terms, 5) screen the identified articles with titles and abstract to exclude articles which are clearly irrelevant to the study, 6) screen the remaining full-text articles following inclusion and exclusion criteria,

7) extract data from individual studies, 8) assess study quality, 9) conduct a quantitative (e.g., meta-analysis, meta-regression) or qualitative synthesis of results (Mallett *et al.* 2012; Shamseer *et al.* 2015).

1.6.2. The meta-analysis

Following a systematic review, the researchers may then conduct a meta-analysis of the identified articles to estimate the pooled effect of interventions (Borenstein 2009). A single study sometimes fails to detect a statistically significant treatment effect on outcomes even when such an effect exist. This can be due to the number of participants/experimental animals being too small to detect such an effect. We could conduct a study with a large number of participants/experimental animals to overcome the effect of small sample sizes. However, a larger study is more expensive and sometimes logically challenging. The meta-analysis approach, on the other hand, can serve as an attractive alternative by allowing us to synthesize results from a number of studies to draw more conclusive and precise conclusion (Altman *et al.* 2001).

Meta-analysis is the statistical synthesis of the results of the studies identified through the systematic review to estimate a pooled effect (Chiappelli 2010). Results from a number of comparative studies evaluating the same or similar treatment effects are considered in the analysis (Altman *et al.* 2001). To obtain a pooled effect, analyses employ the fixed-effect or the random effect meta-analysis approaches (Borenstein 2009). The fixed-effect meta-analysis is based on the assumption that the treatment effect is common for all studies

in the analysis. Therefore, the variation among results of studies is considered as sampling error. Nevertheless, in many systematic review and meta-analysis studies this assumption cannot be justified. This is because, although only comparable studies are included for the meta-analysis, we cannot assume that all the studies are identical. The effect size could be higher (or lower) as a result of the unique characteristics of studies, for example, experimental animal strains, the age of animals, or the room temperature of the day. In contrast to the fixed-effect meta-analysis, the random-effects meta-analysis assumes that the treatment effect could vary between studies. Therefore, the analysis assumes different true treatment effects underlying different studies (Borenstein 2009). The goal of random-effects analysis is therefore to estimate the average effect in the studies (Walker *et al.* 2008). In many cases, the random-effects model can be regarded as a more reasonable option than the fixed-effect model.

1.6.3. Meta-regression and other analyses

The traditional approach of meta-analysis is to combine results of studies to yield a single pooled effect of the interventions using random-effects or fixed-effects meta-analysis. In cases where the size and heterogeneity of the data allow, further statistical analyses such as a meta-regression analysis can be conducted to identify influential predictors of the outcome variable (Borenstein 2009; Chiappelli 2010). The meta-regression analysis aims to identify predictors which have any influence on outcome (e.g., cure rate after praziquantel treatment, protection levels of vaccines) (Thompson *et al.* 2002). In other words, meta-regression is performed to investigate whether a particular predictor can explain any part of the

heterogeneity of treatment effects between studies. For example, meta-regression allows us to quantify the drug dose effect on efficacy levels, such as higher dose yielding a better treatment effect. The meta-regression analysis usually requires more than 10 studies to be included in the analysis, in contrast to standard meta-analysis which does not have a minimum number of studies (Higgins *et al.* 2011).

Regression analysis (e.g., linear regression analysis) can be used in primary studies to assess the relationship between one or more predictors and a dependent variable. The essential idea of meta-regression is the same as regression analysis, except that both predictors and dependent variable are at the study levels rather than the individual subject levels (Borenstein 2009). Similar to traditional meta-analysis, studies are weighted by the precision of their results. The inverse variance within each study is commonly used as an indicate of study precision (Borenstein 2009). In cases where the variables needed to calculate inverse variance within a study (e.g., standard error of the mean, standard deviation, or confidence interval of results) are not available, using sample size for weighting has also been suggested by Hunter and Schmdt as an alternative approach (Hunter *et al.* 2004; Brannick *et al.* 2011). In cases where the sample size is used for weighting, studies with larger sample sizes are assumed to yield better precision, and therefore have a greater power in the analysis. Using the inverse variance of each study does not make this assumption, each study is directly weighted according to the precision of the results and, therefore, this approach has been regarded as more accurate and sophisticated than the Hunter and Schmidt method (Hunter *et al.* 2004; Brannick *et al.* 2011). Nevertheless, the Hunter and Schmdt approach is still

valuable in realistic scientific research conditions, by allowing researchers to include more studies in the analyses (Brannick *et al.* 2011).

As the number of meta-analysis studies has been increasing dramatically in recent years, different statistical methods other than meta-analysis and meta-regression have started to be used. For example, there are meta-analysis studies using classification and regression tree models (CART models) (Dusseldorp *et al.* 2014). Using non-parametric test such as CART models investigate patterns in the data without making an assumption of normality, allowing us to analyse results from a greater variety of studies than meta-regression models.

1.6.4. Limitations of a systematic review and meta-analysis

There are limitations of systematic review approaches. Although systematic review approaches are designed to minimize bias, there are still potential biases which could occur at different steps of the review procedure. The most commonly reported risks of bias in systematic review studies are: publication bias, language bias, and selection bias. Various aids to minimize the bias are mentioned when such approaches have been proposed.

For studies using published articles there is always a risk of publication bias in any systematic review and meta-analysis (Sutton *et al.* 2000; Juni *et al.* 2002). Publication bias occurs when the results of published studies are systematically different from those of unpublished studies (Fujian *et al.* 2015). This systematic difference can occur because studies with statistically significant results or positive results are more likely to be published

than those with non-significant or negative results (Song *et al.* 2010; Fujian *et al.* 2015). Furthermore, those studies with significant findings are more likely to be published without delay: this type of publication bias is also specifically called as a time lag bias (Altman *et al.* 2001). Publication bias can be minimized by identifying and including unpublished studies (Fujian *et al.* 2015). Alongside this, the regular update of systematic review results is also important to minimize the time lag bias (Altman *et al.* 2001).

Systematic reviews also have a risk of language bias (Sutton *et al.* 2000; Juni *et al.* 2002). Language bias is a systematic bias that can occur when results of studies published in a particular language (usually English) are different from those of studies published in other languages (Morrison *et al.* 2012). Language bias can be minimized by including studies that are published in multiple different languages. However, non-English articles can be identified only if they at least published their titles and/or abstracts in English. Therefore, there is still a risk of missing non-English articles if they are published solely in the original language. In cases where it is clear that the disease or health problems of interest are more commonly found and reported from non-English speaking areas, it is worth considering collaborating with native-speakers of targeted languages, or to amend research questions to minimize the influence of language bias. For example, conducting a systematic review about *S. japonicum* infection, which is mainly endemic in China and Southeast Asia (WHO 2015), is difficult without native Chinese speakers in the review team as studies of this infection are frequently reported in Chinese journals.

Selection bias can be caused by following strict and detailed articles selection criteria (e.g., sample size, follow-up time) without exploring the relevant studies prior to developing such criteria. This could cause the risk of excluding relevant studies without consideration. A strict articles selection criteria is sometimes used for the initial search because of its convenience (Sinha *et al.* 2006). However, to minimize or avoid selection bias, it is preferable to start with a systematic review using more relaxed inclusion and exclusion criteria which allow a broad range of studies of varying methodological approaches and sample characteristics (e.g., outcome measures, characteristics of study participants, strains of experimental animals) to be considered. This initial review can be followed by the development of more detailed inclusion and exclusion criteria to identify more homogeneous set of similar studies.

All meta-analyses rely on data collected through a systematic review. The limitations of systematic reviews as described above are applicable to any statistical analysis performed after the review. In addition, scientific articles often report the results at the population or group level but not the individual subject level. Although results at the subject level may be reported in small studies, they are normally not reported in large-scale epidemiological studies. Therefore, meta-analysis studies normally use population or group level results for the analysis. Therefore, predictors identified by meta-regression analyses may not keep the same degrees of influence on the variability among individual participants within the same study (Thompson *et al.* 2002). In the near future, as data publishing become more comprehensive, a new type of meta-analysis may be conducted by synthesizing individual

subject or experimental animal data. However, in the meantime, extra care must be taken when discussing results from a meta-analysis to translate them into individual level phenomena.

1.7. Outline of the thesis

Schistosomiasis is a disease with a long history as a human infection. There is a large of scientific studies published which relate to immunization and drug treatment of schistosomiasis. Therefore, systematically reviewing these studies and updating reviews are important to keep our knowledge about schistosomiasis up to date. Current schistosomiasis control programmes rely heavily on the mass administration of the drug praziquantel. Widespread use of MDA might be putting selection pressure on the parasites that could eventually lead to the evolution of resistance (Norton *et al.* 2010; Humphries *et al.* 2012). Close monitoring of praziquantel efficacy levels is essential for maintaining sustainable schistosomiasis control programmes. In addition, a better understanding of the immune reaction against schistosomiasis in human will much contribute to the development of a vaccine against schistosomiasis, and the control of the disease.

Recently schistosomiasis control efforts undertaken in affected areas, especially a large number of national schistosome control programmes conducting MDA, have been more intensive than ever (Tuhebwe *et al.* 2015). In addition, recently praziquantel has proved to be effective for infant and preschool children (Mutapi *et al.* 2011). These considerations make it timely to conduct a systematic review and meta-analysis to identify factors affecting the efficacy of praziquantel treatment, and also the influence of praziquantel treatment on immunity of people from affected areas.

The overall aim of this thesis is to answer key questions which relate to vaccine development, praziquantel treatment and host immunity for schistosomiasis: attenuated schistosome parasite vaccine efficacy levels in animal models, and praziquantel treatment efficacy and its influence on immunity in humans were investigated. My PhD research has four main objectives: 1) to identify predictors that determine protection levels after treatment with attenuated *S. mansoni* vaccines in the mouse model, 2) to quantify the influence of host and schistosome parasite species on attenuated parasite vaccine efficacy, 3) to explore the direction of change (increase/decrease) in schistosome parasite-specific antibody isotypes after praziquantel treatment in humans, 4) to identify predictors of praziquantel efficacy in humans. In this thesis, the attenuated schistosome parasite vaccines in animal models are investigated, with a particular consideration to schistosome parasite-species and host species differences. The schistosomiasis cure rate with praziquantel treatment, and the influence of praziquantel treatment on host immunity is also explored. For all the analyses, data are collected through a systematic review. An overview of the chapters within this thesis follows.

In Chapter 2, meta-regression models using scientific article as a random effect are used to identify the predictors that have any influence on the levels of protection provided by the attenuated *S. mansoni* vaccine in mouse host models. The direction and magnitude of the effects of identified influential predictors on the level of protection are quantified.

In Chapter 3, random effects meta-analysis (forest plots) are used to quantify the pooled effect of the attenuated *S. mansoni* and/or *S. haematobium* vaccine on the levels of protection in baboon and rat models. The effect of homologous or heterologous schistosome parasite species for vaccination and challenge infection on protection levels are explored.

In Chapter 4, Classification and Regression Tree models are used to explore patterns in the direction of change in the schistosome parasite (whole worm or soluble egg antigens) specific antibody isotypes after praziquantel treatment in humans.

In Chapter 5, meta-regression models using scientific article as a random effect are used to identify factors that influence the schistosomiasis (*S. mansoni* or *S. haematobium* infection) cure rate after praziquantel treatment in humans. The direction and magnitude of the influence of identified predictors on cure rate is quantified.

Chapter 2: A meta-analysis of experimental studies of attenuated *Schistosoma mansoni* vaccines in the mouse model.

2.1. Introduction

The majority of control programmes use the antihelminthic drug praziquantel for MDA. This low-cost and efficacious drug has achieved a significant reduction in disease prevalence and infection intensity in many endemic areas (Midzi *et al.* 2008; Evans *et al.* 2011; Liu *et al.* 2011; WHO 2015). However, there are multiple reports of re-infection after chemotherapy (Leenstra *et al.* 2006; Tukahebwa *et al.* 2013; Webster *et al.* 2013). In addition, praziquantel can clear only adult worms and has little or no effect on existing eggs and immature worms (Xiao *et al.* 2009). This means there is need for additional complementary interventions, one of which is vaccination.

Slowly developing acquired immunity plays a crucial role in the reduction of infection prevalence and intensity in older age groups in endemic areas (Woolhouse *et al.* 1999; Mitchell *et al.* 2011). This suggests that exposure to schistosome antigens can promote protective immunity in humans, however, to date there is no licensed schistosome vaccine (McWilliam *et al.* 2012; Mutapi *et al.* 2013). Currently there are two leading vaccine

candidates, the 28 kDa *S. haematobium* GST (Sh28GST, Brand name: Bilhvax) which is now in phase 3 clinical trials (Mountford *et al.* 2005; ClinicalTrials.gov 2012; Riveau *et al.* 2012), and a recombinant 14KDa, fatty acid-binding protein from *S. mansoni* (rSM14) has successfully passed phase one clinical trials has now proceeded into the phase two clinical trials (Santini-Oliveira *et al.* 2016). Alongside recombinant antigen vaccine studies, the attenuated live cercariae vaccine has been studied extensively in mouse models (Wilson *et al.* 1999; Hewitson *et al.* 2005). Properly prepared attenuated cercariae live long enough to invade the host skin and stimulate protective acquired immunity against subsequent challenge infection but die in the host's body before they mature into adult worms (Smithers 1962). Attenuated schistosome cercariae vaccination experiments in animals use cercariae which are weakened by ionizing radiation (X-ray or gamma ray), ultraviolet, heat, or chemical treatment. Host animals are immunized with attenuated parasites either once or several times before challenge infection with non-attenuated pathogenic cercariae. A certain number of days after the challenge infection, immunized animals and control animals are perfused to quantify the level of protection due to immunization by comparing the number of adult worms recovered from both groups.

A large number of mouse experimental studies using attenuated *S. mansoni* cercariae for vaccination have been published since the 1960s (Bickle 2009), however such studies have never been systematically analyzed. The aim of this chapter was to conduct a meta-analysis to identify measurable experimental conditions (predictors) that affect the level of protection

against challenge infection of vaccinated animals. In addition, levels of each predictor associated with maximum levels of protection were estimated.

2.1.1. Objective of the study

The objectives of current study were:

1) to identify influential factors on efficacy levels of attenuated *S. mansoni* vaccine in mouse model,
2) to estimate optimal levels of each predictor to maximize the levels of protection.

2.2. Material and Methods

2.2.1. Systematic review

An electronic literature search was performed using Science Citation Index Expanded, Conference Proceedings Citation Index and BIOSIS Citation Index, all of which were provided through Web of Knowledge (www.webofknowledge.com). Alongside these, EMBASE (www.elsevier.com), OVID MEDICINE (www.ovid.com), and CAB abstracts were searched simultaneously through OvidSP (ovidsp.tx.ovid.com). Reference lists of all articles identified by the electronic search were searched manually for additional relevant reference. In addition, ProQuest Dissertations & Thesis Full Text (www.proquest.com) was searched as a source of pre-published and grey literature. The search terms were chosen to be as inclusive as possible and were; "cercaria*" AND ("irradiat*" OR "attenuat*") AND ("vaccin*OR schistosom*"). In addition, "Attenuate*" AND "schistosome*" AND "vaccin*" were also used for the search. This search was completed in July 2013. After duplicated articles were removed a total of 1013 articles were identified. Titles and abstracts were screened to exclude those that were not relevant to an attenuated schistosome vaccine animal model. Full texts of potentially relevant articles were reviewed for further selection. Non-English articles were included, and several Chinese and German articles were identified and translated into English by a native Chinese speaker and German speaker respectively for the analysis.

A study was considered eligible if it met all of the following inclusion criteria:

1) vaccination with attenuated cercariae

2) use of ionizing radiation for attenuation
3) use of percutaneous immunization and challenge (i.e. the natural transmission route for schistosome infection)
4) challenge infection using normal (non-attenuated) cercariae
5) worm burden measured after the challenge infection via perfusion
6) outcome (fraction of protection) reported or could be calculated.

In this study, a fraction of protection means the proportion of reduction in worm burden in vaccinated mice compared to that of control mice group. For articles which reported worm count after challenge infection, the following equation was used to calculate the outcome: fraction of protection = [(average number of worms per mouse retrieved from control group – average number of worms per mouse retrieved from vaccinated group) / average number of worms per mouse retrieved from control group]. In the case of articles which failed to report worm counts (allowing calculation of this quantity), only those that stated that they used the same equation as above were included.

Studies were excluded if they met any of the following exclusion criteria:

1) immunizing attenuated cercariae developed to adulthood
2) hosts were transgenic or genetically-engineered
3) hosts had an *in vivo* depletion of immune cells
4) attenuated cercariae were prepared by any means other than ionizing irradiation
5) a non-cercarial vaccine was used (e.g. adult worm, schistosomula, subunit)

6) an artificial infection was conducted prior to vaccination

7) hosts were treated with anthelminthic drugs.

Some articles reported results from multiple separate experiments such as use of different doses of attenuated parasite. In these cases, results from each experiment were recorded as an observation. A list of potential predictors (given in Table 2.1) was drawn up and these quantities were extracted from each article. These potential predictors have been suggested their importance by review articles and also their quantities been reported by many experimental studies (Dean 1983). When an article reported a dose range rather than an exact dose the mid-value was used for the analysis.

Table 2.1: Possible predictors investigated and their units/ codes

Variable name	Unit/ Code
Number of immunizing parasites (total and number per dose)	log10 (number of parasites)
Number of challenge parasites	log10 (number of parasites)
Number of immunizations	Count
Irradiation dose	Krad
Host age	Weeks
Host sex	Male/Female/mixed
Time between the last immunization and challenge	Days
Time between challenge and perfusion	Days

2.2.2. Statistical analysis

Meta-regression was used to identify the influential predictors and effect of dose on protection. Multiple observations (1 to 56) were recorded from single articles and therefore article was included as a random effect in the models. The models were built using a backwards stepwise procedure with 8 potential predictors (listed in Table 2.1). Model selection started with a model with all 8 potential predictors. The least significant predictor, which had a largest p-value, was then removed from the model. In subsequent steps, the least significant remaining predictor was removed from the model until all the predictors in the model were statistically significant ($p<0.05$). The effect of the number of immunizing parasites was explored in two ways in the two separate models: as an average number of immunizing parasites per dose or as a total number of immunizing parasites. Correlations between variables were examined visually by scatter plot graphs for all possible predictor combinations to check for multicollinearity (data not shown). Then, all the possible combinations of two-way interactions of potential predictors were examined using a meta-regression model with two-way interactions. The outcome variable (fraction of protection) was transformed as –ln(1- fraction of protection) to reduce the skewness of residuals (Vittinghoff 2012). Logarithmic transformation is commonly used to transform a highly skewed distribution into one that is less skewed, therefore transforming the data into the one that is closer to a normal distribution, helping meet the model assumption of normality. Although using confidence intervals and standard errors is the most common weighting method for meta-regression (Borenstein 2009), many studies in this dataset failed to report either confidence intervals or standard deviations and there were no comparable

studies which enabled us to justify imputing them. Two kinds of information were available on the size of the studies: the number of control animals and the number of vaccinated animals. The majority of studies used similar numbers of control and vaccinated animals; however, there were several articles which used a higher number of vaccinated animals than control animals. To account for the impact of these unbalanced studies, the number of control animals was used as the more conservative weighting option.

2.2.3. Missing values and outliers

Several outliers were excluded from the analysis. They were six observations with animals kept longer than 300 days or less than seven days after the last immunization and four observations that used more than 10,000 cercariae for immunization. After excluding outliers 745 observations were kept for further selection.

When the numbers of control animals were not reported in an article and only the numbers of vaccinated animals were given, numbers of control animals were then imputed by a linear regression imputation method between numbers of vaccinated and control animals for all studies (Little *et al.* 2002). When the observation was missing for both the number of control and vaccinated animals (4 observations from 4 articles), the average number of control animals of the remaining data set was used for imputation, which was 10 control animals.

2.2.4. Statistical software

Papers identified by systematic review were recorded by Thomson Reuters EndNote and the extracted data were entered into a Microsoft Excel 2010 spreadsheet for further analysis. IBM SPSS Statistics Version 19.0 and Minitab. Inc. MINITAB 16 were used for statistical analysis. GraphPad Software GraphPad Prism version 6.03 was used for graphical expression.

2.3. Results

A total of 1,013 potentially relevant articles were identified through systematic review. From these, a total of 755 observations from 105 articles (articles are listed in the Appendix A.1) met the search criteria and also used the mouse as a host and *S. mansoni* for vaccination and challenge infection. Although the mouse is not a natural host for schistosome infection, it is the most commonly used animal for in vivo attenuated schistosome parasite research.

Among eight potential predictors (Table 2.1), three predictors were found to have statistically significant effects ($p<0.05$) on the outcome value -ln(1-fraction of protection) following the backwards stepwise selection: the log10 transformed total number of immunizing parasites [$F(1, 712)=70.74$, $p<0.001$], the irradiation dose [$F(1, 721)=26.25$, $p<0.001$], and the time between the last immunization and challenge [$F(1, 699)=5.56$, $p=0.019$] (Table 2.2). The reported ranges of each predictor were: the total number of immunizing parasites (50-5,000 cercariae), the irradiation dose (3-160 krad) and the time between the last immunization and challenge (7-230 days). All identified predictors were significant ($p<0.05$) in the model no matter with or without outliers in the model. The number of immunizing parasites was significant in the model regardless of the version of this variable used, i.e. the average number of immunizing parasites per dose or total number of immunizing parasites. In both cases the models were initially considered with the number of immunizations. When the total number of immunizing parasites was used as a predictor, the number of immunizations was not significant. Therefore, for the final model, the total

number of immunizing parasite was used as a predictor with number of immunizations excluded from the model.

Table 2.2: Results from meta-regression multivariable analyses. Positive coefficients indicate the predictor's positive dose effect on fraction of protection whereas negative coefficients indicate predictor's negative influence on fraction of protection.

Predictors	Coefficient	Standard error	F-value (df)	p-value
$\log_{10}$ (number of immunizing parasites per dose)	0.50	0.060	70.74 (1, 712)	<0.001
Irradiation dose	-0.0039	0.00077	26.25 (1, 721)	<0.001
Time between the last immunization and challenge	-0.0016	0.00066	5.56 (1, 699)	0.019

The interaction between $\log_{10}$ transformed total number of immunizing parasites and the time between the last immunization and challenge was statistically significant [$F(1, 718)=4.31$, $p=0.038$]. However this interaction was excluded from the final model for the following reasons: 1) the model with the interaction showed biologically implausible fitted values of fraction of protection for some predictors, 2) the model with/without interaction showed similar fitted values for the fraction of protection around the most frequent values of predictors.

Fitted graphs for each predictor are shown in Figure 2.1 with the outcome variable back-transformed to fraction of protection. Fitted graphs for each predictor were generated by fixing other predictor values at their modes: 500 immunizing parasites, 28 days for the time between the last immunization and challenge, and 20 krad for irradiation dose (solid line in Figure 2.1). The fitted graph of total number of immunizing parasites and fraction of protection showed the lowest level of predicted protection was 36% with 50 cercariae which increased up to 77% with 5,000 cercariae (solid line in Figure 2.1A). The minimum level of protection predicted for 3 krad irradiation was 64% which decreased to 33% with 160 krad irradiation (solid line in Figure 2.1B). Similarly, the estimated level of protection 7 days after the last immunization was 63% which reduced to 47% by 230 days after the last immunization (solid line in Figure 1C). Fitted graphs showed that the total number of immunizing parasites had a positive impact on the fraction of protection whereas irradiation dose and the time between the last immunization and challenge had negative impacts (Figure 2.1). Besides this, to estimate the highest protection, fitted graphs for each predictor were generated with other predictor values at their optimal level: 5,000 immunizing parasites, 7 days for the time between the last immunization and challenge, and 3 krad for irradiation dose (dashed line in Figure 2.1). The results showed that the highest protection estimated by the model results was 79% at 7 days after the last immunization, for mice immunized with 5,000 cercariae attenuated with 3krad (dashed line in Figure 2.1). This 79% protection decreased over time but stayed as high as 70% by 230 days after the last immunization (dashed line in Figure 2.1C).

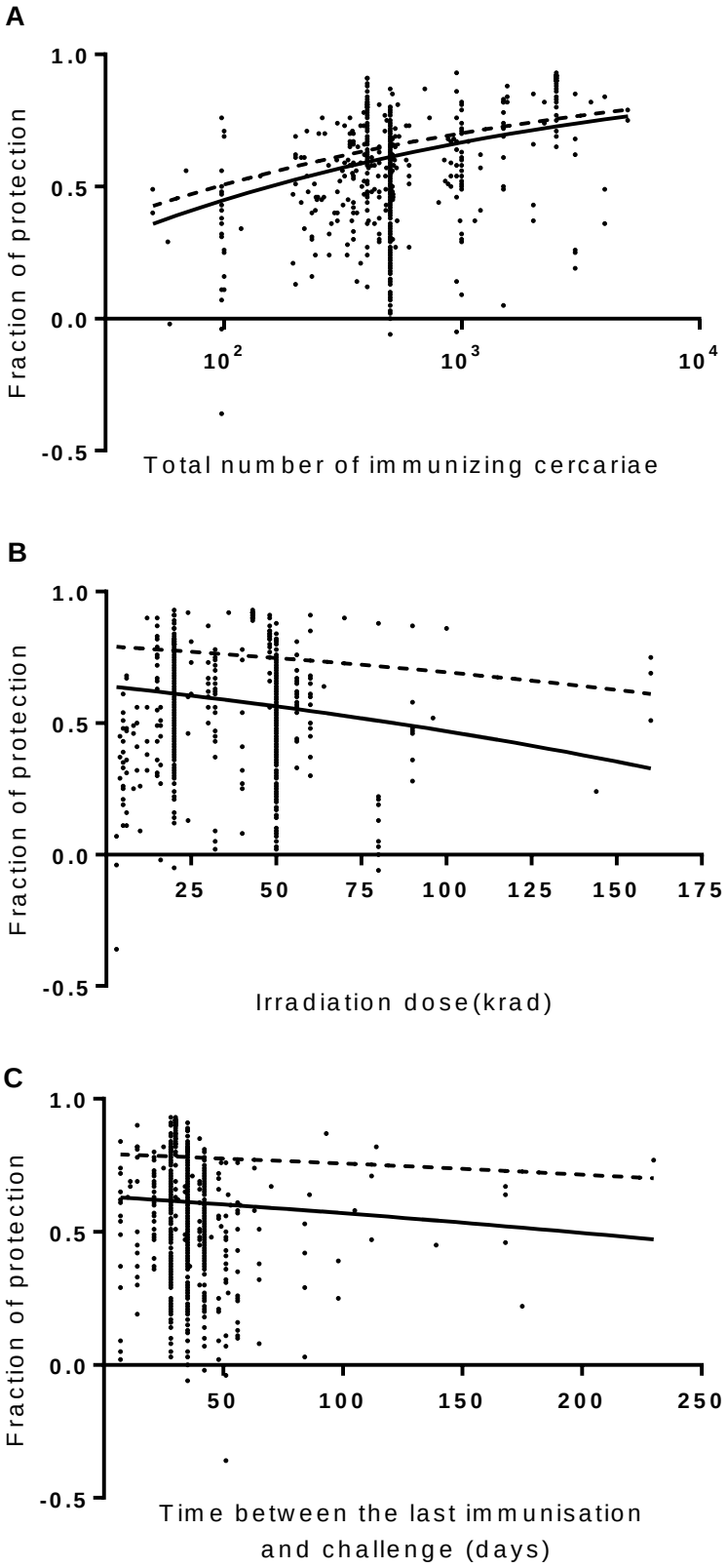

A
1.0
0.5
0.0
-0.5
Fraction of protection
10^2
10^3
10^4
Total number of immunizing cercariae
B
1.0
0.5
0.0
-0.5
Fraction of protection
25
50
75
100
125
150
175
Irradiation dose(krad)
C
1.0
0.5
0.0
-0.5
Fraction of protection
50
100
150
200
250
Time between the last immunisation
and challenge (days)

Figure 2.1: Fitted graphs for identified predictors from a meta-regression model. Identified predictors' effects on fraction of protection in mouse model: (A) the number of immunizing cercariae over the range 50-5000 cercariae (B) the irradiation dose over the range 3-160 krad (C) the time between the last immunization and challenge over the range 7-230 days. Data points indicate reported fraction of protection for each observation. Negative fractions indicate that vaccination was associated with increase of schistosome worm burden. Lines are fitted graphs generated from meta-regression (see text). Dashed lines in the graphs show the highest achievable level of protection which was estimated by analyses over the observed range.

2.4. Discussion

Irradiated *S. mansoni* cercariae vaccines have been tested experimentally against schistosome infection for decades, with important insights obtained from the individual experiments (Bickle 2009). Although the translation of the irradiated parasites vaccine in humans has not been pursued for schistosomiasis, a precedent for this type of approach for human vaccination has been set by malaria vaccine which uses live attenuated *Plasmodium falciparum* sporozoites (Sanaria®PfSPZ Vaccine) and has completed its phase 1 clinical trials and is now undergoing phase 2 clinical trials (Seder *et al.* 2013; WHO 2016). This study represents a meta-analysis of the experimental irradiated cercariae vaccine studies to identify robust variables that affect the levels of protection to inform human vaccine research and development.

The meta-regression models identified three predictors of the reduction in worm burden: these were the total number of irradiated cercariae per immunization, the time between the last immunization and challenge, and the irradiation dose for parasite attenuation. I identified a positive correlation between the number of irradiated cercariae per immunization and the level of protection within the range of 50-5,000 cercariae used in the original studies. The models suggested that the optimally prepared irradiated cercariae vaccine could achieve a protection as high as 79% against challenge infection. As fitted graphs have shown, this is predicted for a single vaccination with 5,000 cercariae attenuated with 3 krad irradiation. This protection declines over time, but remains high for at least 8 months after the last immunization. Approximately 70% protection against challenge infection could be achieved

after 8 months. This trend was consistent in the mouse experimental hosts with *S. mansoni* vaccination studies. The number of immunizing cercariae represents the antigen dose, my results show a positive dose dependency of schistosome attenuated vaccine for higher protection. Studies of live attenuated vaccine for malaria infection also reported a similar positive correlation between the dose of immunizing parasites and the level of protection against future infection. Recently, as part of the phase1 clinical trial of the human malaria vaccine using live attenuated sporozoites (Sanaria® PfSPZ Vaccine), a dose-escalation trial was conducted using 7,500-135,000 irradiated *Plasmodium falciparum* sporozoites per immunization. The participants group that received the highest dose per immunization achieved the highest levels of protection against challenge infection (Epstein *et al.* 2011; Epstein *et al.* 2013; Seder *et al.* 2013). The results showed that the number of immunizing cercariae is also an important factor for higher protection in mouse model studies.

The result from the meta-regression model showed a decrease in the fraction of protection with an increased time between the last immunization and challenge. This period between immunization and challenge represents the time to secondary encounter with the same antigen. When the initial encounter with the antigen takes place via infection or vaccination, the number of B and T cell produced against the antigen increases dramatically (Kaech *et al.* 2007; Harty *et al.* 2008; Sallusto *et al.* 2010; Farber *et al.* 2014). Only a small fraction of those cells will survive as antigen-specific memory T and B cells or as long-lived plasma cells and they will be maintained for a long time (Kaech *et al.* 2007; Harty *et al.* 2008; Sallusto *et al.* 2010; Farber *et al.* 2014). The duration of immune memory in humans after

the vaccination is still controversial (Crotty *et al.* 2004). However, there are several reported estimates for the length of immune memory after the last booster vaccination; 15 years for combined hepatitis A and B vaccine (Van Damme *et al.* 2012), 22 years for hepatitis B vaccine (McMahon *et al.* 2009), over 30 years for poliovirus vaccine (Bottiger *et al.* 1998; Crotty *et al.* 2004), and over 60 years for smallpox vaccine (Crotty *et al.* 2003; Crotty *et al.* 2004). A longitudinal immuno-epidemiological study of schistosomiasis has been conducted by Butterworth *et al.* which reported that the protection induced by chemotherapy can remain for as long as 21 months after the treatment (Butterworth *et al.* 1985). However, other studies reported treated participants' re-infection within one year (Garba *et al.* 2013; Tukahebwa *et al.* 2013). One of the difficulties in evaluating the length of protective immunity in humans is that, in contrast to experimental animals, humans encounter a variety of antigens that could stimulate their immune systems through their daily life. In addition, people infected and being treated for schistosomiasis normally live in schistosomiasis endemic areas. Their encounters with infectious cercariae may work as "natural booster" to stimulate protective immunity. In current study, the times between the last immunization and challenge (7-230 days) were relatively short compared with the life span of humans and schistosome parasites. This reflects that the average lifespan of a mouse is much shorter than that of the schistosome parasite (Kohn 1971; Fulford *et al.* 1995). The decrease in the fraction of protection over time was captured with my models even within this relatively short time range. This result would suggest that boosting vaccines may be necessary for long lasting protection against schistosomes.

There are several different cercariae attenuation methods as I described in the introduction. Within these, ionizing radiation (X-ray of gamma ray) is the most commonly used attenuation method for attenuated schistosome cercariae preparation. Two relatively high irradiation doses around 20 or 50 krad have been reported as the optimal doses for parasite attenuation (Minard *et al.* 1978; Bickle *et al.* 1979) and, in fact, many past studies have applied these irradiation doses. However, my results suggest that a lower irradiation dose could improve protection. The lower irradiation doses enable attenuated parasites to live longer in the vaccinated host. After vaccination, irradiated cercariae have been reported to be present around the skin exposure site for approximately 4 days and then gradually moved to the lungs where they transformed from cercariae into lung stage schistosomulae (Mangold *et al.* 1984). It has been reported that the immunizing parasite has to reach the lungs and transform to lung stage schistosomula to elicit protective immunity against challenge infection (Mangold *et al.* 1984; Dean *et al.* 1992), which may not be the case for cercariae attenuated with high doses of ionizing radiation. Several studies have reported that non-attenuated challenge cercariae in vaccinated mice slowly move to the lungs and then gradually disappear (Wilson *et al.* 1986; Dean *et al.* 1992). Several studies report that cercariae exposed to extremely high irradiation doses will die in the host skin before they migrate inside the host body (Hsu *et al.* 1981; Mangold *et al.* 1984). Mountford *et al.* reported that hosts needed to be exposed to both highly irradiated cercariae, that die in the host skin, and lung-stage schistosome parasites to elicit protective immunity (Mountford *et al.* 1992). One of the possible reasons for the high levels of protection observed when using irradiated cercariae vaccine is that hosts are exposed to a wide variety of antigens which are

expressed by different parasite life stages. Parasites which were attenuated with lower irradiation dose can survive long enough to express a variety of antigens from different life stages (Curwen *et al.* 2004). The results of my meta-analysis suggest that for recombinant vaccine development the combination of antigens which are unique to the different schistosome life stages may be an important factor in achieving a better protection.

In this study, I identified three predictors for effective immunization against schistosome infection using attenuated cercariae: the total number of immunizing parasites, the irradiation dose, and the time between the last immunization and challenge. The study results suggested that the optimally prepared irradiated cercariae vaccine could achieve a protection as high as 79% against challenge infection. Within the reported dose range, the model estimated that maximum protection could be achieved with the highest number of immunizing cercariae (5,000) and the lowest irradiation dose (3 krad). This protection slowly declines but remains high for at least 8 months after the last immunization. This achievable protection is much higher than the partial protection reported by the *S. mansoni* purified antigens that failed to achieve consistent protection above 40% in mice (Bergquist *et al.* 1998; Mountford *et al.* 2005; Wilson *et al.* 2006). Although none of the radiation attenuated cercariae vaccine studies achieved complete protection against challenge infection, it is thought that partial protection induced by immunization can significantly reduce both schistosome related morbidity and parasite transmission (Burke *et al.* 2009; Mitchell *et al.* 2012). This meta-analysis shows there is the high potential for an attenuated

cercarial vaccine, while also providing insights which are important for schistosome vaccine development.

Chapter 3: The influence of host and schistosome parasite species on the attenuated parasite vaccine efficacy: a meta-analysis

3.1. Introduction

There are three major schistosome species that cause human infections: *S. mansoni*, *S. haematobium*, and *S. japonicum*. Infections by these three species are widely reported from different parts of the world. Since *S. mansoni* and *S. haematobium* tend to overlap in their geographical distributions (WHO 2015), people in endemic areas are at risk of co-infection with these parasites. Therefore, it is important to know whether a single vaccine can be effective against multiple schistosome parasite species. Homologous and heterologous schistosome parasite species for vaccination and challenge infection have been studied in mouse hosts. In this chapter, a meta-analysis of these studies was conducted to investigate the influence of homologous and heterologous schistosome parasite species for vaccination and challenge infection on protection levels.

In Chapter 2, I demonstrated the importance of the number of immunizing parasites, the irradiation dose for attenuation, and the time between the immunization and challenge infection (Table 2.2) for the protection levels against challenge infection in the mouse host model (Fukushige *et al*. 2015). Beside mouse host studies, there are a number of attenuated

schistosome parasite vaccine studies that have been conducted in baboon and rat hosts using *S. mansoni* or *S. haematobium* parasites. However these studies have not been systematically analysed to date. Here, I conduct a meta-analysis on these studies incorporating the three predictors that were identified in the previous chapter. In addition to this, I conduct a meta-analysis to estimate the pooled effect of the attenuated parasite vaccine across these studies.

The objectives of this study were:

1) to determine the influence of homologous and heterologous parasite species for vaccination and challenge infection on the efficacy of the attenuated schistosome parasite vaccine in the mouse model;
2) to investigate the influence of the three previously identified predictors (Table 2.2) in baboon and rat model studies;
3) to estimate the pooled effect of the attenuated *S. mansoni* and *S. haematobium* vaccines in baboon model studies, and the attenuated *S. mansoni* vaccine in rat model studies.

3.2. Materials and Methods

3.2.1. Systematic review and data extraction

The procedure for the systematic review is the same as described in Chapter 2 with the following exceptions. In this review, studies with baboons that used schistosomula with non-percutaneous immunization (injection) were kept in the analysis. A preliminary analysis was conducted to investigate the influence of schistosome parasite life cycle stage (cercariae/schistosomula) on protection levels, using univariate meta-regression models. The analyses confirmed that there was a negligible effect of life cycle stage on protection levels ($p>0.05$) in baboon host studies (results not shown). *S. mansoni* studies with rat hosts were included only if they used percutaneous cercariae immunization and challenge infection like in the mouse host with *S. mansoni* studies in Chapter 2. Articles often reported results from multiple separate experiments such as the use of different doses of attenuated parasite. In such cases, each experimental result was recorded as an observation. A total of 29 observations from 11 articles using baboon hosts with *S. mansoni* or *S. haematobium* (Group 3 in Figure 3.1), and 47 observations from 11 articles using rat hosts with *S. mansoni* (Group 4 in Figure 3.1) met the search criteria and were included in this study. In addition, I included a total of 70 observations from 10 articles using mouse as the host and *S. mansoni*, *S. haematobium*, *S. japonicum*, or *S. bovis* for vaccination and/or immunization but not homologous *S. mansoni* immunization and challenge infection (Group 2 in Figure 3.1).

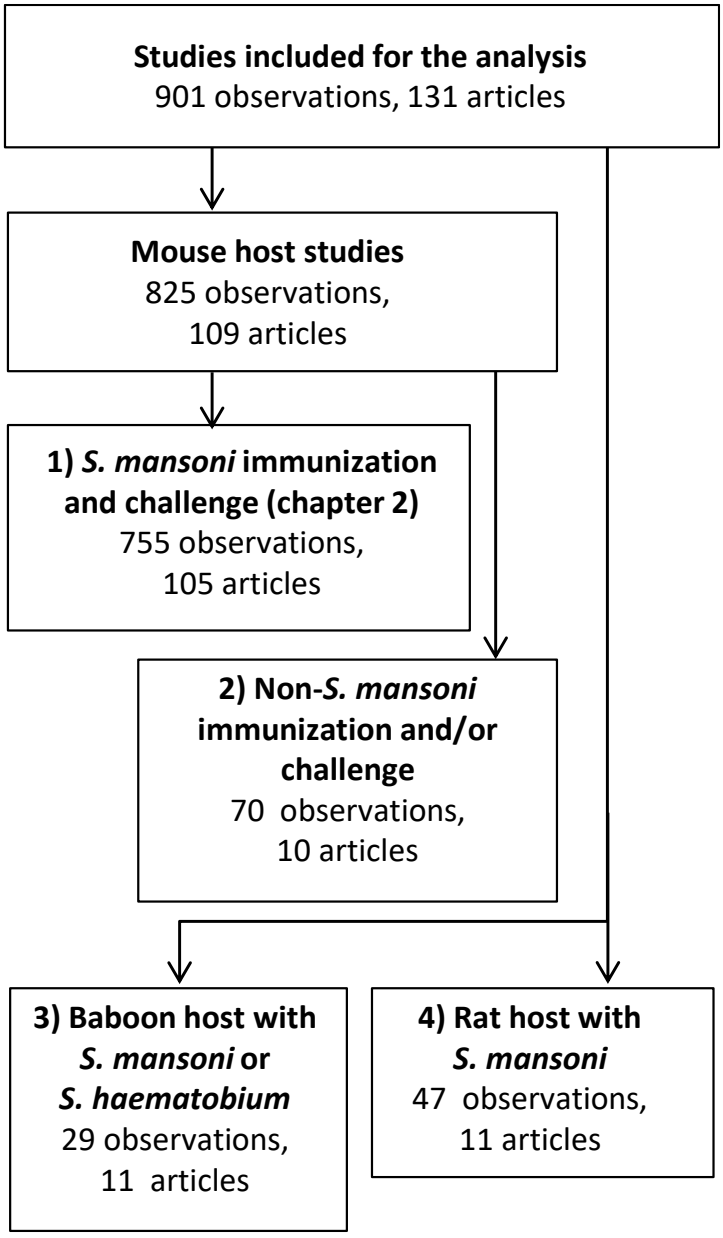


Figure 3.1: Data selection for the meta-analysis. Observations were divided into four groups according to host species and parasite species used in the studies: 1) mouse model using *S. mansoni*, 2) mouse model where immunization and/or challenge infection used non-*S. mansoni* schistosome species, 3) baboon model with homologous immunization and challenge infection using either *S. mansoni* or *S. haematobium*, 4) rat model with

immunization and challenge infection using *S. mansoni*. There were six articles included both in group 1 and 2, because they reported results using both homologous *S. mansoni* immunization and challenge (Group 1), and immunization and/or challenge infection with non-*S. mansoni* species (Group 2).

3.2.2. Statistical analysis

3.2.2.1. Meta-regression models

To test the effect of host species on the efficacy of the attenuated schistosome parasite vaccine, I fitted a model with host species, the number of immunizing parasites, the irradiation dose for attenuation, and the time between the immunization and challenge infection as predictors. The latter three predictors (Table 2.2) were those I identified using mouse host and *S. mansoni* immunization and challenge studies in Chapter 2. For this analysis the data for *S. mansoni* with mouse host studies (Group 1 in Figure 3.1), baboon host studies (Group 3 in Figure 3.1) and rat host studies (Group 4 in Figure 3.1) were used. As in the analyses in Chapter 2, the outcome variable (fraction of protection) was transformed as –ln(1- fraction of protection) to reduce the skewness of residuals (Vittinghoff 2012). In addition, the number of parasites was also transformed as $\log_{10}$ (number of immunizing parasites per dose) for the analysis.

I ran three additional meta-regression models using three previously identified predictors (number of immunizing parasites, irradiation dose, and the time between immunization and challenge; Table 2.2) to explore their influence on vaccine efficacy in different animal host

models. Studies using the following combinations of host and schistosome parasite species were used for these three models: baboon with *S. mansoni*, baboon with *S. haematobium* (Group 3 in Figure 3.1), and rat with *S. mansoni* (Group 4 in Figure 3.1). To account for the fact that some articles reported multiple experimental results (observations), all articles were assigned a unique article ID, which was included as a random effect in the model. The observations were weighted according to their sample size (the number of control animals). One study failed to report the number of control animals (rat host *S. mansoni*). Therefore this observation was weighted using the median number of control animal among rat studies (n=6).

Alongside this, to investigate the influence of parasite species on protection levels, I fitted a meta-regression model with the three predictors identified from mouse hosts and *S. mansoni* data (Table 2.2) as well as three additional predictors: 1) immunizing parasite species, 2) challenge parasite species, and 3) heterologous or homologous schistosome species challenge infection using the mouse host data (Group 1 and 2 in Figure 3.1). To explore the influence of homologous or heterologous schistosome parasite species used in immunization and challenge infection further, a graph was generated showing reported percentage of protected values together with schistosome parasite species for vaccination and challenge infection.

3.2.2.2. Random effects meta-analysis: forest plots

Forest plots were generated using Review Manager (RevMan 5) for baboon and rat host

studies to estimate the pooled effect of attenuated *S. mansoni* and *S. haematobium* vaccine effect on the infection intensity (by comparing the number of schistosome worms retrieved after challenge infection between vaccinated and control animals). Studies were included in this analysis only if they reported the standard deviation (SD), standard error of the mean (SE) or confidence interval (CI) of retrieved worm count from both vaccinated and control animals. Article IDs of these studies were included as a random effect in the meta-analysis models. These models were used to estimate both the standardized mean difference (Hedges' adjusted g) of each study as well as the pooled effect of irradiated *S. mansoni* vaccination in these baboon and rat host studies (Borenstein 2009; Deeks *et al.* 2010). Study names in the forest plot were organized by the name of the author followed by the year of publication. In cases where an article reported multiple observations, observations were distinguished by adding a letter after the first author's name and the year of publication. There are multiple studies where a single control animal group was used to compare with multiple vaccinated animal groups, in such cases the number of control animals was divided by the number of vaccinated groups to balance the weight of the observation in the meta-analysis (Higgins *et al.* 2011).

3.2.3. Statistical software

Articles identified by the systematic review were recorded using Thomson Reuters EndNote and the extracted data were entered in a spread sheet using Microsoft Excel 2010. B. Tummers, DataThief III. 2006 was used to extract data from published graphs. IBM SPSS Statistics Version 19.0 and Minitab. Inc. MINITAB 16 were used for the meta-regression

analysis. Review Manager (RevMan) [Windows] version 5.3. was used to generate the forest plots and estimate the pooled mean. GraphPad Software GraphPad Prism version 6.03 was used to draw graphs.

3.3. Results

3.3.1. The influence of host species on protection levels

A total of 1,013 potentially relevant articles were identified through a systematic literature review. From these, a total of 29 observations from 11 articles using baboon hosts with *S. mansoni* or *S. haematobium* (Group 3 in Figure 3.1), and 47 observations from 11 articles using rat hosts with *S. mansoni* (Group 4 in Figure 3.1) met the search criteria and included in the analyses. There were 5 observations from a single article using baboon hosts with *S. haematobium* that failed to report the number of immunizing parasites. Otherwise, values of all three potential predictors were reported by all articles used for the analysis.

The influence of host species (mouse, rat, or baboon) on protection levels of attenuated *S. mansoni* vaccination was statistically significant using meta-regression analysis [$F(2, 246)=17.22$, $p<0.001$] when species added to previously identified predictors (Table 2.2). The results suggested significant lower protection levels in baboon hosts than mouse hosts.

The effect of immunizing dose could not be explored for baboon host with *S. haematobium* studies because all of them used the same number of parasites for immunization (Table 3.1). The number of immunizing parasites was shown to have a positive influence on the fraction protected for rat host studies with *S. mansoni* [$F(1, 43)=7.37$, $p<0.001$, coefficient=1.05] (Table 3.2). Time between immunization and challenge both had non-significantly negative effects in rat host studies with *S. mansoni*, baboon hosts with *S.mansoni*, and baboon hosts with *S. haematobium* studies (Table 3.2). Irradiation dose had non-significantly negative

effects in rat host studies with *S. mansoni*, baboon hosts with *S. haematobium* studies but had non-significant positive effects in baboon hosts with *S. mansoni* studies (Table 3.2).

Table 3.1: Frequencies of experimental animal host and schistosome parasite species reported in attenuated parasite vaccine studies with reported range of each predictor.

Host species/ schistosome species	N*	Number of immunizing parasites	Irradiation dose (krad)	Time between the last immunization and challenge (days)
Rat/*S. mansoni*	47	300-3,000	2-80	14-252
Baboon/*S. mansoni*	16	8,664-45,000	6-60	7-61
Baboon/*S. haematobium*	13	24,000	3-60	56-266

* Number of observations.

Table 3.2: Results from random-effects meta-regression models. Positive coefficients indicate a positive effect of the predictor on the fraction protected, whereas negative coefficients indicate a negative effect of the predictor on the fraction protected.

Host/ Parasite	Predictors	Coefficient	Standard error	F-value (df)	p-value
Rat/*S. mansoni*	Number of immunizing parasites per dose (log10 transformed)	1.05	0.22	7.37 (1, 43)	<0.001
	Irradiation dose	-0.0052	0.0055	0.90 (1, 38)	0.34
	Time between the last immunization and challenge	-0.0029	0.0019	2.4 (1, 42)	0.13
Baboon/ *S. mansoni*	Number of immunizing parasites per dose (log10 transformed)	0.26	0.43	0.36 (1, 10)	0.56
	Irradiation dose	0.0059	0.0055	1.14 (1, 8)	0.32
	Time between the last immunization and challenge	-0.013	0.0088	2.06 (1, 10)	0.18
Baboon/ *S. haematobium*	Number of immunizing parasites per dose (log10 transformed)	-	-	-	-
	Irradiation dose	-0.014	0.0088	2.4 (1, 4)	0.19
	Time between the last immunization and challenge	-0.0049	0.0025	3.9 (1, 9)	0.078

3.3.2. Meta-analysis results

3.3.2.1. Rat host

A total of 47 experimental results from 11 rat host with *S. mansoni* studies met all inclusion criteria and were included in the random effect meta-analysis (Panel A in Figure 3.2). The number of experimental results from a single article varied from 1 to 24. All studies included in this analysis reported a positive influence of vaccination on the reduction of the number of worms retrieved after the challenge infection. The protection levels in the vaccinated group against challenge infection ranged from 17% to 88%. The random effect meta-analysis results showed the number of worms retrieved in the vaccinated group is significantly lower than that of control groups ($Z=10.72$, $p<0.00001$, the overall pooled standardized mean difference=-2.93). The results also showed high heterogeneity of protection levels between studies ($I^2=71\%$) (Higgins *et al.* 2003).

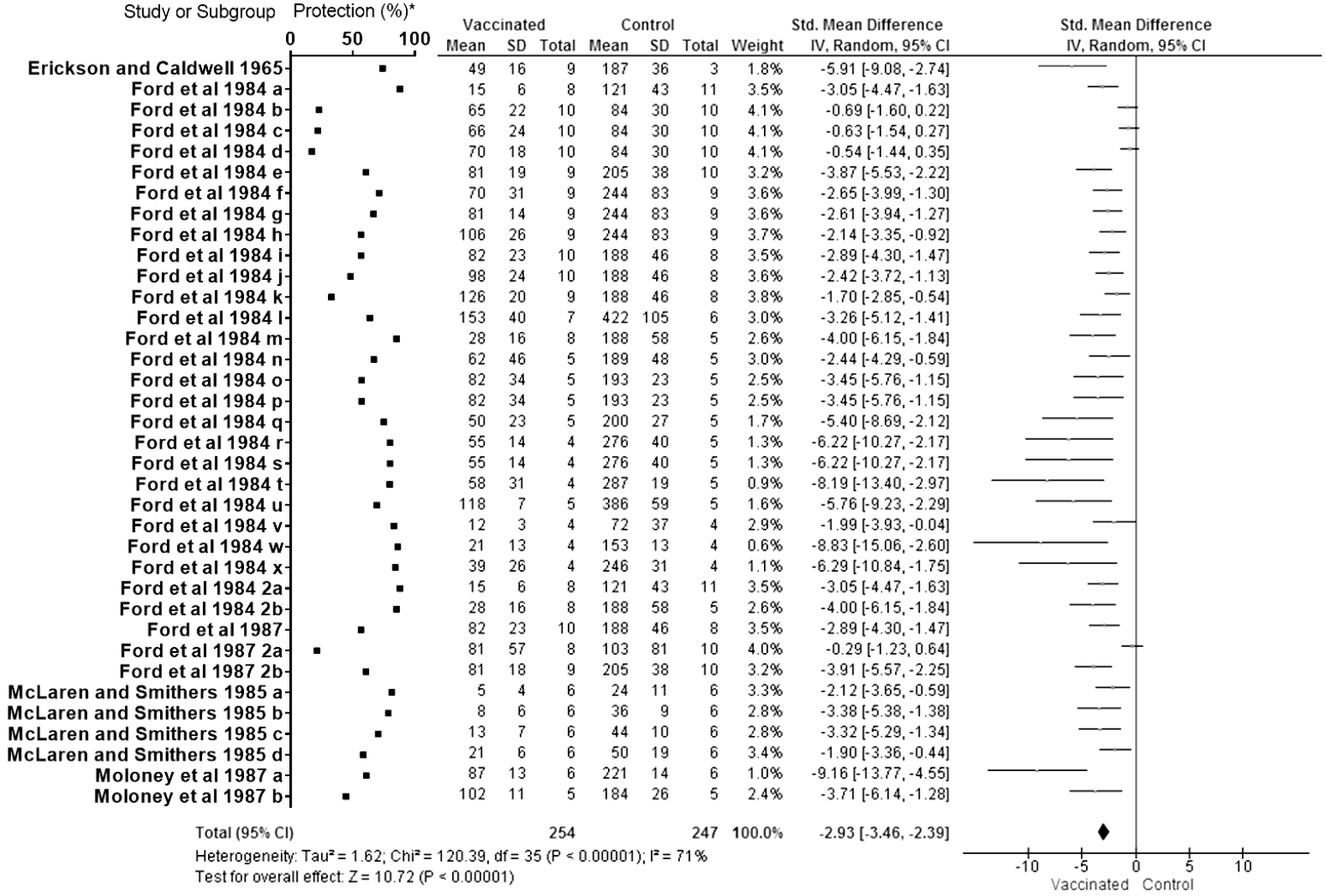
3.3.2.2. Baboon host

There were 16 experimental results from nine articles included in the random effects meta-analysis of baboon hosts and *S. mansoni* studies (Panel B in Figure 3.2). The number of experimental results extracted from a single article varied from one to five observations. All of these experimental results reported a smaller number of worms retrieved in vaccinated baboons compared to non-vaccinated control baboons, and the random effect meta-analysis results showed the number of worms retrieved in the vaccinated baboon group was significantly lower than that of control groups ($Z=5.10$, $p<0.00001$, the overall pooled standardized mean difference=-1.38). The protection levels among vaccinated groups against

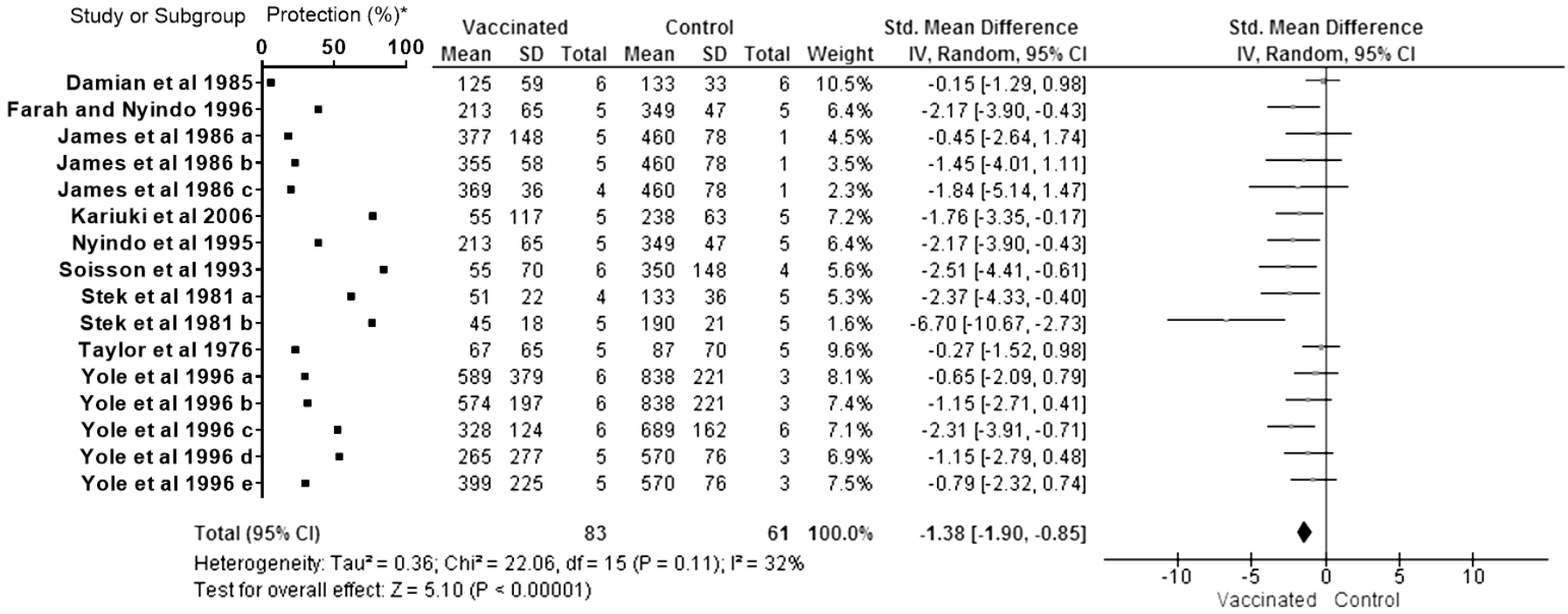
challenge infection ranged from 23% to 76%. The results also showed moderate heterogeneity of standardized mean difference between studies (I^2=32%) (Higgins *et al.* 2003).

Similarly, all 13 experimental results from two articles that reported results from baboon hosts with *S. haematobium* immunization and challenge infection, reported a positive influence of vaccination on protection levels (4% to 91%) (Panel C in Figure 3.2). The random effect meta-analysis confirmed that the number of worms retrieved in the vaccinated baboon group was significantly lower than that of control groups (Z=2.27, p=0.02, the overall pooled standardized mean difference=-1.39). The results also showed low heterogeneity of standardized mean difference between studies (I^2=25%) (Higgins *et al.* 2003).

(A)


Study or Subgroup
Protection (%)*
0
50
100
Vaccinated
Control
Std. Mean Difference
Std. Mean Difference
Mean SD Total Mean SD Total Weight IV, Random, 95% CI
IV, Random, 95% CI
Erickson and Caldwell 1965 49 16 9 187 36 3 1.8% -5.91 [-9.08, -2.74]
Ford et al 1984 a 15 6 8 121 43 11 3.5% -3.05 [-4.47, -1.63]
Ford et al 1984 b 65 22 10 84 30 10 4.1% -0.69 [-1.60, 0.22]
Ford et al 1984 c 66 24 10 84 30 10 4.1% -0.63 [-1.54, 0.27]
Ford et al 1984 d 70 18 10 84 30 10 4.1% -0.54 [-1.44, 0.35]
Ford et al 1984 e 81 19 9 205 38 10 3.2% -3.87 [-5.53, -2.22]
Ford et al 1984 f 70 31 9 244 83 9 3.6% -2.65 [-3.99, -1.30]
Ford et al 1984 g 81 14 9 244 83 9 3.6% -2.61 [-3.94, -1.27]
Ford et al 1984 h 106 26 9 244 83 9 3.7% -2.14 [-3.35, -0.92]
Ford et al 1984 i 82 23 10 188 46 8 3.5% -2.89 [-4.30, -1.47]
Ford et al 1984 j 98 24 10 188 46 8 3.6% -2.42 [-3.72, -1.13]
Ford et al 1984 k 126 20 9 188 46 8 3.8% -1.70 [-2.85, -0.54]
Ford et al 1984 l 153 40 7 422 105 6 3.0% -3.26 [-5.12, -1.41]
Ford et al 1984 m 28 16 8 188 58 5 2.6% -4.00 [-6.15, -1.84]
Ford et al 1984 n 62 46 5 189 48 5 3.0% -2.44 [-4.29, -0.59]
Ford et al 1984 o 82 34 5 193 23 5 2.5% -3.45 [-5.76, -1.15]
Ford et al 1984 p 82 34 5 193 23 5 2.5% -3.45 [-5.76, -1.15]
Ford et al 1984 q 50 23 5 200 27 5 1.7% -5.40 [-8.69, -2.12]
Ford et al 1984 r 55 14 4 276 40 5 1.3% -6.22 [-10.27, -2.17]
Ford et al 1984 s 55 14 4 276 40 5 1.3% -6.22 [-10.27, -2.17]
Ford et al 1984 t 58 31 4 287 19 5 0.9% -8.19 [-13.40, -2.97]
Ford et al 1984 u 118 7 5 386 59 5 1.6% -5.76 [-9.23, -2.29]
Ford et al 1984 v 12 3 4 72 37 4 2.9% -1.99 [-3.93, -0.04]
Ford et al 1984 w 21 13 4 153 13 4 0.6% -8.83 [-15.06, -2.60]
Ford et al 1984 x 39 26 4 246 31 4 1.1% -6.29 [-10.84, -1.75]
Ford et al 1984 2a 15 6 8 121 43 11 3.5% -3.05 [-4.47, -1.63]
Ford et al 1984 2b 28 16 8 188 58 5 2.6% -4.00 [-6.15, -1.84]
Ford et al 1987 82 23 10 188 46 8 3.5% -2.89 [-4.30, -1.47]
Ford et al 1987 2a 81 57 8 103 81 10 4.0% -0.29 [-1.23, 0.64]
Ford et al 1987 2b 81 18 9 205 38 10 3.2% -3.91 [-5.57, -2.25]
McLaren and Smithers 1985 a 5 4 6 24 11 6 3.3% -2.12 [-3.65, -0.59]
McLaren and Smithers 1985 b 8 6 6 36 9 6 2.8% -3.38 [-5.38, -1.38]
McLaren and Smithers 1985 c 13 7 6 44 10 6 2.8% -3.32 [-5.29, -1.34]
McLaren and Smithers 1985 d 21 6 6 50 19 6 3.4% -1.90 [-3.36, -0.44]
Moloney et al 1987 a 87 13 6 221 14 6 1.0% -9.16 [-13.77, -4.55]
Moloney et al 1987 b 102 11 5 184 26 5 2.4% -3.71 [-6.14, -1.28]
Total (95% CI) 254 247 100.0% -2.93 [-3.46, -2.39]
Heterogeneity: Tau² = 1.62; Chi² = 120.39, df = 35 (P < 0.00001); I² = 71%
Test for overall effect: Z = 10.72 (P < 0.00001)
-10
-5
0
5
10
Vaccinated
Control

(B)


Study or Subgroup
Protection (%)*
0
50
100
Vaccinated
Control
Std. Mean Difference
Std. Mean Difference
Mean SD Total Mean SD Total Weight IV, Random, 95% CI
IV, Random, 95% CI
Damian et al 1985 125 59 6 133 33 6 10.5% -0.15 [-1.29, 0.98]
Farah and Nyindo 1996 213 65 5 349 47 5 6.4% -2.17 [-3.90, -0.43]
James et al 1986 a 377 148 5 460 78 1 4.5% -0.45 [-2.64, 1.74]
James et al 1986 b 355 58 5 460 78 1 3.5% -1.45 [-4.01, 1.11]
James et al 1986 c 369 36 4 460 78 1 2.3% -1.84 [-5.14, 1.47]
Kariuki et al 2006 55 117 5 238 63 5 7.2% -1.76 [-3.35, -0.17]
Nyindo et al 1995 213 65 5 349 47 5 6.4% -2.17 [-3.90, -0.43]
Soisson et al 1993 55 70 6 350 148 4 5.6% -2.51 [-4.41, -0.61]
Stek et al 1981 a 51 22 4 133 36 5 5.3% -2.37 [-4.33, -0.40]
Stek et al 1981 b 45 18 5 190 21 5 1.6% -6.70 [-10.67, -2.73]
Taylor et al 1976 67 65 5 87 70 5 9.6% -0.27 [-1.52, 0.98]
Yole et al 1996 a 589 379 6 838 221 3 8.1% -0.65 [-2.09, 0.79]
Yole et al 1996 b 574 197 6 838 221 3 7.4% -1.15 [-2.71, 0.41]
Yole et al 1996 c 328 124 6 689 162 6 7.1% -2.31 [-3.91, -0.71]
Yole et al 1996 d 265 277 5 570 76 3 6.9% -1.15 [-2.79, 0.48]
Yole et al 1996 e 399 225 5 570 76 3 7.5% -0.79 [-2.32, 0.74]
Total (95% CI) 83 61 100.0% -1.38 [-1.90, -0.85]
Heterogeneity: Tau² = 0.36; Chi² = 22.06, df = 15 (P = 0.11); I² = 32%
Test for overall effect: Z = 5.10 (P < 0.00001)
-10
-5
0
5
10
Vaccinated
Control

(C)

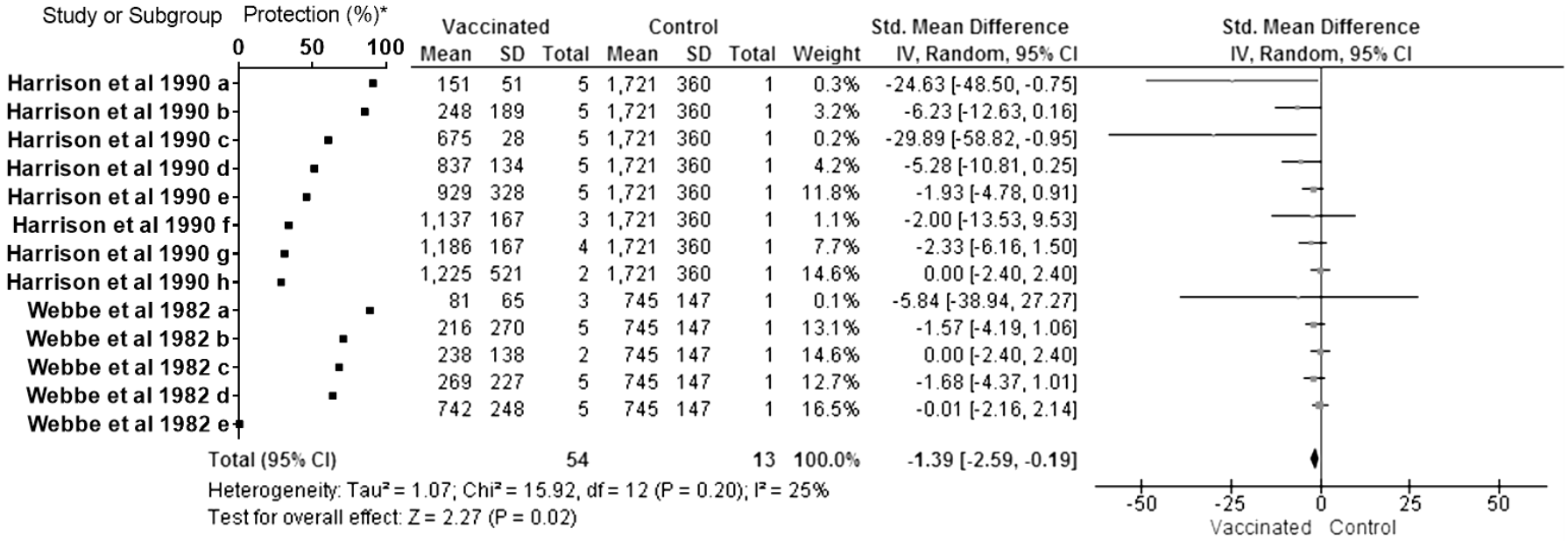

Study or Subgroup
Protection (%)*
0
50
100
Vaccinated
Control
Std. Mean Difference
Std. Mean Difference
Mean SD Total Mean SD Total Weight IV, Random, 95% CI
IV, Random, 95% CI
Harrison et al 1990 a 151 51 5 1,721 360 1 0.3% -24.63 [-48.50, -0.75]
Harrison et al 1990 b 248 189 5 1,721 360 1 3.2% -6.23 [-12.63, 0.16]
Harrison et al 1990 c 675 28 5 1,721 360 1 0.2% -29.89 [-58.82, -0.95]
Harrison et al 1990 d 837 134 5 1,721 360 1 4.2% -5.28 [-10.81, 0.25]
Harrison et al 1990 e 929 328 5 1,721 360 1 11.8% -1.93 [-4.78, 0.91]
Harrison et al 1990 f 1,137 167 3 1,721 360 1 1.1% -2.00 [-13.53, 9.53]
Harrison et al 1990 g 1,186 167 4 1,721 360 1 7.7% -2.33 [-6.16, 1.50]
Harrison et al 1990 h 1,225 521 2 1,721 360 1 14.6% 0.00 [-2.40, 2.40]
Webbe et al 1982 a 81 65 3 745 147 1 0.1% -5.84 [-38.94, 27.27]
Webbe et al 1982 b 216 270 5 745 147 1 13.1% -1.57 [-4.19, 1.06]
Webbe et al 1982 c 238 138 2 745 147 1 14.6% 0.00 [-2.40, 2.40]
Webbe et al 1982 d 269 227 5 745 147 1 12.7% -1.68 [-4.37, 1.01]
Webbe et al 1982 e 742 248 5 745 147 1 16.5% -0.01 [-2.16, 2.14]
Total (95% CI) 54 13 100.0% -1.39 [-2.59, -0.19]
Heterogeneity: Tau² = 1.07; Chi² = 15.92, df = 12 (P = 0.20); I² = 25%
Test for overall effect: Z = 2.27 (P = 0.02)
-50
-25
0
25
50
Vaccinated
Control

Figure 3.2: Forest plot of attenuated schistosome parasite vaccine efficacy in baboon or rat model.(A) *S. mansoni* immunization and challenge infection in rat host, (B) *S. mansoni* immunization and challenge infection in baboon host, (C) *S. haematobium* immunization and challenge infection in baboon host. *Protection was calculated using following equation: protection (%) = [(average number of worms per experimental animal retrieved from control group – average number of worms per experimental animal retrieved from vaccinated group)*100 / average number of worms per experimental animal retrieved from control group].

3.3.3. The influence of homologous or heterologous schistosome parasite species immunization and challenge infection on the protection levels

Among six potential predictors, “challenge parasite species”, and “heterologous or homologous schistosome species challenge infection” were confirmed to have a significant influence on protection levels [$F(3, 745)=3.42$, $p=0.017$, and $F(1, 745)=21.17$, $p<0.001$ respectively, see Figure 3.3] in addition to the previously identified predictors (number of immunizing parasites, irradiation dose, and the time between immunization and challenge infection, Table 2.2) using mouse hosts with a variety of schistosome parasite species studies (group 1 and 2 in Figure 3.1). The immunizing parasite species was not statistically significant in the model. Homologous schistosome parasite species immunization and challenge infection studies showed significantly higher protection levels in *S. bovis*, *S. haematobium*, and *S. mansoni* attenuated vaccine studies (Figure 3.4). The *S. japonicum* studies were not included in the graph because only one study reported the protection levels with heterologous schistosome parasite species challenge infection using *S. japonicum* parasites for immunization.

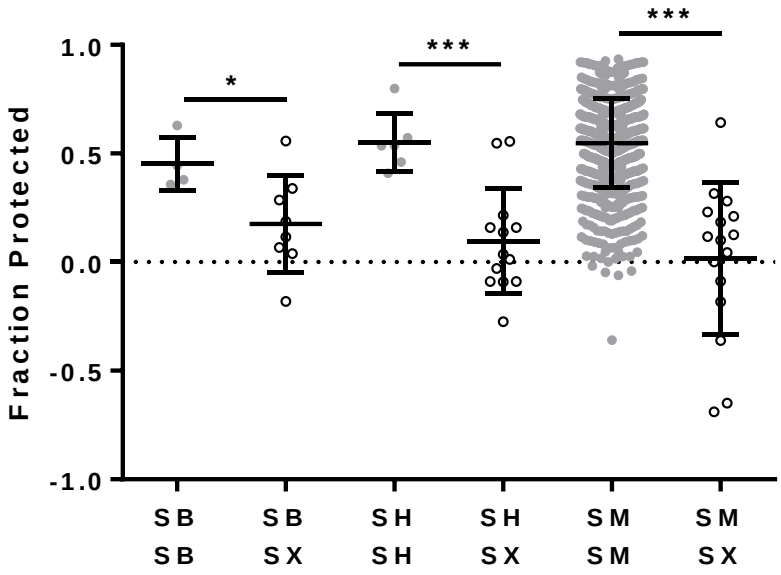


Figure 3.3: Fraction protected by different immunizing parasite species in mouse model. Schistosome parasite species are shown on x-axis. The upper letters show immunizing parasite species and the lower ones show challenge parasite species (SB: *S. bovis*, SH: *S. haematobium*, SM: *S. mansoni*, SX: any schistosome parasite species different from immunizing parasite species). Data points indicate the reported fraction protected for each observation. Each bar represents mean and standard deviation of fraction protected. Independent sample t-tests were conducted for each pair that used the same parasite species for immunization. NS non-significant, * significant at $p<0.05$, *** significant at $p<0.001$.

3.4. Discussion

Whilst the discovery of an efficacious vaccine for schistosomiasis has been a goal for many years, presently there is no licensed schistosome vaccine for human use. A series of animal studies have reported that high levels of protection can be achieved using attenuated schistosome parasites for vaccination (Fukushige *et al.* 2015). I conducted a meta-analysis using these reports to determine the effects of different factors on the efficacy of attenuated schistosome parasites as vaccines. In particular, I reviewed studies of baboon hosts with *S. mansoni* or *S. haematobium*, rat hosts with *S. mansoni*, and mouse hosts with a variety of different schistosome parasite species. The results for baboon hosts indicated that protection against schistosome challenge infections can be induced by both attenuated *S. mansoni* and *S. haematobium* vaccines. Similarly, in rat hosts, the significant impact of attenuated *S. mansoni* vaccination on the reduction of challenge infection intensity was confirmed. My results also demonstrated higher protection levels for homologous than for heterologous combination of schistosome parasite species used for immunization and challenge infection in mouse host. Altogether, these results emphasize the high potential of attenuated schistosome parasite vaccine for human use, and the species specificity of attenuated schistosome parasite vaccines.

Mouse hosts have been extensively used attenuated parasite vaccine studies. However, it is unclear to what extent observations from mouse host models translate into predictions about human protective immunity against schistosomiasis (Abdul-Ghani *et al.* 2010). It has been reported that schistosome infections of mice are different to those in humans. For example,

Cheever (1969) reported that the infection intensity expressed as worm burden per unit of body weight was much higher in mouse host models than in human cases (Cheever 1969). In fact, the mouse is not a natural host for schistosome parasites (Cheever *et al.* 1994). On the other hand, natural schistosome infections have been reported in several wild animals including wild baboons (Fenwick 1969; Taylor *et al.* 1972; Muller-Graf *et al.* 1997). Nonhuman primates, especially baboons, have been regarded as the most accurate non-human models of human clinical manifestations of schistosomiasis (Siddiqui *et al.* 2008). All baboon study articles that have been included in this analysis reported protective effects of attenuated schistosome vaccines against challenge infections. Furthermore, the meta-analysis results confirmed the significant effect of attenuated schistosome parasite vaccination on the protection against challenge infection in both baboon with *S. mansoni* and baboon with *S. haematobium* studies. These results suggest that an attenuated schistosome parasites vaccine has a high potential of having a protective effect in humans.

My previous investigation of mouse host with *S. mansoni* studies (Chapter 2) identified three predictors of protection levels against future infections: the number of immunizing parasites, the irradiation dose for attenuation, and the time between the immunization and challenge infection (Table 2.2). Whilst the same set of predictors was used to run the meta-regression analysis for studies of baboon hosts with either *S. mansoni* or *S. haematobium*, I could not confirm statistical significance ($p<0.05$) for any of these previously identified predictors. All baboon hosts with *S. haematobium* studies used the same number of parasites for immunization, and therefore immunizing dose could not be explored in this group (Table

3.1). Furthermore, all baboon hosts with *S. mansoni* studies used a large number of attenuated schistosome parasites for immunization (>8,000). Nevertheless, analyses showed a positive coefficient of immunizing dose for baboon host with *S. mansoni* studies (coefficient=0.26), although this result was not statistically significant. Further studies are required to estimate and immunizing parasite dose to achieve high levels of protections.

The influence of irradiation dose on protection levels was not statistically significant in either rat host or baboon host studies. The rat hosts with *S. mansoni* and baboon hosts with *S. haematobium* study results showed a negative coefficient (rat/*S. mansoni* coefficient=-0.0052, baboon/*S. haematobium* coefficient=-0.014). In contrast, baboon hosts with S. *mansoni* studies showed positive coefficients of irradiation dose on protection levels (baboon/*S. mansoni* coefficient=0.0059), although they were not statistically significant. Unlike mouse and rat host studies, baboon studies used two different schistosome parasite life cycle stages for immunization (cercariae and schistosomula). However, the univariate analysis results showed that there was no significant effect of immunizing parasite life cycle stages on protection levels. Furthermore, the negative irradiation dose effect on survival time of schistosome parasites have been reported both for cercariae and schistosomula in murine model (Bickle *et al.* 1979; Hsu *et al.* 1981; Mangold *et al.* 1984). In addition, studies using baboon hosts with *S. mansoni* and *S. haematobium* have reported broad irradiation dose ranges (baboon/*S. mansoni*: 6-60 krad, baboon/*S. haematobium*: 3-60 krad) which could affect the survival time of parasites after vaccination. The reason why the analysis could not detect a statistically significant effect of irradiation dose could be due to the small sample

sizes. It also could possibly be due to variation the time and /or distribution of immunizing parasites required inside the host in order to stimulate protective immunity. Together, these results suggest that there is a possibility to develop efficacious vaccines using highly attenuated *S. mansoni* parasites.

In the previous chapter, I demonstrated that the protection induced by vaccines gradually decreased over time, but remained at high levels for at least eight months after vaccination in mouse hosts immunized and infected with *S. mansoni* cercariae. In the current study, although the studies using rat hosts with *S. mansoni* and baboon hosts with *S. haematobium* reported follow up dates of more than six months, I could not detect a significant influence of time between vaccination and challenge infection. Both rat and baboon host studies showed a non-significant negative coefficient for time between the vaccination and challenge infection (rat/*S. mansoni* coefficient=-0.0029, baboon/*S. mansoni* coefficient=-0.013, baboon/*S. haematobium* coefficient=-0.0049). This might suggest there is also a slow decline of protection levels after the vaccination in rat and baboon hosts. Using the rat host with *S. mansoni*, Phillips *et al.* (1980) reported that protection levels reduced by about half between two months and eight months after immunization (Phillips *et al.* 1980). Similarly, Harrison et al. (1990) reported that protection among baboon hosts vaccinated with attenuated *S. haematobium* reduced by about one third between two months and seven months after immunization (Harrison *et al.* 1990). Further studies are required to estimate the length of protection which can be achieved by a single vaccination. Furthermore, these results

together with results from Chapter 2 would suggest that boosting vaccines may be necessary for long lasting protection against schistosomiasis.

Studies of the mouse host that used variety of schistosome parasite species for immunization and challenge were analysed to explore the influence of heterologous schistosome parasite species on levels of protection. The use of different parasite species for immunization and challenge was statistically significant when it was included in the meta-regression analysis together with the three previously identified predictors (Figure 3.3). This result suggests species specificity of the attenuated cercariae vaccine in the mouse model. Supporting this result, Bickle *et al.* (1985) reported higher protection levels of homologous schistosome parasite species immunization and challenge infection compared to that of heterologous immunization and challenge infection in the mouse hosts model (Bickle *et al.* 1985). More recently, Cesari *et al.* (2010) reported low levels of cross-reactivity to *S. mansoni* membrane antigens in patients infected with *S. haematobium* (Cesari *et al.* 2010). A comparative study shown that there are differences in the adult proteome between different schistosome species, with some of these differences then translating to differential antigen recognition patterns (Higon *et al.* 2011). These results highlight the importance of considering which schistosome species is prevalent in a region when developing an efficacious vaccine in human use.

There were studies with multiple vaccinated animals groups were compared with a single control animal group. In these cases, the number of control animals was divided by the

number of vaccinated groups to balance the weight to the observation in the meta-analysis (Higgins *et al.* 2011). When a single study report data for multiple subgroups, combining data across subgroups to make a single pair of comparison (i.e., vaccinated versus control) is a commonly used approach in meta-analysis (Borenstein 2009; Higgins *et al.* 2011). However, this approach was not being used in current analysis. For random effects meta-analysis in this chapter, subgroups were treated as independent observations. Therefore, there is a risk that the analyses overestimated the vaccination effect. Nevertheless, all the studies identified and included in the meta-analysis reported a positive protective effect of vaccination. Therefore, presenting the variability of protection levels between observations would be more informative than combine subgroups into one. These variability of protection within a same study were reported to be due to the number of vaccinated parasites per dose, the number of vaccination, irradiation dose, and/or the time between the last immunization and challenge infection (Ford *et al.* 1984; Ford *et al.* 1984; Mclaren *et al.* 1985; Moloney *et al.* 1987).

The results of this study confirm the potential of attenuated schistosome parasite vaccines, in multiple species. There is still a need for more studies to estimate the optimal immunizing parasite dose and the potential duration of protection from these vaccines. The findings also emphasize the influence of challenge schistosome parasite species on protection levels. The attenuated schistosome parasite vaccines could be more efficacious against homologous species challenge infection than heterologous species challenge infection. As both *S. haematobium* and *S. mansoni* are co-endemic in parts of the African continent (Garba *et al.*

2010; Meurs *et al.* 2012; Gouvras *et al.* 2013), a multi-species vaccine for these two species has been an ambition for many years. In the context of other findings, these results suggest the potential importance of antigens that are unique to different schistosome species as a key factor in the efficacy of a multi-species vaccine.

Chapter 4: Factors influencing the direction of change in schistosome specific antibodies after praziquantel treatment: a systematic review and meta-analysis.

4.1. Introduction

It is well documented in the literature that naturally acquired immunity against schistosome infections reduces both prevalence and infection intensity in the older age groups in endemic areas (Woolhouse *et al.* 1999; Mitchell *et al.* 2011). One reason why this immunity takes time to develop is thought to be because the schistosome parasite is capable of evading host immunity and has an average life span of several years (Harris *et al.* 1984; Walter *et al.* 2006). That is, while antigens from schistosome adult worms have been reported to be essential for the development of protective immunity, they only become accessible to the host immune system when the worms die (Mitchell *et al.* 2012). In fact, praziquantel treatment for schistosome infection has been reported to enhance host protective immunity by exposing to the parasite's hidden antigens (Harnett *et al.* 1986; Mutapi *et al.* 2005; Doenhoff *et al.* 2008).

Multiple schistosome parasite-specific antibodies such as IgA and IgE have been reported to be associated with resistance to future infections (Rihet *et al.* 1991; Vereecken *et al.* 2007). There is a considerable number of studies that have been conducted to evaluate the influence of praziquantel treatment on schistosome-specific antibody levels among schistosome infected populations. In 2001, Mutapi reviewed these studies and reported high levels of heterogeneity in the type and magnitude of change in antibody level after chemotherapy between different human populations (Mutapi 2001). To date, many potential factors have been suggested to explain this variation, such as pre-treatment infection intensity (Rujeni *et al.* 2012), level of schistosome endemicity (Rujeni *et al.* 2012), age (Abebe *et al.* 2001; Mutapi *et al.* 2003), sex (Abebe *et al.* 2001), and co-infection with human immunodeficiency virus (HIV) (Joseph *et al.* 2004). However, more work is needed to confirm the roles of these factors. Therefore, the objective of this study is to identify predictors that influence the direction of change in antibody isotypes after praziquantel treatment by conducting a systematic review and meta-analysis.

The objectives of this study were:

1) to explore the influence of praziquantel treatment on the levels of schistosome specific antibody isotypes;
2) to identify factors that influence the levels of schistosome specific antibody isotypes after praziquantel treatment.

4.2. Material and Methods

4.2.1. A systematic review

An electronic literature search was conducted using several databases: Web of Science Core Collection, BIOSIS Citation Index, and MEDLINE all of which were searched through Web of Science (www.webofknowledge.com). In addition, EMBASE, Global Health and Ovid Medicine were searched through Ovid (ovidsp.tx.ovid.com). The search terms were: "schistosom*" AND ("antibod*" OR "IgA" OR "IgE" OR "IgM" OR "IgG*") AND ["albendazole" OR "metrifonate" OR "artesunate" OR "antihelmint*" OR "chemotherap*" OR "praziquantel" OR "oxamniquine" OR ("drug" AND "treatment")]. This electronic literature search was completed in January 2014. After removing duplicates, a total of 1,366 unique articles were identified for consideration in the present study. Titles and abstracts of articles were screened to exclude those that were clearly not relevant. Full texts of potentially relevant articles were then reviewed for further selection. Full texts of the relevant articles were sourced through the Web of Science, the Ovid, the Google Scholar (scholar.google.com), the University of Edinburgh library, and the Inter Library Loan of the University of Edinburgh. Non-English articles were included in this study, and several Chinese articles were identified and translated into English by a native Chinese speaker for the systematic literature review.

An article was included in this study if it met all of the following inclusion criteria:

1) human study (either sex),
2) infection with *S. mansoni* and/or *S. haematobium*,

3) participants treated with praziquantel,
4) number of participants reported,
5) schistosome-specific antibody levels reported before and after praziquantel treatment,
6) follow-up studies conducted within 14 to 180 days after praziquantel treatment,
7) schistosome soluble worm antigen (WWA) and/or soluble egg antigen (SEA) used to measure antibody isotype levels,
8) participants potentially exposed to schistosome infection for longer than 1 year before the study,
9) participants ages could be categorized at child (0-10 years old), adolescent (11-21 years old) or adult (≥21 years old).

Articles were excluded based on the following exclusion criteria:

1) participants had a previous history of antihelminthic treatment prior to the study participation,
2) participants were treated with any antihelminthic drug other than praziquantel (e.g., oxamniquine),
3) participants were specially selected because of co-infection with HIV,
4) purified schistosome antigens were used to measure antibody isotype levels,
5) participants were temporary visitors to endemic areas (i.e., travellers).

Although studies using non-praziquantel antihelminthic drugs were initially considered for this study, they were excluded based on exclusion criteria (2). This was because: 1) the majority of identified studies used praziquantel for the treatment, 2) only small number of studies were published using non-praziquantel antihelminthic drugs. There were seven studies with oxamniquine (Mendes *et al.* 1982; Butterworth *et al.* 1985; Khalife *et al.* 1986; Dunne *et al.* 1992; Caldas *et al.* 2000; Vendrame *et al.* 2001; Gomes *et al.* 2002), one study with levamisole (Snyman *et al.* 1998), and one study with metrifonate (Feldmeier *et al.* 1983). In addition, there was a study that treated participants with either metrifonate or oxamniquine (Stevens *et al.* 1983).

Schistosome specific antibody isotype levels before (baseline) and after (follow-up) treatment with praziquantel were extracted from the selected articles. For those articles that reported results only in graphical format, the software DataThief III (2006) was used to extract the raw data, whenever the graph format allowed it. In addition to antibody levels, the following information was extracted from each article: the year of publication, article title, names of authors, study area (country, region and village) schistosome parasite species, co-infection status, co-infecting pathogen species, number of participants, age or age range, sex, height, weight, days between the treatment and follow-up, pre- and post- treatment infection intensity and prevalence, praziquantel dose, and cure rate. Several articles reported results from multiple different groups of participants in the same study area, such as from different age groups of participants. In such cases, the result from each group was recorded as one observation. For the purpose of analysis, they were treated as independent

observations. For articles that reported results from multiple follow-up time points, the first follow-up after 14 days was selected and included in this study. A total of 92 observations from 26 articles (published 1988-2013) met all the inclusion criteria and were considered for the final meta-analysis (Table 4.1).

Table 4.1: Summary of 26 articles selected after systematic review. S.m=*Schistosoma mansoni*; S.h=*Schistosoma haematobium*; SEA=schistosome soluble egg antigen; WWA=schistosome soluble worm antigen.

Reference	Parasite species	Antigen type	Antibody isotype	Follow up (days)
Abebe *et al.* 2001	S.m	SEA	IgA, IgE, IgG1, IGG2, IgG3, IgG4, IgG, IgM	35
Ali *et al.* 1994	S.m, S.h	SEA/WWA	IgA	90
Feldmeier *et al.* 1988	S.m, S.h	WWA	IgE, IgG	150
Fouda *et al.* 2007	S.m	WWA	IgE	180
Grogan *et al.* 1996	S.h	SEA/WWA	IgE, IgG4	35
Hamadto *et al.* 1990	S.m	SEA/WWA	IgA, IgE, IgG, IgM	49
Hussein *et al.* 1996	S.m	SEA/WWA	IgG, IgM	60
Ismail *et al.* 1992	S.m, S.h	SEA/WWA	IgG, IgM	90
Joseph *et al.* 2004	S.m	SEA/WWA	IgE, IgG1, IgG2, IgG3, IgG4	35
Mutapi *et al.* 1998 (a)	S.m, S.h	SEA	IgA, IgG1	84
Mutapi *et al.* 1998 (b)	S.h	SEA	IgA, IgE, IgG1, IgG2, IgG3, IgG4	126
Nagaty *et al.* 1996	S.m, S.h	SEA/WWA	IgA, IgE, IgG, IgM	180
Nassr *et al.* 2002	S.m	WWA	IgG1, IgG4	90
Naus *et al.* 1998	S.h	SEA/WWA	IgE, IgG1, IgG2, IgG3, IgG4,IgM	30

Reilly *et al.* 2008	S.h	WWA	IgG1, IgG3	42
Satti *et al.* 2004	S.m	WWA	IgE, IgG4	21
Satti *et al.* 1996 (a)	S.m	SEA/WWA	IgE, IgG1, IgG2, IgG3, IgG4, IgM	90
Satti *et al.* 1996 (b)	S.m	WWA	IgA	90
Snyman *et al.* 1997	S.h	WWA	IgE, IgG	21
Snyman *et al.* 1998	S.h	WWA	IgE, IgG	90
Tweyongyere *et al.* 2009	S.m	SEA/WWA	IgE, IgG1, IgG2, IgG3, IgG4	42
van Lieshout *et al.* 1999	S.m	WWA	IgE, IgG1, IgG3, IgG4, IgG, IgM	63
Vereecken *et al.* 2007	S.m	SEA/WWA	IgA, IgE, IgG1, IgG2, IgG3, IgG4, IgM	42
Walter *et al.* 2006	S.m	SEA/WWA	IgA, IgE, IgG1, IgG2, IgG3, IgG4, IgM	35
Wilson *et al.* 2013	S.m, S.h	WWA	IgE	63
Zinyowera *et al.* 2006	S.h	SEA/WWA	IgA, IgE, IgG	42

Potential predictors were selected according to their biological importance, as suggested by earlier studies (Abebe *et al.* 2001; Rujeni *et al.* 2012) and if they were reported by the majority of articles included in this study. The following predictors were considered: age groups (0-10 years old, 11-21 years old, ≥21 years old), pre-treatment infection intensity (light or heavy), schistosome species (*S. mansoni*, *S. haematobium*, or co-infection), disease

prevalence (low/moderate or high), and days between chemotherapy and follow-up (Table 4.2).

Table 4.2: List of potential predictors investigated and their measurement units/ codes

Potential predictors (units)	Codes		
Age (years)	0-10	11-20	≥21
Infection intensity	Light	Heavy	
S. mansoni (eggs/1g faeces)	1-99	≥100	
S. haematobium (eggs/10ml urine)	<50	≥50	
Prevalence (%)	Low/Moderate	High	
	<50	≥50	
Schistosome species	*S. mansoni*	*S. haematobium*	Co-infection
Days after chemotherapy (days)	Continuous		

4.2.2. Statistical analysis

The majority of studies included for investigation used the enzyme-linked immunosorbent assay (ELISA) method to quantify antibody isotype levels and reported optical density (OD). However, OD values cannot be directly compared between studies conducted by different research groups (Voller *et al.* 1978). Therefore, the outcome variable was categorized according to the direction of change in antibody levels from pre-treatment baseline to levels at follow-up. That is, pre-treatment and post-treatment antibody isotype levels were compared within the same population and the outcome was categorized as “increase” if the post-treatment level was higher than the pre-treatment level, and “decrease” if it was lower.

There were seven observations that reported the exact same value of antibody isotype levels for both pre- and post- treatment (Hamadto *et al.* 1990; Van Lieshout *et al.* 1999; Walter *et al.* 2006). They were categorized into “decrease” group in this study for analyses purposes. All the observations were grouped according to schistosome parasite antigens (whole worm antigen or whole egg antigen) that were used to measure antibodies and analysed separately.

There were 29 observations from four articles that failed to report pre-treatment infection intensity of study participants. In these cases, pre-treatment infection intensity was obtained from scientific publications from the larger populations that contain the study populations (articles listed in Appendix C.2). Similarly, there were three observations from two articles that did not report the schistosome infection prevalence in the study area. In these cases prevalence was obtained from using scientific publications or governmental reports from the same area or larger area that contain the study area (Appendix C.2).

A preliminary analysis was conducted using mixed effects logistic regression models. In these models, article ID was used as a random effect. In addition, each observation was weighted according to its sample size (number of participants). The results indicated that two predictors, age and infection intensity, have a significant influence on the direction of change of the antibody isotypes after praziquantel treatment. Furthermore, the results indicated that there is an association between these two predictors. However, the mixed effects logistic regression model results were not considered further. This was mainly

because the majority of the models had very high information criterion values, indicating instability (results are not shown).

In schistosomiasis endemic areas, infection intensity peaks in the young age group, giving an age-dependent convex curve (Woolhouse 1998). To take this non-linear association into account, a combination predictor for age and infection intensity was generated, with format age/infection intensity as shown in Table 4.3. All the observations were categorized into any of these age/infection intensity categories.

Table 4.3: Combined predictor: age/ infection intensity

Age category (years)	Pre-treatment infection intensity	Predictor name
0-10	Light	child/light
0-10	Heavy	child/heavy
11-20	Light	adolescent/light
11-20	Heavy	adolescent/heavy
≥21	Light	adult/light
≥21	Heavy	adult/heavy

4.2.3. Classification and Regression Tree (CART) models

Classification and Regression Tree (CART) models were used to identify influential predictors of the direction of change of the schistosome specific antibody after praziquantel treatment (Breiman 1984; Loh 2011). Confidence intervals or standard errors are the most common weighting methods for meta-regression (Borenstein 2009) and for meta-CART (Dusseldorp *et al.* 2014). However, in this analysis the measures of antibody levels are ELISA OD values, which cannot be compared directly when coming from different research groups (Voller *et al.* 1978). Therefore, in this analysis, the sample size (the number of participants) was used for weighting. The number of participants across studies varied from 7 to 148. The potential predictors used for the analysis were: age/ infection intensity (as in Table 4.3), schistosome species (*S. mansoni*, *S. haematobium*, or co-infection), days between treatment and follow-up, and disease prevalence (low/moderate or high) (Table 4.2).

The CART analysis was conducted to build a tree using the standard three steps: 1) growing a maximum sized tree, 2) pruning the tree to generate sub-trees, and 3) identifying the optimal sized tree (Breiman 1984). Initially, the maximum-sized complex trees were grown with data from all study variables for each antibody isotypes. All potential predictors were compared using the Gini index to identify the optimum split of the dependent variable (increase or decrease in my study). Based on the Gini index, the strongest predictor variable and its splitting value, that is sub-groupings for categorical variables and cut-off value for continuous variables, were used to split the original data (i.e., root node) into two subgroups (i.e., daughter nodes). The subgroups were then divided repeatedly into smaller subgroups

following the same procedure until they represented the most homogeneous subgroups achievable (i.e., terminal node). In this study, terminal nodes of these maximum-sized trees were set to be pure or with only a single observation. Then a series of subtrees was generated by pruning the initial maximum-sized trees. To estimate the optimal subtree among the different sized subtrees, 10-fold cross-validation analysis was conducted for each subtree followed by the selection based on the one standard error (SE) rule (Breiman 1984). Briefly, the cross-validation analysis is used to estimate the risk of misclassification using a randomly selected subset (i.e., test samples) of the original dataset (i.e., learning sample) (Breiman 1984). The optimal tree is the one that yields the minimal risk estimate. However, the noisy nature of the data and the instability of the cross-validation procedure can lead to the selection of unstable and large trees (Lewis 2000). Therefore, following the one SE rule, the smallest tree that has a cross-validation risk estimate of less or equal to the minimal risk plus one SE of the minimal error, was selected as the optimal tree (Breiman 1984; Lemon *et al.* 2003) (Appendix C.4 and C.5).

4.2.4. Software

Articles identified by the systematic review were recorded using Thomson Reuters EndNote and the extracted data were entered into a spreadsheet using Microsoft Excel 2010. B. Tummers, DataThief III. 2006 (http://datathief.org/) was used to extract data from published graphs. IBM SPSS Statistics Version 21.0 and IBM SPSS modeller Version 15.0 were used for statistical analysis. GraphPad Software GraphPad Prism version 6.03 was used to create graphs.

4.3. Results

Following a systematic review, a total of 92 observations from 26 articles (published 1988-2013) met all inclusion criteria and were considered for the final analysis. There was a high degree of heterogeneity in the direction of change of antibodies (increase/decrease) after praziquantel treatment depending on antigen type and antibody isotypes (Figure 4.1). Two anti-SEA antibody isotypes (IgG, IgM) showed a significant trend of decrease after the praziquantel treatment (X^2=8.07, p=0.005, X^2=4.48, p=0.034 respectively) (Figure 4.1). In contrast, five anti-WWA antibody isotypes (IgA, IgE, IgG1, IgG2, IgG4) showed a significant trend of increase (X^2=12.25, p<0.001, X^2=8.26, p=0.004, X^2=6.55, p=0.011, X^2=7.14, p=0.008, X^2=10.71, p=0.001) (Figure 4.1).

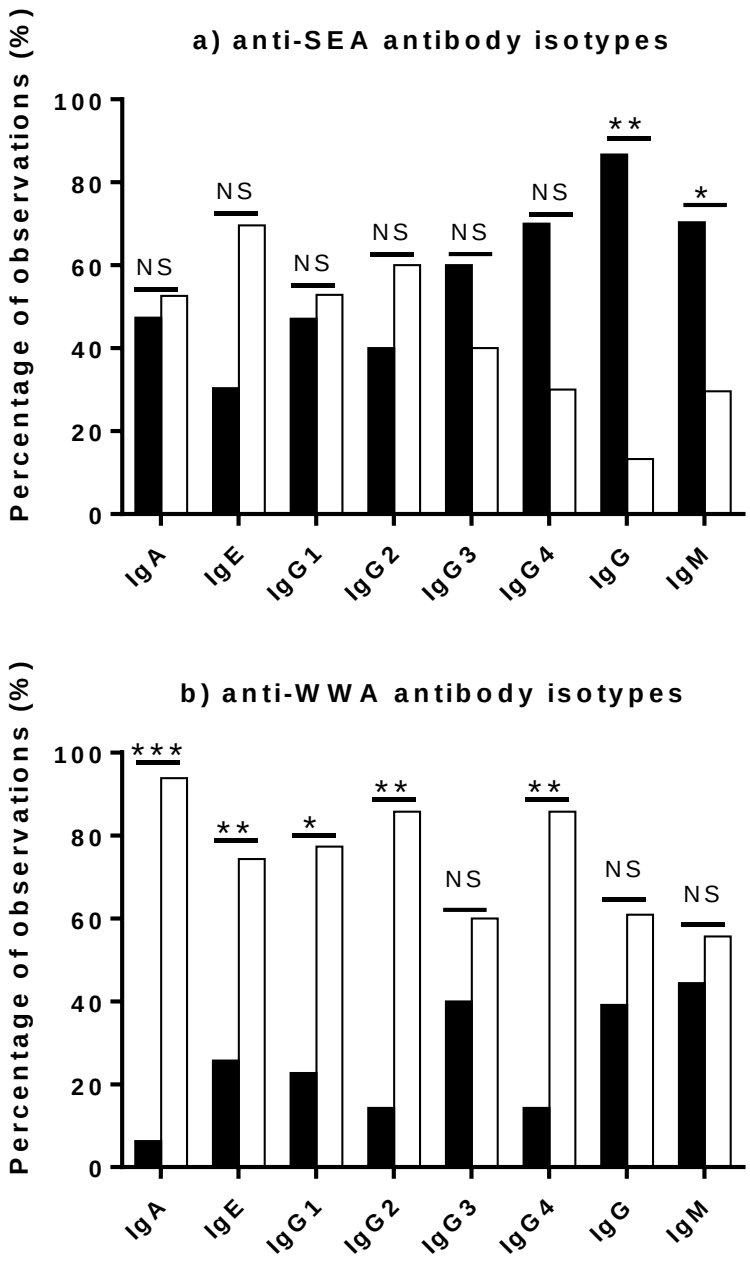


Figure 4.1: The percentage of observations with increasing or decreasing levels of (a) anti-SEA, and (b) anti-WWA antibody isotypes after praziquantel treatment for eight antibody isotypes. The graph shows the fraction of observations that reported decrease (filled bar) or increase (unfilled bar) of each antibody isotype. Chi-square tests were conducted for each pair of anti-SEA or anti-WWA antibody isotype. NS non-significant, * significant at $p<0.05$, ** significant at $p<0.01$, *** significant at $p<0.001$.

The CART analysis identified optimal trees for anti-SEA (IgA, IgE, IgG1, IgG2, IgM) and for anti-WWA (IgG, IgM) (Table 4.4, Figure 4.2, Figure 4.3). The cross-validation analysis identified the lowest risk of misclassification at the root node, for a number of anti-SEA antibodies (IgG3, IgG4, IgG) and anti-WWA antibodies (IgE, IgG1, IgG2, IgG3, IgG4), suggesting that there are no stable trees for these cases. In addition, there was no tree generated for anti-WWA IgA, this could be due to high homogeneity of the observations: all observations except one observation reported an increase of anti-WWA IgA after praziquantel treatment.

Table 4.4: Predictors identified by the classification and regression tree model analyses

Predictors	Anti-SEA antibodies	Anti-WWA antibodies
Days after treatment	IgE	
Age/ infection intensity	IgA, IgG1, IgG2, IgM	IgG, IgM
No predictor: mostly decrease	IgG3, IgG4, IgG	
No predictor: mostly increase		IgA, IgE, IgG1, IgG2, IgG3, IgG4

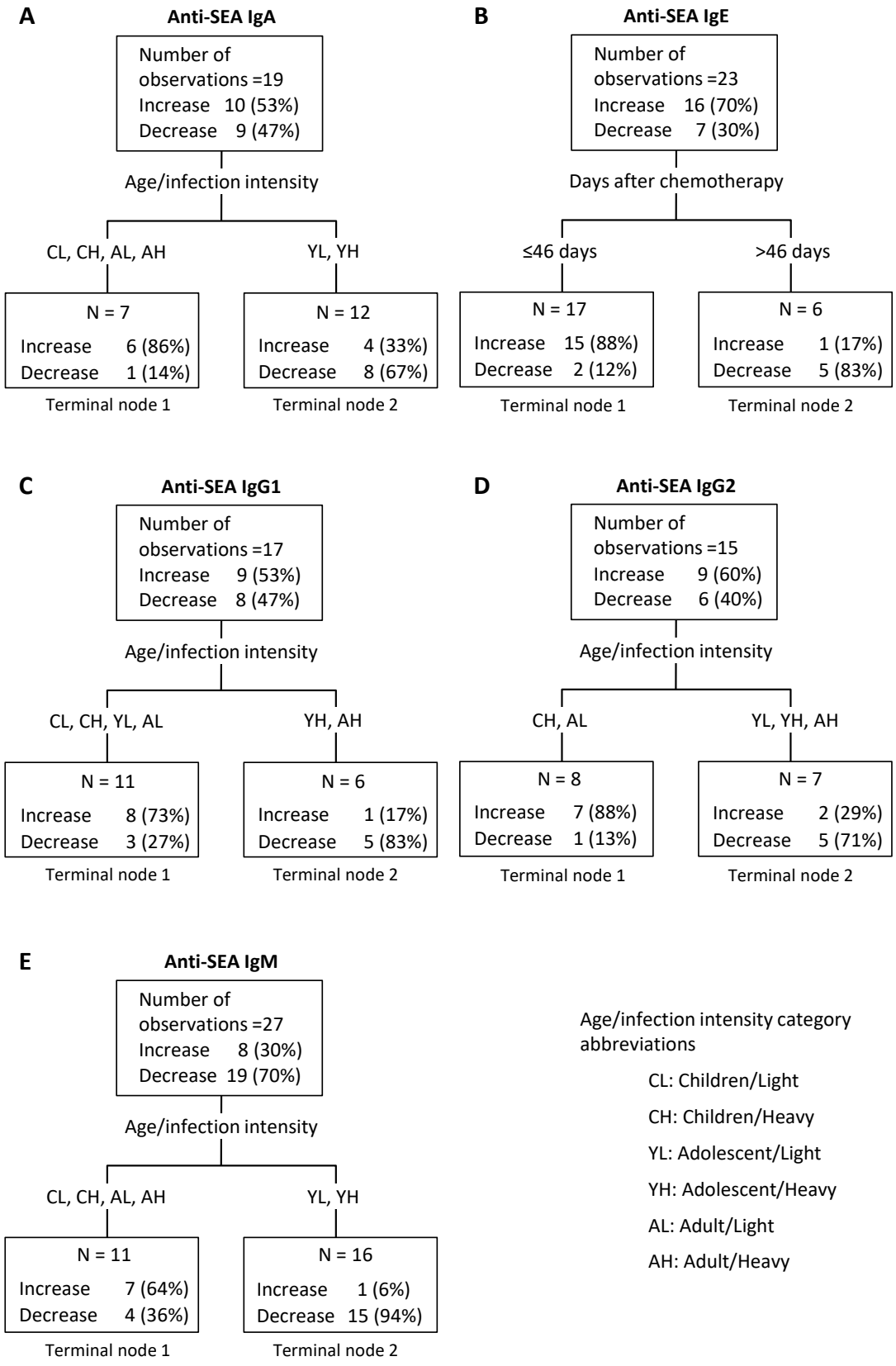

A
Anti-SEA IgA
Number of observations =19
Increase 10 (53%)
Decrease 9 (47%)
Age/infection intensity
CL, CH, AL, AH
YL, YH
N = 7
Increase 6 (86%)
Decrease 1 (14%)
Terminal node 1
N = 12
Increase 4 (33%)
Decrease 8 (67%)
Terminal node 2
B
Anti-SEA IgE
Number of observations =23
Increase 16 (70%)
Decrease 7 (30%)
Days after chemotherapy
≤46 days
>46 days
N = 17
Increase 15 (88%)
Decrease 2 (12%)
Terminal node 1
N = 6
Increase 1 (17%)
Decrease 5 (83%)
Terminal node 2
C
Anti-SEA IgG1
Number of observations =17
Increase 9 (53%)
Decrease 8 (47%)
Age/infection intensity
CL, CH, YL, AL
YH, AH
N = 11
Increase 8 (73%)
Decrease 3 (27%)
Terminal node 1
N = 6
Increase 1 (17%)
Decrease 5 (83%)
Terminal node 2
D
Anti-SEA IgG2
Number of observations =15
Increase 9 (60%)
Decrease 6 (40%)
Age/infection intensity
CH, AL
YL, YH, AH
N = 8
Increase 7 (88%)
Decrease 1 (13%)
Terminal node 1
N = 7
Increase 2 (29%)
Decrease 5 (71%)
Terminal node 2
E
Anti-SEA IgM
Number of observations =27
Increase 8 (30%)
Decrease 19 (70%)
Age/infection intensity
CL, CH, AL, AH
YL, YH
N = 11
Increase 7 (64%)
Decrease 4 (36%)
Terminal node 1
N = 16
Increase 1 (6%)
Decrease 15 (94%)
Terminal node 2
Age/infection intensity category abbreviations
CL: Children/Light
CH: Children/Heavy
YL: Adolescent/Light
YH: Adolescent/Heavy
AL: Adult/Light
AH: Adult/Heavy

Figure 4.2: Classification and Regression Tree Models identifying profiles of observations that had higher (increase) or lower (decrease) anti-SEA antibody isotype levels after praziquantel treatment. (A) anti-SEA IgA, (B) anti-SEA IgE, (C) anti-SEA IgG1, (D) anti-SEA IgG2, or (E) anti-SEA IgM. The hierarchy of the Classification and Regression Tree Model starts from the terminal nodes at the top. Abbreviations for age/infection intensity groups are listed in the text box and described in Table 4.3.

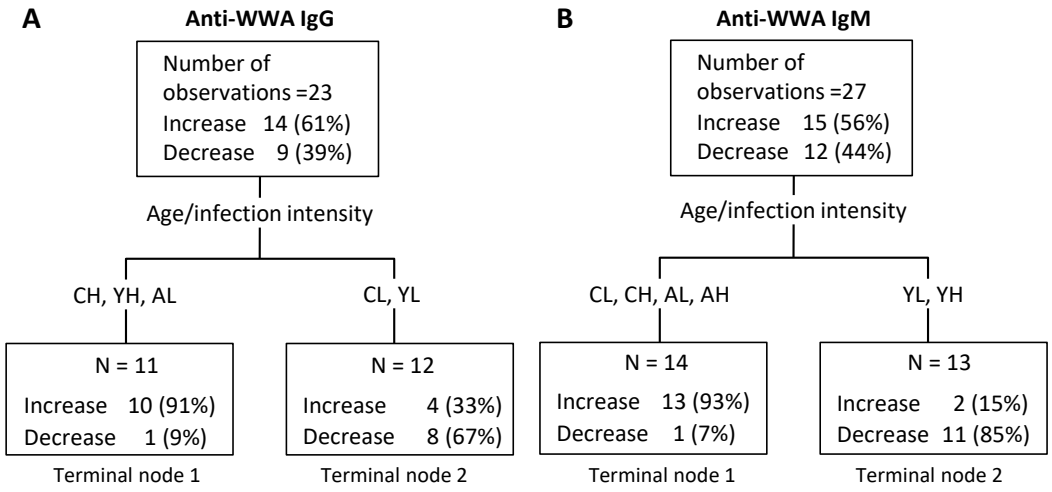


Figure 4.3: Classification and Regression Tree Models identifying profiles of observations that had higher (increase) or lower (decrease) anti-WWA antibody isotype levels after praziquantel treatment. (A) anti-WWA IgG or (B) anti-WWA IgM. The hierarchy of the Classification and Regression Tree Model starts from the terminal nodes at the top. Abbreviations for age/infection intensity groups are as listed in the text box in Figure 4.2, also described in Table 4.3.

In the CART analysis, where the direction of change in antibody isotype was a binary outcome (increase/decrease), age/infection intensity was identified as the most influential variable for direction of change for anti-SEA antibodies (IgA, IgG1, IgG2, IgM) and for anti-WWA (IgG, IgM) antibodies (Panel A, C, D, E in Figure 4.2 and Figure 4.3). For anti-SEA (IgA, IgM) antibodies, both adolescent/light and adolescent/heavy groups were categorized into the nodes dominated by observations of decrease in antibody levels (terminal node 2 in panel A, E in Figure 4.2), whereas the remaining age/infection intensity groups were grouped into the nodes dominated by observations of increase (terminal node 1

in panel A, E in Figure 4.2). For anti-SEA IgG1, adolescent/heavy and adult/heavy groups were categorized into the node which dominated by observations of decrease in antibody levels (terminal node 2 in panel C in Figure 4.2). For anti-SEA IgG2, adolescent/light, adolescent/heavy, and adult/heavy groups were categorized into the node which dominated by observations of decrease in antibody levels (terminal node 2 in panel D in Figure 4.2).

For anti-WWA IgG, age/infection intensity groups of children/heavy, adolescent/heavy, and adult/light were categorized into the node dominated by observations of increase (terminal node 1 in panel A in Figure 4.3) in contrast to the children/light and adolescent/light groups which were categorized into the node dominated by observations of decrease in antibody level (terminal node 2 in panel A in Figure 4.3). For anti-WWA IgM, children/light, children/heavy, adult/light and adult/heavy age/infection intensity groups were categorized into the node dominated by observations of increase in levels (93% of observations) (terminal node 1 in panel B in Figure 4.3) whereas adolescent/light and adolescent/heavy groups were categorized into the node dominated by observations of decrease in levels (terminal node 2 in panel B in Figure 4.3).

The first split from the root node of anti-SEA IgE was according to days after praziquantel treatment. The percentage of observations that reported increases of anti-SEA IgE less than 46 days after praziquantel treatment was 88% (terminal node 1 in panel B in Figure 4.2) while only 17% of observations reported increases more than 46 days after praziquantel treatment (terminal node 2 in panel B in Figure 4.2).

4.4. Discussion

This meta-analysis aimed to identify variables that influence the direction of change in schistosome-specific antibody isotype levels after praziquantel treatment in humans. Praziquantel is currently the recommended drug for treatment of schistosomiasis (WHO 2015). Some field studies have reported that praziquantel treatment can enhance the development of host protective immunity against future re-infection (Harnett *et al.* 1986; Mutapi *et al.* 1998; Doenhoff *et al.* 2008). Furthermore, schistosome parasite-specific antibodies are thought to play an important role in this protective immunity (Dunne *et al.* 1992; Zhang *et al.* 1997). The reasons underlying the observed substantial heterogeneity in the post-treatment response (Mutapi 2001) remain unclear. The study results are important as they increase our understanding of human immunity against schistosomiasis, in particular, the influence of the mass drug administration programmes on the protective immunity against future re-infection.

A higher number of studies reported an increase of anti-WWA antibodies after praziquantel treatment, than a decrease for any antibody isotypes (Table 4.4). In particular, the analyses indicated that there was a significantly higher proportion of studies that reported an increase than a decrease in anti-WWA (IgA, IgE, IgG1, IgG2, IgG4) antibodies (Figure 4.1). Among these antibodies, anti-WWA IgA stood out as it was reported to increase in all studies, except one, which reported the exact same anti-WWA IgA levels for both pre- and post-praziquantel treatment. Praziquantel treatment has been reported to damage adult worm tegument, and therefore allows host immunity to detect schistosome worm antigens that

would otherwise not be accessible until those worms die naturally (Harnett *et al.* 1986; Mutapi *et al.* 2005). Mutapi *et al.* (Mutapi *et al.* 2005).have reported that praziquantel treatment enhances the host immunological recognition of *S. haematobium* defined antigens. My results show that the praziquantel treatment could enhance the host immune recognition of schistosome worm antigens.

In contrast with the anti-WWA antibodies, studies of anti-SEA antibody isotypes did not show a significant tendency to increase rather than a decrease of their levels after praziquantel treatment (Figure 4.1). There were two anti-SEA antibodies (IgG, IgM), for which a significant proportion of studies reported a decrease after praziquantel treatment. Besides this, anti-SEA (IgG3, IgG4) antibodies were also dominated by studies that reported a decrease after chemotherapy. The reason for this could potentially be that praziquantel treatment which reduces the number of mature adult worms would reduce egg output (Cheever *et al.* 1994), inducing a decrease in some anti-SEA antibody levels after the treatment. However, there are multiple anti-SEA antibodies (IgA, IgE, IgG1, IgG2) for which a majority of observations report a post-treatment increase than a decrease. These results suggest that there is a high degree of heterogeneity in the direction of change (increase or decrease) among different anti-SEA antibody isotypes and also among different populations after praziquantel treatment (Figure 4.1). This result indicates that praziquantel treatment had different effects on different antibody isotypes, in particular anti-SEA antibodies in different populations.

The analysis revealed significant increases in anti-WWA IgA following praziquantel treatment. Previously, high anti-schistosome WWA IgA levels have been reported to be negatively associated with *S. mansoni* re-infection after praziquantel treatment (Vereecken *et al.* 2007). A study with purified *S. mansoni* adult worm antigen rSm28-GST demonstrated that rSm28-GST specific IgA levels increased with age (6-66 years old) which in turn was associated with a reduction of *S. mansoni* worm fecundity (Grzych *et al.* 1993; Liu *et al.* 1996). Supporting this report, a mathematical modelling study also suggested that naturally acquired protective immunity develops by age is mainly targeting worm fecundity (Mitchell *et al.* 2012). The reduction in worm fecundity can reduce the disease severity dramatically even when treated patients are re-infected later on in life. This is because the morbidity of schistosomiasis is mainly caused by parasite eggs remaining trapped in host tissues, which provokes an inflammatory immune response in the host (Silveira *et al.* 2002; Gryseels *et al.* 2006; Colley *et al.* 2014). These results in combination with the previous studies reported above, suggest that praziquantel treatment can boost the levels of anti-WWA IgA, which might contribute to reducing morbidity during future infections.

Schistosome specific IgE is the antibody isotype that is most commonly associated with protection against re-infection after praziquantel treatment in humans (Colley *et al.* 2014). High levels of anti-SEA IgE and/or anti-WWA IgE antibodies have been reported to be associated with low re-infection rates of *S. mansoni* (Dunne *et al.* 1992), *S. haematobium* (Hagan *et al.* 1991) and *S. japonicum* (Zhang *et al.* 1997). My CART analysis demonstrated that there is a transient increase in anti-SEA IgE antibody, followed by a decrease below

pre-treatment levels 46 days after chemotherapy. On the other hand, anti-WWA IgE levels showed a tendency to increase after treatment within the reported follow-up times (21-180 days, 74% of the observations). Schistosome anti-WWA IgE antibody levels have been reported to increase with age in endemic areas, potentially contributing to reducing prevalence and infection intensity in the older age groups (Hagan *et al.* 1991; Webster *et al.* 1997). Hence, the tendency for anti-WWA IgE levels to be elevated during the 180 days after treatment revealed by my analysis suggests that chemotherapy could enhance protection against re-infection for some populations for at least 6 months after the post-treatment. That is, the immunological consequences of praziquantel treatment have a potential to reduce susceptibility to future re-infection in some populations.

The majority of studies reported a decrease in anti-SEA IgG4 (70% of observations) after praziquantel. High levels of anti-SEA IgG4 have been associated with high infection intensity, severity of inflammatory granuloma, and fibrosis (Boctor *et al.* 1990; Grogan *et al.* 1996; Silveira *et al.* 2002). Therefore, in some studies, anti-SEA IgG4 has been considered a marker for disease burden (Abd El-Aal *et al.* 2005). Thus, decrease of anti-SEA IgG4 might be reflecting the efficacy of praziquantel treatment at the reducing infection intensity and consequently disease.

The analysis revealed a significant proportion of studies showing an increase rather than decrease in anti-WWA IgG4 (86% of observations reported increase) after treatment. Longitudinal and cross-sectional population studies have demonstrated the association of

both anti-SEA IgG4 and anti-WWA IgG4 with human susceptibility to re-infection after treatment in schistosomiasis endemic areas (Grogan *et al.* 1997; Oliveira *et al.* 2012). In particular, IgG4 has been suggested as a possible blocking antibody that inhibits the action of protective IgE in both *S. haematobium* and *S. mansoni* infections (Hagan *et al.* 1991; Demeure *et al.* 1993; Colley *et al.* 2014). Field studies have reported that the ratio of IgE to IgG4 has a positive influence on resistance to future *S. mansoni* re-infection (Pinot de Moira *et al.* 2010). There were multiple studies that reported the direction of change for both IgE and IgG4 after praziquantel treatment. However, I found no evidence for any possible changes in the ratios of IgE to IgG4 after chemotherapy in this study, as the ratio of IgE to IgG4 change depends on the magnitude of change in both antibodies. More epidemiological research is required to determine how the ratio of these two antibodies changes after chemotherapy. Studies are also required to clarify the association between the IgE to IgG4 ratio and re-infection rate after chemotherapy.

The CART analysis results showed that for several antibody isotypes, the direction of change after chemotherapy can be partially explained by the combination of participants' age and pre-treatment infection intensity. For anti-SEA (IgA, IgM) and anti-WWA IgM, the majority of observations reported a decrease in levels among 11-20 years old participants with any pre-treatment infection intensity levels (Figure 4.2 and 4.3). Although the reasons for this association between adolescent age group and antibody isotypes direction of change is still unclear, there are multiple factors unique for adolescents. The adolescents often harbour the greatest burden of schistosome infection prevalence in many endemic areas

(Fulford *et al.* 1992; Mutapi *et al.* 2006; Colley *et al.* 2014). In endemic areas, people get infected by schistosome parasites as early as during their first year of age (Ruganuza *et al.* 2015). From then on, infection intensity and prevalence increase as the frequency of contact with natural water sources, which may be contaminated with infectious schistosome parasites, increases as they age (Sow *et al.* 2011). The adolescents have been reported to have most frequent water contact compared to other age groups in some populations in endemic areas (Kloos *et al.* 1983). Therefore, adolescents are thought to be carrying a high schistosome infection burden, while they are still developing acquired protective immunity against infection. The role of anti-SEA (IgA, IgM) and anti-WWA IgM in protective immunity are also still unclear. More epidemiological studies investigating the dynamics of these antibody levels over age within populations in endemic areas would help to clarify the association with host age. Furthermore, epidemiological cohort studies also could be conducted to investigate the influence of praziquantel treatment on these antibodies and also their association with re-infection after treatment.

In this study, none of the identified CART analysis results had pure-terminal nodes with only increase or decrease studies. This finding suggests that there could be influential predictors on direction of changes in schistosome-specific antibodies which could not be identified by current analyses. For example, Mutapi *et al.* have reported that *S. haematobium* infected children have significantly decreased levels of anti-SEA IgA after praziquantel treatment (Mutapi et al. 1998). In contrast, reports from *S. mansoni* infections show that there is an increase or no change in the levels of anti-SEA IgA in children before and after praziquantel

treatment (Abebe *et al.* 2001; Vereecken *et al.* 2007). These studies suggest although the analyses could not detect the influence of schistosome parasite species, praziquantel treatment might influence the antibody levels in the different way according to parasite species.

There are limitations of CART model. First of all, although multiple observations were extracted from a single article, CART analysis does not allow the random effects to be taken into account unlike other statistical analysis used in this thesis (e.g., random-effects meta-analysis, random-effects meta-regression). In addition, CART analysis was developed with the aim to analyze large data sets with a large number of potential predictors. In general, even a relatively small data sets normally yield maximum sized trees of 30-40 terminal nodes before tree pruning (Breiman 1984). Nevertheless, as the data was small in this study, the number of terminal nodes in maximum sized trees was only between 4 to 11. Therefore, even for these antibody isotypes with their optimal tree identified at the root node (without any subgroupings), there might be undetected predictors that could be identified with a bigger data set.

This meta-analysis has revealed that more studies reported an increase of anti-WWA antibodies isotypes than a decrease after praziquantel treatment. However, the analyses have also showed a considerable variability among different antigens, antibody isotypes, and populations in the direction of schistosome-specific antibody isotype levels change following treatment with praziquantel, confirming the work of Mutapi in 2001 (Mutapi

2001). Although the combination of age and infection intensity, and the number of days after treatment were identified as influential predictors for some antibody isotypes, there is no single predictor that consistently affects all antibody isotypes in the same way. These results could suggest that praziquantel treatment has diverse effects on protective immunity against re-infection. My results also demonstrated that the antibody isotypes that have been reported to have protective effect against future re-infection (anti-WWA IgA, IgE) can be stimulated by praziquantel treatment in the majority of cases for at least as long as 6 months after the treatment. These results therefore reinforce the reported immunizing effect of praziquantel treatment, while at the same time, highlighting the need for further studies to explain the observed heterogeneity in changes in antibody isotype levels following praziquantel treatment.

Chapter 5: Identifying factors that influence cure rates during schistosomiasis treatment with praziquantel: a systematic review and meta-analysis.

5.1. Introduction

Since its discovery by German pharmaceutical companies Bayer AG, Leverkusen and E. Merck, Darmstadt in 1972 (Gönnert *et al.* 1977), praziquantel has been used as the first drug of choice for treating schistosome infection (WHO 2015). This efficacious, low-cost drug has achieved a significant reduction in disease prevalence, infection intensity, and morbidity in many endemic areas (Midzi *et al.* 2008; Evans *et al.* 2011; Liu *et al.* 2011; WHO 2015). To date, there is no convincing evidence of the development of parasite resistance to praziquantel, even in China, where praziquantel has been used extensively for schistosome control for more than 30 years (Lamberton *et al.* 2010; Liu *et al.* 2011; Wang *et al.* 2012; Huyse *et al.* 2013) or in an Egyptian village where praziquantel treatment has been constantly provided for over 10 years (Botros *et al.* 2005; Othman *et al.* 2015). Recent meta-analysis studies have also concluded that praziquantel is still effective against schistosome infections (Liu *et al.* 2011; Danso-Appiah *et al.* 2013). The relatively long generation time of schistosome parasites is thought to make it difficult for the parasite to

develop resistance against praziquantel (Ward *et al.* 1988; Biolchini Cde *et al.* 2006; Nour 2010).

However, there is still a chance that schistosome parasites will develop resistance against praziquantel (Bickle 2009). Recent schistosomiasis control efforts, including the large number of national schistosome control programmes conducting MDA (Cleland *et al.* 2014; Omedo *et al.* 2014; Tuhebwe *et al.* 2015) may be putting an extra selection pressure on schistosome parasites (Norton *et al.* 2010). As such, attempts at eradication using MDA are leading to fears that the selection pressures on the parasite may eventually cause the evolution of resistance (Norton *et al.* 2010; Humphries *et al.* 2012). Close monitoring of praziquantel efficacy is important since this is the only drug that is effective against all three major human schistosome parasite species: *S. mansoni*, *S. haematobium* and *S. japonicum*.

Nevertheless, there are multiple epidemiological studies that have reported low praziquantel efficacy levels from different schistosomiasis endemic areas (Tchuente *et al.* 2004; Guidi *et al.* 2010; Keiser *et al.* 2014), in contrast to other studies that have reported high efficacy levels (Sousa-Figueiredo *et al.* 2010; Wilson *et al.* 2013). There are several factors that have been suggested, which could have an influence on the praziquantel efficacy levels. This includes schistosome parasite related factors (Garba *et al.* 2013; Gower *et al.* 2013), host related factors (Stelma *et al.* 1995; Utzinger *et al.* 2000), and drug administration strategy related factors (Gryseels *et al.* 1987; Muhumuza *et al.* 2014) (Table 5.1). However, it is still not very clear which factors have a major influence on the cure rate. Therefore, I conducted

a meta-analysis of published praziquantel efficacy studies to identify the factors that influence the levels of efficacy of praziquantel treatment by taking into consideration differences in host characteristics and drug administration strategy.

Table 5.1: Potential factors that have been reported to influence schistosome infection cure rate after praziquantel treatment.

Influential factor	Reference
Schistosome parasite species	(Garba *et al.* 2013)
Pre-treatment infection intensity	(Utzinger *et al.* 2000)
Schistosome infection prevalence	(Stothard *et al.* 2013)
Age of participants	(Van Lieshout *et al.* 1999)
Praziquantel treatment dose	(Gryseels *et al.* 1987)
Snack provision with treatment	(Muhumuza *et al.* 2014)
Number of parasitological samples collected	(Utzinger *et al.* 2001)
Previous praziquantel treatment in the same area	(Norton *et al.* 2010)

5.1.1. Study objectives

The objectives of this study were:

1) to identify factors influencing the cure rate of praziquantel treatment;
2) to investigate whether the cure rate of praziquantel treatment has been sustained over the period of study (treatment years 1979-2013).

5.2. Material and Methods

5.2.1. Systematic review

A systematic literature review was conducted to identify articles that reported the effectiveness of praziquantel in schistosomiasis endemic areas. An electronic literature search was conducted using Citation Index Expanded, Conference Proceedings Citation Index, BIOSIS Citation Index, and MEDLINE, all of which were provided through Web of Knowledge (www.webofknowledge.com). Alongside these, EMBASE (www.elsevier.com), OVID MEDICINE (www.ovid.com), and CAB abstract were searched simultaneously though OvidSP (ovidsp.tx.ovid.com). The search terms were chosen to be as inclusive as possible and were; "schistosom*" AND "praziquantel" AND ("treatment" OR "efficacy" OR "cure" OR "egg reduction rate" OR "chemotherapy"). This search was completed in November 2014. After duplicated articles were removed, a total of 4,558 potentially relevant articles were identified. The titles and abstracts were reviewed to exclude those articles that were clearly not related to efficacy levels for *S. mansoni* or *S. haematobium* infections in humans. Then the remaining 807 potentially relevant articles were reviewed by the full text. Non-English articles were included, and several Chinese, French, German, Italian, Portuguese, Russian, and Spanish articles were translated into English by native speakers of each language to be considered in this study. These translations were double checked using google translate (https://translate.google.co.uk/). In addition, Japanese articles were included and reviewed without translation.

A study was considered eligible if it met all of the following inclusion criteria:

1) used human participants
2) was based on *S. mansoni* or *S. haematobium* infections
3) had all participants treated with praziquantel
4) reported the cure rate and/or schistosomiasis prevalence both before and after praziquantel treatment
5) had the praziquantel treatment completed within a single day, which includes both single and two praziquantel treatments
6) had the follow up study conducted within 90 days after treatment
7) provided participants age that could be categorised as either child (0-19 years old) or adult (≥20 years old)
8) reported the number of participants.

Studies were excluded based on the following exclusion criteria:

1) used non-human animal subjects
2) were performed in vitro
3) reported from less than 10 participants (e.g., a clinical case report)
4) targeted acute schistosomiasis cases
5) were studies based on schistosome parasite species other than *S. mansoni* or *S. haematobium*
6) were studies based on mixed schistosome parasite species infection
7) were review article or meeting abstracts

8) had participants specially selected based on their being co-infected with other diseases such as HIV, malaria, or soil-transmitted helminths
9) used different antihelminthic drug (e.g., oxamniquine) together with praziquantel
10) reported cure rates not based on parasitological results (e.g., antibody levels)
11) had specially selected participants who received any antihelminthic drug treatment prior to the praziquantel treatment
12) had participants that were not originally from endemic areas (e.g., travellers, foreign military)
13) had participants that were originally from endemic areas but had moved to non-endemic areas prior to the study (e.g., immigrants).

Only studies that had participants that were currently living in and were originally from endemic areas were considered in the analyses. This was to reduce the heterogeneity of schistosomiasis infection history among participants. Articles often reported results from multiple separate groups of participants such as individuals from different villages, or in different age groups. In these cases, results from each group was recorded as an observation. A list of potential predictors (given in Table 5.2) was drawn up and information on these variables was extracted from each article. The potential predictors were selected based on their biological importance as suggested by previous studies (Stothard *et al.* 2013; Zwang *et al.* 2014). Pre-treatment infection intensity, which is quantified by the number of schistosome eggs in urine or stool samples, was initially considered as a potential predictor. However, pre-treatment infection intensity could not be used in the statistical analysis for a

number of reasons: 1) almost the half of studies included in this analysis failed to report pre-treatment infection intensity, 2) studies reported pre-treatment infection intensity using different metrics (arithmetic mean, geometric mean, median, range or category), which made it difficult to synthesize results, 3) preliminary univariate analysis on a subset of studies where pre-treatment infection intensity could be included indicated no statistically significant effect on cure rate.

There were two articles (three observations) that did not report praziquantel treatment dose. In these cases, as all of them reported there was a single treatment, the WHO recommendation treatment dose (40 mg/kg body weight) was imputed based on the assumption that the study followed this recommendation (WHO 2002). This 40 mg/kg body weight praziquantel dose was also the most commonly used dose among these articles that reported the treatment dose. There were 49 articles (94 observations) that did not report treatment year. In these cases the average interval between praziquantel treatment provision and publication among articles which reported treatment year (i.e., two years) was used to estimate treatment year from publication year, and used for the analysis. Remaining potential predictors (schistosome parasite species, age of participants, time between treatment year and follow up, and country) were reported by all studies included in the analysis.

Table 5.2: List of potential predictors investigated and their units/ description.

Variable name	Units/ code
Treatment dose	Praziquantel dose in mg/kg body weight
Schistosome parasite species	*S. mansoni,* *S. haematobium*
Age	Child (0-19 years old), Adult (≥20 years old)
Time between treatment and follow up	Time between the praziquantel treatment and follow up in days
Treatment year	Year in which the subjects were treated with praziquantel
Country	Name of the country where study was conducted

5.2.2. Statistical analysis (meta-regression models)

Meta-regression with sequential sums of squares was applied to identify the influential predictors of cure rate. The models were built using the forward stepwise selection procedure with 6 potential predictors (Table 5.2). Briefly, the stepwise selection procedure is a process of building a model by adding or removing potential predictors based on the p-values of statistics (in this analysis, p-values of F statistics were used) (Kutner 2005). Multiple observations (up to 20) were sometimes reported from a single article and therefore article was included as a random effect in the models. Associations between variables were examined visually for all possible predictor combinations (data not shown). Although using the levels of precision of each study, such as a standard error of cure rate, for weighting is

the most common weighting method for meta-regression (Borenstein 2009), many studies in my dataset failed to report either confidence intervals, standard errors, or standard deviations of cure rate. Instead of imputing these missing values, the size of the studies (the number of participants for each observation) was used for weighting.

Subgroup analysis was conducted to investigate the effect of treatment year and country on the cure rate. For this analysis, studies that used praziquantel treatment dose 40 mg/kg body weight were selected. These studies were grouped according to schistosome parasite species (*S. mansoni* or *S. haematobium*) and age group (children or adult) into four subgroups: 1) children with *S. mansoni*; 2) children with *S. haematobium*; 3) adults with *S. mansoni*; 4) adults with *S. haematobium*. For each subgroup, four independent meta-regression models were run using treatment year and country as predictors as follows:

1) Cure rate = f(T) + Re + error
2) Cure rate = f(C) + Re + error
3) Cure rate = f(T + C) + Re + error
4) Cure rate = f(T + C + T*C) + Re + error

T: Treatment year

C: Country

Re: Random effect (article)

5.2.3. Statistical software

Articles identified by the systematic review were recorded using Thomson Reuters EndNote and the extracted data were entered into a spreadsheet using Microsoft Excel 2010. B. Tummers, DataThief III. 2006 (http://datathief.org/) was used to extract data from published graphs. IBM SPSS Statistics Version 19.0 and Minitab. Inc. MINITAB 16 Statistical Software were used for the meta-regression analysis. GraphPad Software GraphPad Prism version 6.03 was used for graphical presentation.

5.3. Results

5.3.1. Systematic review results

A total of 224 observations were extracted from 107 articles published from 1981 to 2014 that met all inclusion criteria and were included for the meta-analysis (articles are listed in Appendix D.1). The number of observations reported by a single article ranged from 1 to 20 observations. Cure rates reported by these articles ranged from 16.4% to 100%, with overall average cure rate weighted by number of participants of each observation of 73.0% (SE of mean 11.6%) (Figure 5.1).

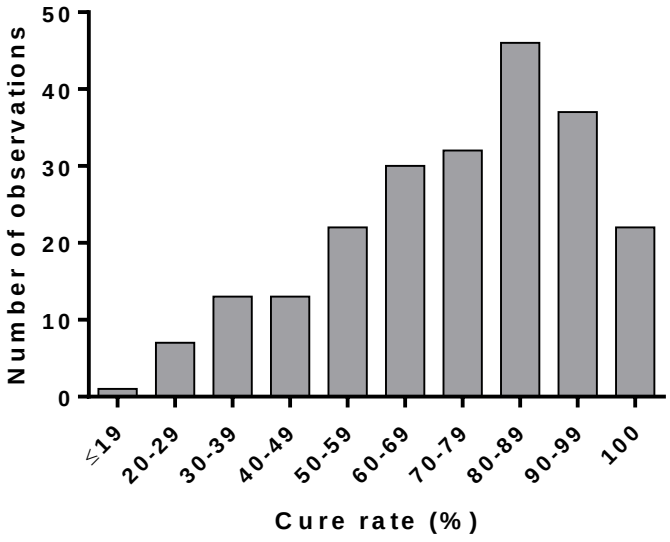
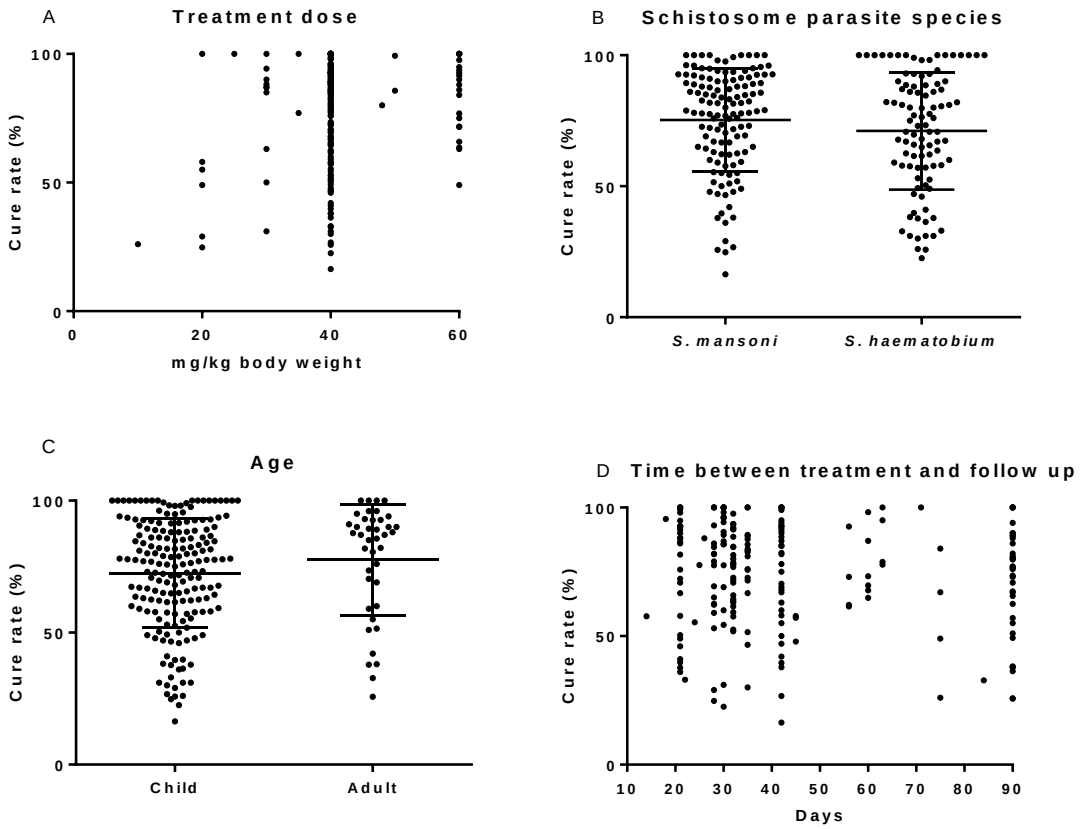


Figure 5.1: The distribution of reported parasitological cure rates following praziquantel treatment.

The reported praziquantel dose ranged from 10 to 60 mg/kg body weight (panel A in Figure 5.2). The majority of the studies (79%, 177 observations from 97 articles) reported the use of a treatment dose of 40 mg/kg body weight, that is, the current WHO recommendation treatment dose (WHO 2015). Although overall reported praziquantel treatment dose ranged 10-60 mg/kg body weight, studies with *S. mansoni*, and studies of adults reported narrower treatment dose range (20-60 mg/kg body weight, 20-40 mg/kg body weight respectively). Reported cure rate ranged from 23% to 100% for *S. haematobium* infection, and from 16% to 100% *S. mansoni* infection (panel B in Figure 5.2). More number of studies were conducted with children, and their cure rate ranged from 16% to 100% (panel C in Figure 5.2). Similarly, cure rate reported from adult participants ranged from 26% to 100% (panel C in Figure 5.2). The reported time between treatment and follow up ranged from 14 to 90 days after praziquantel treatment. Forty-two days and 90 days were the most commonly used time interval between treatment and follow up (37 observations for each) (panel D in Figure 5.2). Treatment year ranged from 1979 to 2013, reported cure rates over the years ranged from 16% to 100% (panel E in Figure 5.2). Schistosome infection cure rates after praziquantel treatment have been reported from 27 countries (panel F in Figure 5.2). There were multiple countries (Mozambique, Botswana, Togo and Yemen) where only one study published from, in these cases they were grouped into “others” for graphical expression and also for statistical analysis.


A
Treatment dose
Cure rate (%)
100
50
0
0
20
40
60
mg/kg body weight
B
Schistosome parasite species
Cure rate (%)
100
50
0
S. mansoni
S. haematobium
C
Age
Cure rate (%)
100
50
0
Child
Adult
D
Time between treatment and follow up
Cure rate (%)
100
50
0
10
20
30
40
50
60
70
80
90
Days

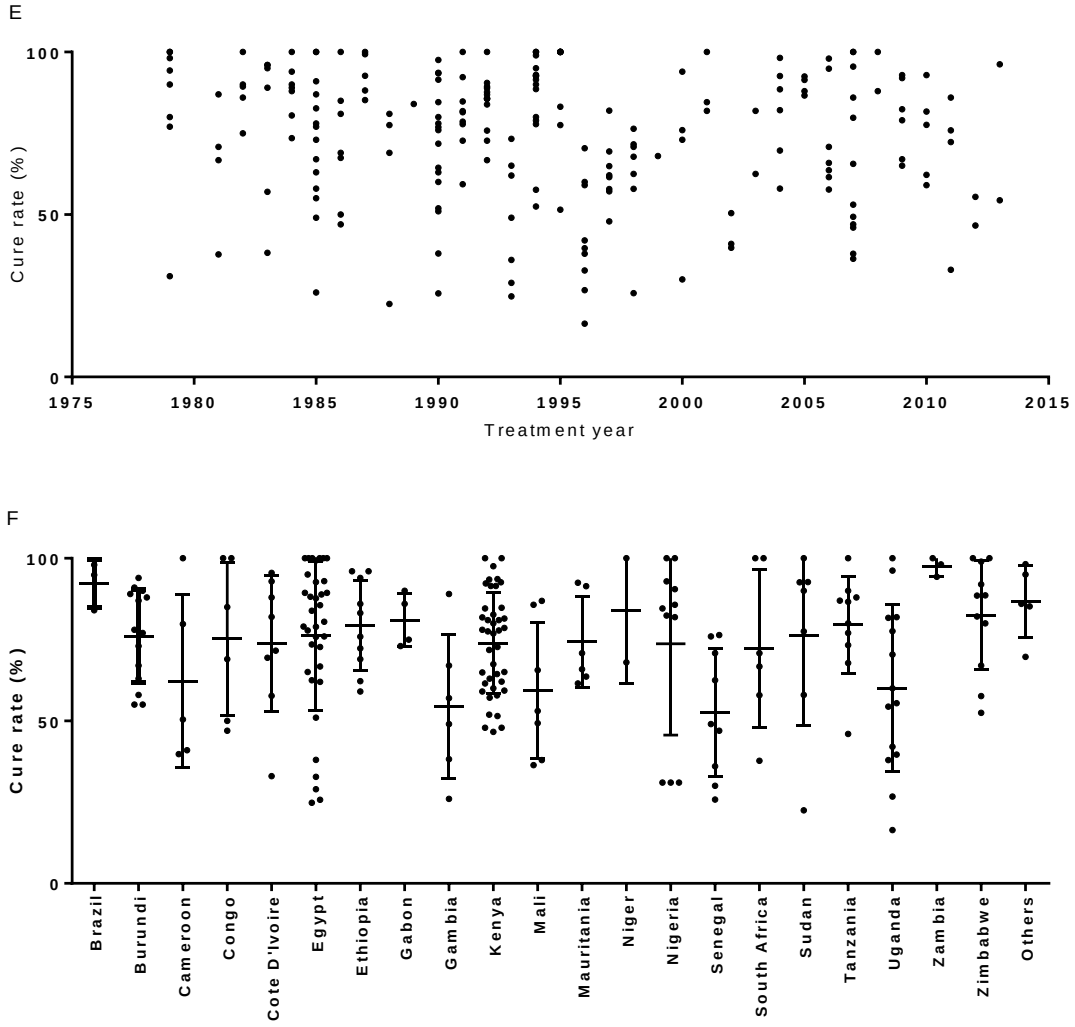


Figure 5.2: Scatter graph of the reported cure rates by predictors. Data points represent the reported cure rate for each observation. A: cure rate by the praziquantel treatment dose over the range 10-60 mg/kg body, B: cure rate by schistosome parasite species (*S. mansoni*, *S. haematobium*), C: cure rate by age category (children: 0-19 years old, adult ≥20 years old), D: cure rate by time after treatment (days), E: cure rate by treatment years from 1979 to 2013, F: cure rate by country. Bars in panel B and C represent the mean and SD. Where only a single study result was reported from a country, the study result was categorized into "others" group in graph in panel F.

The low cure rate (<50%) of schistosome infection after praziquantel treatment have been reported from different studies in different countries, both for *S. mansoni* and *S. haematobium* infections among from both children and adult participants (Table 5.3).

Table 5.3: Studies reported low (<50%) cure rates after praziquantel treatment. Child: 0-19 years old; Adult: ≥20 years old; S.m=*S. mansoni*; S.h=*S. haematobium.* Study names in the table were organized by the name of the author followed by the year of publication. In cases where the same author published multiple articles in the same year, references were distinguished by adding a letter after the first author's name and the year of publication. *Total praziquantel dose (mg/kg body weight) used for the treatment.

Reference	Country	Age	Parasite Species	Praziquantel dose*	Cure rate (%)
Oyediran *et al.* 1981	Nigeria	Child	S.h	30-40	31
Schutte *et al.* 1983	South Africa	Child	S.h	40	37
Wilkins *et al.* 1987 (a)	Gambia	Child	S.h	40	38
Wilkins *et al.* 1987 (b)	Gambia	Child	S.h	10-20	26-49
Polderman *et al.* 1988	Congo	Child	S.m	40	47
Jonge *et al.* 1990	Sudan	Child	S.h	40	23
Abu-Elyazeed *et al.* 1993	Egypt	Adult	S.m	40	26-38
Metwally *et al.* 1995	Egypt	Child	S.m	20	25-29
Guisse *et al.* 1997	Senegal	Child	S.m	40-60	36-49
Abu-Elyazeed *et al.* 1998	Egypt	Adult	S.h	40	33
Olds *et al.* 1999	Kenya	Child	S.m	40	48
Clercq *et al.* 2000	Senegal	Child	S.h	40	26
Clercq *et al.* 2002	Senegal	Child	S.h	40	30
Kabatereine *et al.* 2003	Uganda	Child/ Adult	S.m	40	16-42
Sacko *et al.* 2009	Mali	Child	S.h	40	36-49
Guidi *et al.* 2010	Tanzania	Child	S.h	40	46
Webster *et al.* 2013	Senegal	Child	S.h	40	47
Keiser *et al.* 2014	Cote D'Ivoire	Child	S.h	40	33
Wilson *et al.* 2014	Kenya	Child	S.h	40	47

5.3.2. Meta-regression results

Of the six potential predictors (Table 5.2), three were found to have a significant effect ($p<0.05$) on the response cure rate using forward stepwise selection: the treatment dose [$F(1, 207) = 7.610$, $p=0.006$], the schistosome parasite species [$F(1, 132) = 4.855$, $p=0.029$], and the age category [$F(1, 210) = 3.982$, $p=0.047$] (Table 5.4).

Table 5.4: Results from meta-regression multivariable analyses. Table shows F-values, degrees of freedoms (in parenthesis), and p-values from meta-regression using sequential sums of squares.

Name	Range	F-value (df)	p-value
Treatment dose	10-60 mg/kg body weight	7.610 (1, 207)	0.006
Schistosome parasite species	*S. mansoni* vs. *S. haematobium*	4.855 (1, 132)	0.029
Age	Child (0-19 years old) vs. Adult (≥ 20 years old)	3.982 (1, 210)	0.047

The model results indicated a positive relationship between praziquantel treatment dose and cure rate [$F(1, 207) = 7.610$, $p=0.006$, coefficient=0.541, SE of coefficient=0.196]. The model results suggested a higher cure rate for *S. mansoni* infection than *S. haematobium* infection, and a higher cure rate in adults than children. The fitted line demonstrates that the cure rate increased from 53% to 80% over the reported praziquantel treatment dose (10-60 mg/kg body weight) in children with *S. haematobium* infection (black dashed line in Figure

5.3). The fitted lines also show the estimated cure rate with current WHO recommended dose (40 mg/kg body weight) ranged from 69% to 83% depend on schistosome parasite species and age category (individual lines in Figure 5.3).

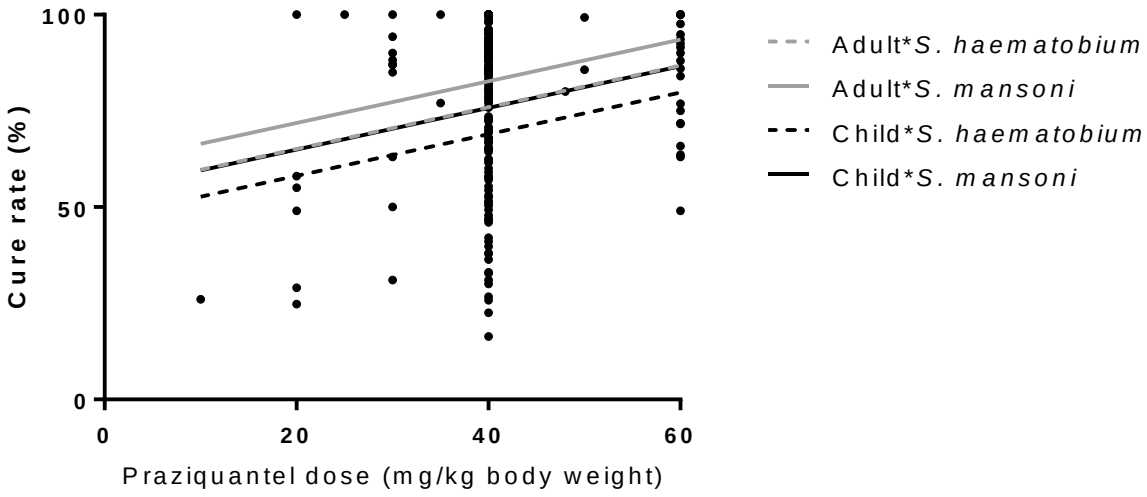


Figure 5.3: A fitted line graph of the effect of praziquantel treatment dose on cure rate. Data points indicate reported cure rate for each observation. Fitted lines for each age and schistosome parasite species combinations over the range 10-60 mg/kg body weight: adult with *S. haematobium* (grey dashed line); adult with *S. mansoni* (grey solid line); child with *S. haematobium* (black dashed line); and child with *S. mansoni* infection (black solid line).

5.3.3. The effect of treatment year and country on cure rate

Neither treatment year nor country were significant predictors of cure rate [F(1, 63) =0.456, p=0.502, and F(1, 86) =1.049, p=0.417 respectively] when they were included in the meta-regression model with the three significant predictors: treatment dose, schistosome

parasite species, and age category (Table 5.4). The subgroup analysis results also suggested negligible effects of treatment year and country on cure rate (Table 5.5). However, when the interaction between treatment year and country was included in the model, both country and interaction between treatment year and country were significant for children infected with *S. haematobium* (Table 5.5). The treatment years reported from each country have a high heterogeneity both in range and frequency (Appendix D.4).

Table 5.5: Effect of treatment year and country on cure rates. Table shows F values, degrees of freedoms (in parenthesis), and p values [in brackets] from a meta-regression using adjusted sums of squares. Article ID and number of participants of each observation were used for random effect and weighting respectively. * Number of observations.

			Univariate regression		Multiple regression		Multiple with interaction		
species	age	N*	Treatment year	country	Treatment year	country	Treatment year	country	Treatment year* country
S. mansoni	child	74	0.021 (1, 22) [0.885]	0.978 (15, 27) [0.502]	0.030 (1, 15) [0.864]	0.948 (15, 27) [0.529]	0.003 (1, 50) [0.953]	1.098 (7, 21) [0.399]	1.093 (7, 21) [0.402]
	adult	29	0.458 (1, 13) [0.510]	0.399 (6, 8) [0.861]	1.631 (1, 8) [0.237]	0.652 (6, 7) [0.691]	0.487 (1, 4) [0.524]	0.189 (3, 4) [0.899]	0.189 (3, 4) [0.899]
S. haematobium	child	71	0.330 (1, 50) [0.568]	1.279 (18, 30) [0.269]	0.376 (1, 26) [0.545]	1.278 (18, 27) [0.275]	0.077 (1, 39) [0.782]	5.058 (12, 39) [<0.001]	5.053 (12, 39) [<0.001]
	adult	5	0.040 (1, 3) [0.855]	could not run the model due to small sample size					

5.4. Discussion

I conducted a meta-analysis of praziquantel efficacy levels using published articles to identify predictors that have any influence on cure rate, as well as to detect any decrease in praziquantel efficacy levels over the 35 year reporting period. Praziquantel has been used as a drug of choice against schistosome infection over the past three decades in many endemic areas (WHO 2015). Although, there is no convincing evidence of the development of parasite resistance against treatment (Botros *et al.* 2005; Othman *et al.* 2015), low cure rates have been reported from different countries over the years. The results from this study are important to detect the parasites' possible resistance. In addition, the results also indicate that there is potential for an alternative praziquantel treatment dose, which could achieve better cure rates in endemic areas where low efficacy levels have been reported using the current standard dose (40 mg/kg body weight). The analyses revealed that there was considerable variability in schistosome infection cure rates after praziquantel treatment over treatment years from 1979 to 2013. Although low cure rates (<50%) have been reported from different African countries over the years (Table 5.3), high cure rates (>90%) have also constantly been reported from the same countries (panel E in Figure 5.2). Statistical analysis suggests that overall there is no significant treatment year effect on cure rates. This result indicates that there was no detectable cure rate reduction over the reporting period. Thus, there is no evidence that schistosome parasites have acquired resistance against praziquantel treatment, suggesting that the praziquantel treatment is still effective against schistosome infection.

Furthermore, subgroup analyses using studies within the selected countries (i.e., Egypt, Kenya, Nigeria, Uganda or Zimbabwe) also did not detect any significant decrease of cure rates over reported treatment years. These results again suggest that praziquantel treatment still maintained its effectiveness for schistosome infection. Nevertheless, there is still a risk of missing the parasites acquiring resistance against praziquantel treatment within each country. This is because, although studies within the same country were selected for subgroup analyses, the majority of these studies were conducted in the different study areas (e.g., different villages). Schistosomiasis transmission is known to be localized in endemic areas as transmission is regulated by the distribution of intermediate fresh water snail hosts. Therefore, disease transmission could vary even within a given endemic area depending on the natural water sources that people use for their daily lives (Clennon *et al.* 2006; Rudge *et al.* 2008). A heavy schistosomiasis burden, which can be indicated by high infection prevalence, has been reported to reduce praziquantel cure rates (Stothard *et al.* 2013). Nevertheless, in my analyses, it was impossible to distinguish between the influence of treatment year and study area on cure rates within each country due to high heterogeneity of study areas. Long term cohort studies of areas undergoing mass drug administration programmes are therefore important for detecting any reduction of praziquantel efficacy levels.

The influence of neither treatment year nor country on cure rates were significant in the main analysis. Nevertheless, the subgroup analyses using data of *S. haematobium* infection among children suggest the significant influence of both country and the interaction between

treatment year and country on cure rates. These results might suggest that the effect of treatment year on cure rates is different depending on the country where a study is conducted (Appendix D.5). In addition, the results also might suggest that cure rates of *S. haematobium* infection among children varies among different countries. However, extra care must be taken when interpreting these results, because the majority of these studies were conducted in different study areas within each country. As discussed above, it is difficult to distinguish the effect of treatment year and study area on cure rates. Furthermore, there were only small number of studies (1-10 observations) reported from each country, which might affect the statistical analyses results (Appendix D.5).

There were a number of studies that reported low schistosome infection cure rates (<50%) after praziquantel treatment (Table 5.3). Some of these studies also discussed possible biological reasons for the causes of these low cure rates. For example, multiple studies mentioned the possible influence of higher pre-treatment infection intensity on lower cure rates (Polderman *et al.* 1988; Guisse *et al.* 1997; Sacko *et al.* 2009; Keiser *et al.* 2014). This is partially because of contamination of dead eggs in the post-treatment parasitological samples, which reduces recorded cure rates (Polderman *et al.* 1988; Sacko *et al.* 2009; Guidi *et al.* 2010; Webster *et al.* 2013; Keiser *et al.* 2014), as it is difficult to differentiate live eggs from dead ones through parasitological diagnostic procedures (Polderman *et al.* 1988; Keiser *et al.* 2014). Thus, although Guidi *et al.* (2010) reported low cure rates after praziquantel treatment (46%), they also reported that the almost all eggs (>95%) detected in the post-treatment samples were dead (Guidi *et al.* 2010). Other studies concluded that

praziquantel treatment was still effective despite low cure rates, because of their high egg reduction rates after the treatment (Schutte *et al.* 1983; Jonge *et al.* 1990; Webster *et al.* 2013). Since praziquantel treatment is only effective for adult schistosome worms and has little or no effect on immature worms (Xiao *et al.* 2009), there is a possibility of participants being categorized as non-cured even after a successful praziquantel treatment, if participants were infected with immature worms. In high transmission areas, people could acquire schistosome infection just before or after praziquantel treatment, and eggs from these infections could be detected during the follow-up study. In these cases, a potential existence of live eggs in the post-treatment parasitological samples can happen even if praziquantel treatment had cleared all infected adult worms. One possible way to minimize dead eggs contaminations to estimate better cure rates is using the optimal interval between praziquantel treatment and parasitological follow up. Webster *et al.* (2013) suggested that the optimal interval between treatment and follow up to estimate cure rates among high pre-treatment infection intensity groups could be longer than three weeks after the treatment to minimize the contamination of dead eggs (Webster *et al.* 2013). On the other hand, Scherrer *et al.* (2009) reported the highest cure rates were estimated at 15-20 days after treatment compared to any shorter or longer (21-44 days) intervals between treatment and follow up (Scherrer *et al.* 2009). Although the main analysis did not demonstrate a significant influence of time between praziquantel treatment and follow up on cure rates within reported follow up days (14-90 days), longer follow up time could cause inaccurate cure rates due to re-infection after treatment. These studies suggest that low cure rates could be reported after praziquantel treatment if participants were infected with immature worms

and/or follow-up parasitological samples were contaminated with dead eggs. Measuring egg reduction rates together with cure rates could be advantageous for the better understanding of the impact of praziquantel treatment on schistosomiasis burden, especially in high transmission areas. Additionally, there could be a potential benefit in investigating schistosome egg viability when estimating the true praziquantel treatment effects, especially among high pre-treatment infection intensity participants.

Although multiple epidemiological studies have reported low cure rates (<50%) after praziquantel treatment and associated it with high pre-treatment infection intensity (Polderman *et al.* 1988; Guisse *et al.* 1997; Sacko *et al.* 2009; Keiser *et al.* 2014), I could not include pre-treatment infection intensity for statistical analysis in the current study. This was mainly because only a limited number of studies reported pre-treatment infection intensity. Furthermore, the preliminary univariate analysis using pre-treatment infection intensity as ordinal categorical variable (heavy/moderate/light) showed that the influence of pre-treatment infection intensity on cure rate was not statistically significant. To maximize the number of studies to be included in the main analysis pre-treatment infection intensity was not considered further.

My results suggest that praziquantel dose has a positive effect on the cure rates within the range of the reported treatment doses of 10-60 mg/kg body weight. Supporting this result, Taylor *et al.* previously reported a similar increase of cure rates with an increase in the praziquantel doses over 10-40 mg/kg body weight both for both for *S. mansoni* and *S.*

haematobium infection (Taylor *et al.* 1988). My results might suggest that a higher praziquantel dose than the current recommendation (40 mg/kg body weight) could potentially improve the cure rates. However, the absence of studies for adults with elevated praziquantel dose (>40 mg/kg body weight) made it difficult to estimate the effect of a higher dose in adults. In addition, the risk of having adverse events after praziquantel treatment has been reported to be higher in elevated doses (>40 mg/kg body weight) (Olliaro *et al.* 2011). On the other hand, no difference in the risk of adverse events have been reported between 40 mg/kg body weight and any lower treatment dose (Zwang *et al.* 2014). Severe adverse events can potentially make it difficult to re-recruit children after the initial treatment in high-prevalence areas (≥50% of individuals infected), where yearly treatment of school children is recommended (WHO 2002; Utzinger *et al.* 2009). Nevertheless, it might be worth considering using elevated praziquantel dose especially in schistosomiasis endemic areas with low praziquantel efficacy levels to achieve better cure rates (Garba *et al.* 2001; Sacko *et al.* 2009). However, when using a higher than recommended dose, extra care must be taken to minimize the occurrence of adverse events, and thus to ensure compliance in regular MDA.

In this analysis, studies that conducted the praziquantel treatment within a single day, which include both single and multiple praziquantel treatments, were selected. This was due to the high heterogeneity in the number of treatments and the days between the treatments among studies conducted multiple treatments over different days. Among the excluded studies, Tchuem *et al.* (Tchuem *et al.* 2013) reported high cure rate (>95% for *S. mansoni* infection,

>80% for *S. haematobium* infection) after praziquantel treatment using a total dose of 80 mg/kg body weight, which was administrated in two treatments in 3 weeks interval. Similarly, N'Goran *et al.* (N'Goran *et al.* 2003) reported high *S. haematobium* infection cure rates (>90%) after the two oral doses of praziquantel (each of 40mg/kg body weight) 4 weeks apart. Furthermore, the same study reported that significantly lower adverse events occurred after the 2nd praziquantel treatment in comparison to the 1st treatment (N'Goran *et al.* 2003). These reports together with my results suggest that using a higher praziquantel dose could improve the schistosome infection cure rates. In addition, should a higher praziquantel dose be used, it is worth considering dividing the treatment doses over different days in order to minimize the adverse events. Further studies are required to estimate the optimal time interval between these proposed treatments.

My results suggest a significantly higher cure rate in adults (≥20 years old) than in children (0-19 years old). In a previous meta-analysis, Stothard *et al.* (Stothard *et al.* 2013) have reported that there was a negligible difference between pre-school children and school aged children in their cure rate levels, suggesting that the effect of host age on praziquantel efficacy levels takes time to become detectable. The effectiveness of praziquantel has been reported to depend on host immune mechanisms that kill adult worms when praziquantel damages parasites' tegument to expose hidden antigens (Sabah *et al.* 1985). In addition, enhanced cure rates have been reported in experimental mice with high levels of schistosome parasite specific antibodies (Doenhoff *et al.* 1987; Fallon *et al.* 1992). Schistosome specific protective immunity is known to slowly develop with age in endemic

areas: this immunity reduces the infection intensity among adults, and could thus also improve the praziquantel treatment efficacy levels (Woolhouse *et al.* 1999; Mitchell *et al.* 2011). The difference in cure rate between these two age groups could be partially due to the immunological differences between adult and children.

Regardless of the immunological differences between adults and children, there is still a possibility that the age category is an indicator of pre-treatment infection intensity. Although an effect of pre-treatment infection intensity on praziquantel efficacy levels has been reported (Utzinger *et al.* 2000; Stothard *et al.* 2013), I could not take this into account in my analysis due to the high heterogeneity of populations that were represented by the study participants. For example, there are some studies that targeted all inhabitants of the study area (e.g., village, region) (Kabatereine *et al.* 2003; Muhumuza *et al.* 2014), whereas other studies targeted participants from high risk populations only (e.g., canal cleaners) (Satti *et al.* 1996; Black *et al.* 2009). Although these populations' characteristics have a potential to influence cure rates, there was not enough information about them. Both schistosome infection intensity and prevalence are known to peak among children in schistosomiasis endemic areas (Wilkins *et al.* 1984; Fulford *et al.* 1992), and age could be a confounder of the pre-treatment infection intensity. Further epidemiological and immunological studies are required to clarify whether the age effect on the cure rate is an artefact of these potential confounding factors, or is representing immunological difference between children and adults.

A number of epidemiological studies have been conducted in areas where both *S. mansoni* and *S. haematobium* infections are endemic. However, these studies have not demonstrated a consistent trend of different cure rate for schistosome parasite species (*S. mansoni* or *S. haematobium*). For example, of the two species, a better cure rate was reported for *S. haematobium* infection in Niger (Garba *et al.* 2013), whereas a better cure rate for *S. mansoni* infection was reported in Cameroon and in Senegal (Tchuente *et al.* 2013; Knowles *et al.* 2015). My results suggest that praziquantel treatment leads to higher cure rates in *S. mansoni* than in *S. haematobium* infection. Supporting this result, a meta-analysis conducted by Stothard *et al.* (Stothard *et al.* 2013) using studies on African children reported higher cure rate of *S. mansoni* infection than *S. haematobium*. The biological mechanism behind this difference between *S. mansoni* and *S. haematobium* is still not clear. Although calcium ion channels of schistosome parasites have been suggested as the molecular target of praziquantel, the mechanism of the action of praziquantel is not yet fully understood (Pica-Mattoccia *et al.* 2008). Therefore, the mechanism that could induce the difference in the levels of susceptibility among different schistosome parasite species is unknown. This difference might be caused by genetic and/or molecular level difference between *S. mansoni* and *S. haematobium* (Valentim et al. 2013). Another possibility is the different distributions of *S. mansoni* and *S. haematobium* in human body, as *S. mansoni* is mainly found in the superior mesenteric veins draining the large intestine whereas *S. haematobium* is found in the venous plexus of bladder (CDC 2016). These differences in the areas of infection in the human body might affect the levels of exposure to praziquantel. Understanding differences in praziquantel efficacy by species is important to adjust mass praziquantel administrative

programmes according to which schistosome parasite species is endemic in the targeting areas. There is still a need for more in vitro and animal studies of the mechanisms that cause the susceptibility difference between *S. mansoni* and *S. haematobium*.

There are multiple studies that have reported a limited sensitivity of current standard parasitological diagnostic methods for both *S. mansoni* and *S. haematobium* infection (Kat Katz, and urine filtration respectively), in particular, for participants with low infection intensity (Degarege *et al.* 2014; Knopp *et al.* 2015; Olliaro *et al.* 2015; Siqueira *et al.* 2015). This could influence the estimated cure rate difference found between *S. mansoni* and *S. haematobium* infection, as parasite eggs could be more easily detected in urine samples than faeces samples after praziquantel treatment (panel A and B in Appendix D.3). In addition, as a single schistosome egg in parasitological samples makes a difference between cured and non-cured participants, low sensitivity of diagnostic methods can easily cause the false negative cases that inflate reported cure rates. Therefore, although parasitological cure rates have been commonly used to evaluate the praziquantel efficacy levels (Stothard *et al.* 2013; Olliaro *et al.* 2015), WHO have recently recommended the use of egg reduction rate (the comparison of pre- and post- praziquantel treatment infection intensity expressed by the parasite egg counts) as a primary outcome measure to assess the praziquantel efficacy levels (WHO 2013). Furthermore, the primary aim of MDA programmes has been to reduce morbidity due to schistosomiasis in endemic areas (Lelo *et al.* 2014). Therefore, praziquantel may still be highly effective in MDA programmes when it can yield high egg reduction rate

even if it is not a complete cure. Further meta-analysis study with egg reduction rate could increase our understanding about praziquantel efficacy levels dynamics over reported years.

There are multiple factors that could not be included in the current study regardless of their potential influence on schistosome infection cure rate. For example, the source and manufacturer of praziquantel could be a confounder of other predictors. This is because variation in the praziquantel quality has been reported after the praziquantel patent expired, in particular as some fake praziquantel has been identified from different countries (Sulaiman *et al.* 2001). Studies that reported low cure rates might have used low-quality praziquantel; however, this could not be confirmed as majority of studies did not report the origin of praziquantel. The other example is providing supplemental snack or drink prior to praziquantel treatment, as this has been reported to improve the praziquantel efficacy levels (Muhumuza *et al.* 2014). There were multiple studies that reported providing a snack and/or juice before the praziquantel treatment (Groning *et al.* 1985; Simonsen *et al.* 1990; Berhe *et al.* 1999; Midzi *et al.* 2008; Sousa-Figueiredo *et al.* 2010; Mitchell *et al.* 2011; Olliaro *et al.* 2011; Navaratnam *et al.* 2012; Sousa-Figueiredo *et al.* 2012; Muhumuza *et al.* 2014), or alternatively, treated participants after their breakfast, lunch or dinner (Burchard *et al.* 1984; Farid *et al.* 1984; Kern *et al.* 1984). However, this could not be taken into account in the analyses as the majority of the studies did not report supplemental feeding status. It is important to develop a definitive guideline about how to report results of epidemiological studies about praziquantel efficacy levels, especially about factors which have been reported to influence cure rates.

There was no significant effect of treatment year on cure rate, which suggests a stable schistosome infection cure rate with praziquantel over the reported treatment years from 1979 to 2013. The results of this study also demonstrated that cure rates could partially depend on praziquantel treatment dose, schistosome parasite species (*S. mansoni* or *S. haematobium*), and age category (child or adult). Results also indicated that current WHO recommended treatment dose (40 mg/kg body weight) can achieve a cure rate in the range 69% to 83 % depending on schistosome parasite species and age group of participants. Although there was no clear evidence of schistosome parasites developing resistant and/or tolerance against praziquantel treatment, the regular monitoring of cure rate is essential for sustainable use of praziquantel, especially in the countries that have been using praziquantel for a long time (such as Egypt and Kenya). Similarly, areas which are currently undergoing MDA (Tuhebwe *et al.* 2015) are in need of close monitoring to detect any susceptibility levels change of schistosome parasite for praziquantel treatment. Close monitoring and possible adjustment of control programmes according to the cure rates would enable us to continue sustainable use of praziquantel for schistosomiasis treatment and control.

Chapter 6: General discussion

6.1. Introduction

Schistosomiasis remains a major public health problem, especially in sub-Saharan African countries (WHO 2015). Current control efforts are mainly focused on mass administration of the antihelminthic drug praziquantel, aiming to reduce prevalence, infection intensity and morbidity of schistosomiasis in endemic areas (WHO 2015). Closely monitoring praziquantel efficacy levels is important as it is the only drug that can treat all three major schistosome parasite species infecting humans: *S. mansoni*, *S. haematobium*, and *S. japonicum*. In addition, a better understanding of the impact of praziquantel treatment on host schistosome specific immunity will be advantageous in further understanding host immune mechanisms, as well as helping to estimate the impact of mass drug administrations (MDAs) on the development of protective immunity against re-infection.

Although extensive efforts have been made towards developing vaccines against schistosomiasis, there are still no vaccines licensed for human use. To date, there are only two candidate vaccines which are now in clinical trials: the 28 kDa *S. haematobium* GST (Sh28GST, Brand name: Bilhvax) which is in phase 3 clinical trials (Mountford *et al.* 2005; ClinicalTrials.gov 2012; Riveau *et al.* 2012), and 14KDa fatty acid-binding protein from *S. mansoni* (rSM14) which is in phase 2 clinical trials (Santini-Oliveira *et al.* 2016). Alongside

these subunit vaccine candidates, attenuated live-schistosome parasite vaccines have been studied extensively in animal models.

This thesis has four main objectives as presented in the Introduction: Chapters 2 and 3 relate to attenuated schistosome vaccines, Chapters 4 and 5 to praziquantel treatment. In this thesis, influential predictors of the efficacy levels of attenuated *S. mansoni* vaccine in murine models were identified (Chapter 2). Furthermore, the analyses using data for different animal hosts (rat and baboon) and different parasite species (*S. mansoni*, *S. haematobium*, *S. japonicum*, and *S. bovis*) quantified the effect of host and parasite species on attenuated schistosome parasite vaccine efficacy levels (Chapter 3). Using meta-regression models, predictors associated with praziquantel treatment efficacy were identified (Chapter 5). Classification and Regression Tree models were also applied to explore the predictors which have an influence on whether schistosome parasite specific antibody isotype levels increase or decrease after praziquantel treatment (Chapter 4).

In this discussion chapter, the key findings from this thesis are summarized and discussed alongside some possible implications for schistosomiasis control programmes. The strengths and limitations of methods used in this thesis are discussed, and suggestions for further research are provided before drawing some final conclusions.

6.2. Key findings and their implications

Currently, the majority of schistosomiasis vaccine development studies rely on recombinant technology to produce vaccines which contain only those antigens of parasites that are considered to stimulate host protective immunity against infection most effectively (de Veer *et al.* 2011). To date, there are two promising recombinant schistosomiasis vaccine candidates which are currently undergoing clinical trials (Sh28GST and rSM14). There are no ongoing clinical trials using attenuated schistosome parasite vaccine candidates, despite their high efficacy levels in animal studies (Chapter 2 and 3). Furthermore, multiple review articles have questioned the feasibility of attenuated parasite vaccines for human use (Waine *et al.* 1997; Hewitson *et al.* 2005; McManus 2005; El Ridi *et al.* 2015). This is in part because attenuated parasite vaccines have a complex composition, some of which may cause unwanted side-effects of vaccination (Soler *et al.* 2007). Thus, the number of articles published on attenuated schistosome parasite vaccines has dramatically reduced in recent years (Fukushige *et al.* 2015). In contrast, there is one attenuated malaria vaccine undergoing clinical trials. The PfSPZ vaccine, which is an attenuated *Plasmodium falciparum* sporozoite vaccine, reported as having high efficacy levels in animal models, has completed its phase 1 clinical trials and is now undergoing phase 2 clinical trials (Seder *et al.* 2013; WHO 2016). Alongside the PfSPZ vaccine, a recent study reported that a genetically attenuated *P. falciparum* sporozoite vaccine has high protective effect in the murine model (Van Schaijk *et al.* 2014).

In addition to the above, there are a number of malaria vaccine candidates. One of the most promising current malaria vaccine candidate is a recombinant protein vaccine that targets the

circumsporozoite protein of *P. falciparum* (RTS,S/AS01 also known as Mosquirix) which has completed its phase 3 clinical trials (Tinto *et al.* 2015). Mosquirix has now been reviewed by the European Medicines Agency (EMA) and the WHO for use in malaria endemic areas (Morrison 2015). This would not only be the first licensed malaria vaccine, but also the first licensed vaccine for human use for any parasitic disease. Besides RTS,S/AS01, there are a number of recombinant malaria vaccine candidates undergoing pre-clinical or clinical trials (WHO 2016). Currently, the majority of schistosomiasis and malaria vaccine development studies use recombinant techniques rather than attenuated parasite techniques (WHO 2016), as there are multiple advantages of recombinant vaccines. For example, once efficient antigens and adjuvants for vaccination are identified, recombinant vaccine can be produced in sufficient quantity at a low cost (Canales *et al.* 1997; Soler *et al.* 2007; Reed *et al.* 2013).

Nevertheless, attenuated schistosome parasite vaccines also have potential advantages over recombinant vaccines. The analyses showed that the optimally prepared irradiated *S. mansoni* cercariae vaccine could produce a protection as high as 79% against challenge infection in mice (Chapter 2). This result indicates the high potential efficacy of attenuated schistosome parasite vaccines, in contrast to recombinant vaccine candidates, which have failed to achieve consistent protection above 40% in mice (Bergquist *et al.* 1998; Mountford *et al.* 2005; Wilson *et al.* 2006). The analyses also showed the schistosome parasite species specificity of attenuated schistosome parasite vaccines (Figure 3.3 in Chapter 3). Thus, these vaccines are likely to be more effective against homologous species infection than

heterologous species infection. This result suggests that different vaccines might have to be developed according to prevalent schistosome species, to achieve better protection. Nevertheless, once we develop an effective attenuated schistosome parasite vaccine in a single schistosome species, we could potentially apply the same techniques for other schistosome parasites species. In addition, one study has reported the strain specificity of attenuated cercariae vaccine. Moloney *et al.* (1985) reported that an attenuated Chinese mainland *S. japonicum* cercariae vaccine was protective against homologous strains challenge, but was not protective against challenge infection with different *S. japonicum* strains (Moloney *et al.* 1985). Unlike recombinant vaccines, attenuated schistosome parasite vaccines could be prepared using parasite strains endemic to the target area, which might be able to achieve better protection.

Findings from attenuated schistosome parasite vaccine studies would improve our understanding of human immunity against schistosomiasis, which could be useful for further vaccine development. In Chapter 2, the negative influence of irradiation dose effect on protection levels suggests that for high protection, the host might have to be exposed to the different antigens from the different parasite life stages. This finding suggests that for recombinant schistosomiasis vaccine development, a mixture of antigens that are unique to different parasite life stages, might be able to achieve better protection levels than the antigens from a single life stage. Supporting this suggestion, schistosome worm antigens have been reported to be essential for the development of naturally acquired immunity among people in endemic areas, who are thought to be exposed to schistosome

eggs/cercariae/schistosomula antigens though natural infections (Mitchell *et al.* 2012). These findings further suggest that the antigen combinations for effective immunization might be different among naïve populations that have no previous contact with schistosome parasites, and people in the endemic areas who have been exposed to parasite antigens through their infection and treatment.

Among the three major human schistosome species, *S. japonicum* is known for its broad host range (Loker 1983; Riley *et al.* 2008). Domestic livestock such as water buffaloes, pigs, and sheep have been considered as major reservoir hosts of *S. japonicum* transmission (Riley *et al.* 2008; Gray *et al.* 2009). Therefore, schistosomiasis transmission blocking veterinary vaccine development against *S. japonicum* infection has mainly been studied in China where *S. japonicum* infection is endemic (Taylor *et al.* 1998; Shi *et al.* 2002). Among these vaccine candidates, an attenuated *S. japonicum* cercarial vaccine has been reported as offering high protection in the major transmission reservoirs, water buffaloes (Shi *et al.* 1990), and pigs (Shi *et al.* 1993; Bickle *et al.* 2001; Lin *et al.* 2011) in field conditions. Vaccinating domestic livestock could reduce the *S. japonicum* transmission burden in endemic areas. Furthermore, if successful, a veterinary vaccine could provide a paradigm for attenuated schistosome vaccine development for human use.

Considering the fact that there are no licensed schistosomiasis vaccines to date, it would be better not to restrict the approaches and techniques of vaccine development. It may be worth considering further development of attenuated schistosome parasite vaccines, with the

ultimate aim being their use in humans. Using diverse approaches in vaccine development, which include both recombinant vaccines and attenuated schistosome parasite vaccines, could enable us to identify an effective vaccine against schistosomiasis.

Schistosomiasis is mainly endemic in remote areas of developing countries where infrastructure is poor. Therefore, a good vaccine for schistosomiasis must have some characteristics that are necessary to provide vaccines for people in such areas. In general, the effective vaccine of any disease must be able to induce high protection against infection with minimal side effects. In addition, a good schistosomiasis vaccine must be affordably priced, be biologically stable at room temperature, and be easy to administrator (Loker *et al.* 2015). Furthermore, a vaccine must be suitable for young children who carry the heaviest disease burden in endemic areas (Fulford *et al.* 1992; Mutapi *et al.* 2006; Colley *et al.* 2014). Immunogenic antigen identification is fundamental, but there will be many hurdles to overcome after that before a schistosomiasis vaccine can be made available to the people who need it the most.

Since there are no schistosomiasis vaccines to date, the major control strategy that has been used over the past three decades is the mass administration of praziquantel in disease endemic areas (Gönnert *et al.* 1977; WHO 2015). This efficacious, low-cost drug has achieved a significant reduction in schistosomiasis burden in many endemic areas (Midzi *et al.* 2008; Evans *et al.* 2011; Liu *et al.* 2011; WHO 2015). Since praziquantel is currently the drug of choice for treatment and control of schistosomiasis (WHO 2015), the monitoring of

its efficacy levels is essential for sustainable disease control. In Chapter 5, results of the most comprehensive meta-analysis of praziquantel cure rate levels of *S. mansoni* and *S. haematobium* treatment to date are presented. Although there have been multiple studies reporting low cure rates after praziquantel treatment (Table 5.3 in Chapter 5), there was no significant reduction in cure rates over the reported years (praziquantel treatment years between 1979 to 2013). These analyses suggest that praziquantel is still effective against schistosome infections. The current WHO recommended praziquantel dose (40 mg/kg body weight) demonstrates that its estimated cure rate range of 69-83% depends on schistosome parasite species (*S. mansoni* vs. *S. haematobium*) and age group (children: 0-19 years old vs. adults: 20 years old or older) of participants. Despite the concerns about possible schistosome resistance to praziquantel, the analyses confirmed that praziquantel remains effective in the treatment of schistosomiasis.

Until recently, infants and preschool-age children (aged 5 years and below) have been excluded from praziquantel treatment. However, multiple studies have reported that praziquantel is safe and efficacious in infants and preschool-age children (Mutapi *et al.* 2011; Coulibaly *et al.* 2012; Stothard *et al.* 2013). In 2010, the WHO recommended that praziquantel be provided to infants and preschool-age children through regular health services (WHO 2011). Although infants and preschool-age children are still being excluded from schistosomiasis MDA programmes, this change in policy could make it possible to extend MDA to this part of the population in the future. In a meta-analysis, Stothard *et al.* (2013) reported that praziquantel cure rate levels among African preschool-age children

were comparable to those for school aged children (Stothard *et al.* 2013). However, further investigations are still needed to clarify the age effect on cure rates as the number of articles published for infants and preschool-age children is still limited (Stothard *et al.* 2013). My analyses indicated that there is an influence of age on cure rates after praziquantel treatment: adults (≥20 years old) showed higher cure rates than children (0-19 years old). More data are needed to confirm and quantify this effect. The close monitoring of praziquantel efficacy levels is essential for sustainable schistosomiasis control, especially if the targeted population is to be expanded to include preschool-age children.

In schistosomiasis endemic areas, it has been reported that infection intensity in older age groups is lower, partially due to naturally acquired protective immunity against re-infection (Woolhouse *et al.* 1999; Mitchell *et al.* 2011). One reason why this immunity takes time to develop is thought to be the long life span of the schistosome parasite which is capable of evading host immunity (Harris *et al.* 1984; Walter *et al.* 2006). While antigens from schistosome adult worms have been reported to be essential for the development of naturally acquired protective immunity among people in endemic areas, they only become accessible to the host immune system when the worms die (Harnett *et al.* 1986; Mutapi *et al.* 2005; Doenhoff *et al.* 2008). Consequently, praziquantel treatment for schistosome infection has been reported to enhance host protective immunity by exposing them to the parasite's hidden antigens (Harnett *et al.* 1986; Mutapi *et al.* 2005; Doenhoff *et al.* 2008). Supporting these reports, my results suggest that praziquantel treatment could increase the levels of anti-WWA IgA and IgE, both of which have been reported to be associated with resistance to

re-infection (Rihet *et al.* 1991; Vereecken *et al.* 2007), in the majority of populations (Chapter 4).

However, Chapter 4 also showed that there was a considerable variability in whether schistosome specific antibody levels increased or decreased after chemotherapy among different human populations. The analyses demonstrated that the combination of pre-treatment infection levels and age of participants influence the change of some antibodies (Figure 4.2 in Chapter 4). In addition, my analyses also showed the influence of days between treatment and follow up on the levels of anti-SEA IgE: an initial increase of anti-SEA IgE followed by a decrease below the pre-treatment levels 46 days after praziquantel treatment (Figure 4.2 in Chapter 4). Nevertheless, neither of these predictors could fully explain the variability of schistosome specific antibody level changes after praziquantel treatment among populations. There are multiple schistosome specific antibodies which have been reported to have positive or negative association with levels of protective immunity against re-infection. For example, high levels of schistosome specific IgE have been associated with protection against re-infection, whereas high levels of schistosome specific IgG4 have been associated with high levels of susceptibility to infection (Rihet *et al.* 1991; Grogan *et al.* 1997; Oliveira *et al.* 2012). Therefore, the variability of schistosome parasite specific antibody isotype level changes might suggest that the praziquantel treatment will influence protective immunity in a different way among different populations. That is, although the analyses suggested that the praziquantel treatment will increase the levels of protective immunity against re-infection in the majority

of populations, there is still a possibility that the same treatment have variable effects on the levels of protection for the other populations. Further studies are required to clarify the effects of praziquantel treatment on protective immunity against re-infection, and also to identify influential predictors of these effects. These results also highlight the importance of developing an effective vaccine for schistosomiasis.

6.3. Methodological limitations and suggestions for future works

6.3.1. Methodological limitations

There are limitations of the systematic review and meta-analysis approaches. As such analyses are based on published articles there is always a risk of publication bias and language bias in any meta-analysis study (Sutton *et al.* 2000; Juni *et al.* 2002). In this thesis, I took an extra step to minimize language bias by including non-English articles into the analysis, which included Chinese, French, German, Italian, Portuguese, Russian, Spanish and Japanese articles. I identified non-English articles which published titles and/or abstracts in English, so there is still a risk of missing non-English articles if they were published solely in the original language. In this thesis, I aimed to make the systematic review procedure as comprehensive as possible by using multiple literature databases, and by contacting authors when a full-text article could not be accessed easily. Although the funnel plot is the most commonly used graphical method to detect publication bias, it was not applied beyond preliminary graphical exploration in this thesis. This is mainly because the funnel plot procedure makes the assumption that there is a single true outcome value (e.g., cure rate, fraction protected) which is common among all studies, which is not the case for the data characteristics in this study (Duval *et al.* 2000; Bax *et al.* 2011). In addition, Sutton *et al.* (2000) have conducted a meta-analysis of meta-analysis articles to investigate the effect of publication bias on the final outcome of studies, and reported that there was only a small impact of publication biases on the statistical conclusions of meta-analysis articles (Sutton *et al.* 2000).

In this thesis, I aimed to maximize the number of studies available for meta-analysis. As reviewed in Chapter 1, using the inversed variance within the study is the most commonly and sophisticated approach to weight studies in meta-analysis and meta-regression (Borenstein 2009). In this approach, studies with smaller within study variance are regarded to be more accurate and have larger power in the analysis. Nevertheless, I found that the majority of studies identified in my study did not report the within study variance of their results. There were two approaches that could be taken to deal with this issue. The first option was to exclude studies that failed to report within study variance from quantitative analyses. This approach enables me to use the inversed variance within the study for weighting. At the same time, the number of studies included would be reduced by more than 50%. The second option, which I adopted in this thesis was to use an alternative weighting of observations for the statistical analyses. I used sample size (i.e., number of animals or participants) as a weighting for all the analyses except the random-effect meta-analysis in Chapter 2. Sample size has been recommended as an alternative weighting methods for meta-regression analysis (Hunter *et al.* 2004; Brannick *et al.* 2011). This weighting approach is based on the assumption that results of larger studies are more likely to be accurate than that of smaller studies. Nevertheless, the association between sample size and accuracy of results could not be confirmed in current study.

Praziquantel has been used for more than 30 years to control the morbidity and prevalence of schistosomiasis in endemic areas (Gönnert *et al.* 1977; WHO 2015). Although the same drug has been used, the design of control programmes has been modified over the time to

increase their treatment impact. For example, providing a supplemental snack or drink prior to treatment has been reported to improve cure rates and also to reduce side effects after treatment (Muhumuza *et al.* 2014). Therefore, a number of recent studies provided a snack for children prior to the treatment (Groning *et al.* 1985; Simonsen *et al.* 1990; Berhe *et al.* 1999; Midzi *et al.* 2008; Sousa-Figueiredo *et al.* 2010; Mitchell *et al.* 2011; Olliaro *et al.* 2011; Navaratnam *et al.* 2012; Sousa-Figueiredo *et al.* 2012; Muhumuza *et al.* 2014). On the other hand, there also were studies that provided praziquantel for children with an empty stomach, believing in that this approach could yield better treatment effect (Kiliku *et al.* 1991). Studies with or without a supplemental snack could have different treatment efficacy, however, I could not consider this factor in the analysis. Because the majority of studies do not reported if they provided any snack before the treatment. Analysing schistosomiasis studies published over decades made it difficult to include all the predictors of interest, as some of potential predictors were not reported by the older studies. Conducting subgroup analysis is possible but risks selection bias. In this thesis, therefore, I focused on predictors whose importance has been recognized for a long time. There is still a risk that I have missed predictors (such as pre-treatment supplemental snack) whose importance has only recently been recognised.

The majority of studies included in the analyses reported the age range of participants, which varied between studies (e.g., 10-20 years old, 5-50 years old). In addition, there were other studies that reported the average age of participants but did not report age range. In this thesis, I used broad age range categories (i.e., child/adolescent/adult or child/adult) to

include as many as possible studies in meta-analyses. Initially, more detailed age group categorization (i.e., infant/child/adolescent/adult/elderly) was attempted but the variability among studies did not allow this approach. There could be differences in disease burden and immunity levels between very young pre-school aged children and school children, or school children and adolescent but such differences could not be investigated in my study.

6.3.2. Recommendations for future studies

There are a number of ongoing mass praziquantel administration programmes in schistosomiasis endemic areas, where people are treated at regular intervals (Cleland *et al.* 2014; Omedo *et al.* 2014; Tuhebwe *et al.* 2015). Unlike experimental naïve animals, people in these endemic areas have been chronically exposed to schistosome parasite antigens. Yole *et al.* (1996) reported high efficacy levels of attenuated *S. mansoni* vaccine in schistosome infection naïve baboons (Yole *et al.* 1996; Kariuki *et al.* 2004). Furthermore, the same research group investigated the influence of previous schistosome infection and/or praziquantel treatment on vaccine efficacy levels, and reported the comparable high protections among all vaccinated baboons (Kariuki *et al.* 2006). This might suggest that attenuated schistosome parasite vaccines have a potential to be an effective vaccine for both schistosome infection naïve human populations (e.g., travellers) and people in endemic areas. However, there are also limitations of animal studies. For example, the time between initial schistosome infection and vaccination in these baboon studies ranged from 12 to 18 weeks (Kariuki *et al.* 2006), which in contrast with the prolonged infection in endemic areas that can last few years even to decades. In addition, people in schistosomiasis endemic areas are often co-infected with other pathogens such as soil transmitted helminths and malaria (Mutapi *et al.* 2000; Alemu *et al.* 2011), which could also influence vaccine efficacy levels. Moreover, a previous study reported that adult worm antigens are essential to develop a protective immunity among people in endemic areas (Mitchell *et al.* 2012), not cercarial antigens. These reports together with my analyses might suggest that although attenuated cercarial vaccines have potential to be an effective vaccine, the vaccine efficacy levels

among schistosome infection naïve populations and people in endemic areas could be different. Further studies are required to estimate the influence of chronic schistosome infection and previous praziquantel treatment on human immunity against schistosomiasis, which might affect the levels of vaccine efficacy.

Schistosomiasis infection intensity and related morbidity have been reported to vary among individuals, even in populations within the same geographical areas (e.g., villages, neighbours sharing the same water sources). This could be due to the variation of water-contact frequencies (Chandiwana *et al.* 1991), age (Mutapi *et al.* 1997), or the levels of acquired immunity (Butterworth *et al.* 1987; Mutapi *et al.* 1997). However, as there was no available data for individual cases in the majority of studies, the average values of the participants were used both for response variables (e.g., cure rate, antibody direction of change) and predictors. A group of participants often consisted of individuals of different age, co-infection status with other pathogens, and with different levels of schistosomiasis burden. Extra care must be taken when translating the results from meta-analysis into real epidemiological situations. When publishing the raw study data becomes more common in the field of epidemiology, more detailed meta-analyses could be undertaken by synthesizing individual-level raw data from multiple studies. This would enable an increase in the number of potential predictors, and could also enhance the general applicability of the findings from the analyses.

Currently MDA programmes with praziquantel, is the leading approach to schistosomiasis

control in endemic areas. A lot of effort has been made to maximize MDA coverage and also to improve praziquantel treatment efficacy. Praziquantel usage in endemic countries has increased from 100 million tablets per year in 2005 to over 250 million tablets per year in 2016 enough to treat approximately 140 million individuals (Fenwick 2015). This increase was achieved by praziquantel donation by pharmaceutical companies, especially Merck KGaA (Fenwick 2015; WHO 2016). Furthermore, in July 2012, a non-profit paediatric praziquantel consortium was launched involving Merck KGaA, Darmstadt, Astellas, Swiss TPH and Lygature (Pediatric Praziquantel Consortium 2016). This Pediatric Praziquantel Consortium focused on the development of paediatric praziquantel formulation to treat schistosomiasis in children aged 3 months to 6 years old (Fenwick 2015; Pediatric Praziquantel Consortium 2016).

These results of MDA programmes as well as the increasing number of studies on the safety and efficacy of praziquantel in preschool children, have been published as a number of scientific papers. These studies present a good opportunity to conduct a systematic review and meta-analysis to investigate the efficacy of praziquantel treatments and their influence on host schistosome specific immunity. Regular updates of systematic review and meta-analysis study results will be important to keep our knowledge about schistosomiasis control up to date.

6.4. Conclusions

In this thesis, systematic reviews and meta-analyses were conducted to answer a series of immunological and epidemiological questions concerning schistosomiasis. The findings of this thesis are expected to contribute towards vaccine development, the planning of sustainable control programmes, and improve the understanding of human protective immunity against schistosomiasis. My analyses showed a high protective effect of attenuated schistosome parasite vaccine in animal models. Schistosome vaccine development will benefit from close examination of the mechanisms through which protection is acquired in attenuated schistosome parasite vaccine studies that show high efficacy in animal models. Nevertheless, it will take time to develop a suitable vaccine for human use in schistosomiasis endemic areas.

My analyses showed a large heterogeneity of praziquantel treatment effects on whether schistosome specific antibody levels increased or decreased among different human populations. The results also showed a trend of increase of some schistosome specific antibodies after praziquantel treatment. Some of these antibodies, namely anti-WWA IgA, IgE and anti-SEA IgE, have been reported their protective effect for re-infection. These results might indicate the positive influence of praziquantel treatment on protective immunity against future infection. Furthermore, despite concerns about parasites acquiring resistance to praziquantel treatment, the analyses showed there has been no reduction in praziquantel cure rate in recent years. This result confirm that praziquantel sustains its effectiveness for schistosomiasis treatment even after decades of usage. The close

monitoring of praziquantel efficacy is an important component of sustaining effective schistosomiasis control programmes in endemic areas.

Appendices

Appendix A: Supplementary materials for Chapter 2.

A.1: The list of articles used for the meta-analysis

List of articles included for the analysis (1-105)

1. Agnew, A.M., Murare, H.M. and Doenhoff, M.J. (1989). Specific cross-protection between *Schistosoma bovis* and *S. haematobium* induced by highly irradiated infections in mice. *Parasite Immunol* 11(4): 341-349.
2. Aitken, R., Coulson, P.S., Dixon, B. and Wilson, R.A. (1987). Radiation-resistant acquired-immunity of vaccinated mice to *Schistosoma mansoni*. *Am J Trop Med Hyg* 37(3): 570-577.
3. Aitken, R., Coulson, P.S. and Wilson, R.A. (1988). Pulmonary leukocytic responses are linked to the acquired-immunity of mice vaccinated with irradiated cercariae of *Schistosoma mansoni*. *J Immunol* 140(10): 3573-3579.
4. Anderson, S., Coulson, P.S., Ljubojevic, S., Mountford, A.P. and Wilson, R.A. (1999). The radiation-attenuated schistosome vaccine induces high levels of protective immunity in the absence of B cells. *Immunology* 96(1): 22-28.
5. Barsoum, I.S., Bogitsh, B.J. and Colley, D.G. (1992). Detection of *Schistosoma mansoni* circulating cathodic antigen for evaluation of resistance induced by irradiated cercariae. *J Parasitol* 78(4): 681-686.
6. Beaudoin, R.L., Armstrong, J.C. and Vannier, W.E. (1980). Production of radiation-attenuated vaccines against malaria and schistosomiasis. *Int J Nucl Med Biol* 7(2): 113-124.
7. Bickle, Q.D. (1982). Studies on the relationship between the survival of *Schistosoma mansoni* larvae in mice and the degree of resistance produced. *Parasitology* 84(Feb): 111-122.
8. Bickle, Q.D. and Andrews, B.J. (1985). Resistance following drug attenuation (ro-11-3128 or oxamniquine) of early *Schistosoma mansoni* infections in mice. *Parasitology* 90(Apr): 325-338.
9. Bickle, Q.D. and Doenhoff, M.J. (1987). Comparison of the live vaccine potential of different geographic isolates of *Schistosoma mansoni*. *J Helminthol* 61(3): 191-195.

10. Bickle, Q.D., Ford, M.J. and Andrews, B.J. (1983). Studies on the development of anti-schistosomular surface antibodies by mice exposed to irradiated cercariae, adults and/or eggs of *S. mansoni*. *Parasite Immunol* 5(5): 499-511.
11. Bickle, Q.D., Sacko, M. and Vignali, D.A.A. (1990). Induction of immunity against *Schistosoma mansoni* by drug (ro11-3128)-terminated infections: analysis of surface-antigen recognition. *Parasite Immunol* 12(6): 569-586.
12. Bickle, Q.D., Taylor, M.G., Doenhoff, M.J. and Nelson, G.S. (1979). Immunization of mice with gamma-irradiated intramuscularly injected schistosomula of *Schistosoma mansoni*. *Parasitology* 79(Oct): 209-222.
13. Cheever, A.W., Hieny, S., Duvall, R.H. and Sher, A. (1983). Lack of resistance to *Schistosoma japonicum* in mice immunized with irradiated *S. mansoni* cercariae. *Trans R Soc Trop Med Hyg* 77(6): 812-814.
14. Constant, S.L., Mountford, A.P. and Wilson, R.A. (1990). Phenotypic analysis of the cellular responses in regional lymphoid organs of mice vaccinated against *Schistosoma mansoni*. *Parasitology* 101 Pt 1: 15-22.
15. Correa-Oliveira, R., James, S.L., McCall, D. and Sher, A. (1986). Identification of a genetic locus, Rsm-1, controlling protective immunity against *Schistosoma mansoni*. *J Immunol* 137(6): 2014-2019.
16. Correa-Oliveira, R., Sher, A. and James, S.L. (1984). Defective vaccine-induced immunity to *Schistosoma mansoni* in P strain mice. I. Analysis of antibody responses. *J Immunol* 133(3): 1581-1586.
17. Coulson, P.S. and Mountford, A.P. (1989). Fate of attenuated schistosomula administered to mice by different routes, relative to the immunity induced against *Schistosoma mansoni*. *Parasitology* 99 Pt 1: 39-45.
18. Coulson, P.S. and Wilson, R.A. (1988). Examination of the mechanisms of pulmonary phase resistance to *Schistosoma mansoni* in vaccinated mice. *Am J Trop Med Hyg* 38(3): 529-539.
19. Coulson, P.S. and Wilson, R.A. (1997). Recruitment of lymphocytes to the lung through vaccination enhances the immunity of mice exposed to irradiated schistosomes. *Infect Immun* 65(1): 42-48.

20. Crabtree, J.E. and Wilson, R.A. (1986). The role of pulmonary cellular reactions in the resistance of vaccinated mice to *Schistosoma mansoni*. *Parasite Immunol* 8(3): 265-285.
21. Dean, D.A., Bukowski, M.A. and Clark, S.S. (1981). Attempts to transfer the resistance of *Schistosoma mansoni* infected and irradiated cercaria-immunized mice by means of parabiosis. *Am J Trop Med Hyg* 30(1): 113-120.
22. Dean, D.A., Cioli, D. and Bukowski, M.A. (1981). Resistance induced by normal and irradiated *Schistosoma mansoni*: ability of various worm stages to serve as inducers and targets in mice. *Am J Trop Med Hyg* 30(5): 1026-1032.
23. Dean, D.A. and Mangold, B.L. (1992). Evidence that both normal and immune elimination of *Schistosoma mansoni* take place at the lung stage of migration prior to parasite death. *Am J Trop Med Hyg* 47(2): 238-248.
24. Dean, D.A., Mangold, B.L., Georgi, J.R. and Jacobson, R.H. (1984). Comparison of *Schistosoma mansoni* migration patterns in normal and irradiated cercaria-immunized mice by means of autoradiographic analysis: evidence that worm elimination occurs after the skin phase in immunized mice. *Am J Trop Med Hyg* 33(1): 89-96.
25. Dean, D.A., Mangold, B.L. and Lewis, F.A. (1995). Comparison of two strains of *Schistosoma mansoni* with respect to the sites and kinetics of immune elimination in irradiated cercaria-immunized mice. *J Parasitol* 81(1): 43-47.
26. Dean, D.A., Murrell, K.D., Xu, S.T. and Mangold, B.L. (1983). Immunization of mice with ultraviolet-irradiated *Schistosoma mansoni* cercariae: a re-evaluation. *Am J Trop Med Hyg* 32(4): 790-793.
27. Delgado, V.S. and Mclaren, D.J. (1990). Evidence that radio-sensitive cells are central to skin-phase protective immunity in CBA/Ca mice vaccinated with radiation-attenuated cercariae of *Schistosoma mansoni* as well as in naive mice protected with vaccine serum. *Parasitology* 100: 45-56.
28. Dent, L.A., Munro, G.H., Piper, K.P., Sanderson, C.J., Finlay, D.A., Dempster, R.K., Bignold, L.P., Harkin, D.G. and Hagan, P. (1997). Eosinophilic interleukin 5 (IL-5) transgenic mice: Eosinophil activity and impaired clearance of *Schistosoma mansoni*. *Parasite Immunol* 19(7): 291-300.
29. El Ridi, R., Tawab, N.A. and Guirguis, N. (1993). *Schistosoma mansoni*: identification

and protective immunity of adult worm antigens recognized by T lymphocytes of outbred swiss mice immunized with irradiated cercariae. *Exp Parasitol* 76(3): 265-277.

30. Erickson, D.G. and Caldwell, W.L. (1965). Acquired resistance in mice and rats after exposure to gamma-irradiated cercariae. *Am J Trop Med Hyg* 14(4): 566-573.
31. Flisser, A., Delgado, V.S. and Mclaren, D.J. (1989). *Schistosoma mansoni*: enhanced efficacy of praziquantel treatment in immune mice. *Parasite Immunol* 11(4): 319-328.
32. Hackett, F., Simpson, A.J.G., Omerali, P. and Smithers, S.R. (1987). Surface-antigens of and cross-protection between 2 geographical isolates of *Schistosoma mansoni*. *Parasitology* 94: 301-312.
33. Hanna, L.S., Mohamed, M.I. and Botros, S.S. (1994). *Schistosoma mansoni*: IgG reactivity to soluble adult worm antigen preparation (SWAP) in sera of immunized mice with irradiated attenuated and virulent cercariae. *J Egypt Soc Parasitol* 24(3): 553-568.
34. Hewitson, J.P., Hamblin, P.A. and Mountford, A.P. (2007). In the absence of CD154, administration of interleukin-12 restores Th1 responses but not protective immunity to *Schistosoma mansoni*. *Infect Immun* 75(7): 3539-3547.
35. Hsu, S.Y.L., Hsu, H.F. and Burmeister, L.F. (1981). *Schistosoma mansoni*: vaccination of mice with highly x-irradiated cercariae. *Exp Parasitol* 52(1): 91-104.
36. Hsu, S.Y.L., Hsu, H.F., Osborne, J.W. and Johnson, S.C. (1982). *Schistosoma mansoni*: is acquired-immunity induced by highly X-irradiated cercariae dependent on the size of the challenging dose. *J Parasitol* 68(2): 199-201.
37. James, E.R. and Dobinson, A.R. (1985). Comparison of the protective resistance induced by 60Co-irradiated cercariae and schistosomula of the WFFS and NMRI strains of *Schistosoma mansoni*. *J Helminthol* 59(4): 313-317.
38. James, S.L. and Sher, A. (1983). Mechanisms of protective immunity against *Schistosoma mansoni* infection in mice vaccinated with irradiated cercariae .3. Identification of a mouse strain, P/N, that fails to respond to vaccination. *Parasite Immunol* 5(6): 567-575.
39. Kambara, T. and Wilson, R.A. (1990). In situ pulmonary responses of T cell and macrophage subpopulations to a challenge infection in mice vaccinated with irradiated cercariae of *Schistosoma mansoni*. *J Parasitol* 76(3): 365-372.

40. Kamiya, H., Smithers, S.R. and McLaren, D.J. (1987). *Schistosoma mansoni*: autoradiographic tracking studies of isotopically-labelled challenge parasites in naive and vaccinated CBA/Ca mice. *Parasite Immunol* 9(4): 515-529.
41. Kelly, E.A. and Colley, D.G. (1986). Effects of immunomanipulations on resistance induced by irradiated *Schistosoma mansoni* cercarial sensitization of C57BL/6 and CBA/J mice. *Am J Trop Med Hyg* 35(4): 803-811.
42. Kelly, E.A. and Colley, D.G. (1988). In vivo effects of monoclonal anti-L3T4 antibody on immune responsiveness of mice infected with *Schistosoma mansoni*. Reduction of irradiated cercariae-induced resistance. *J Immunol* 140(8): 2737-2745.
43. King, C.L., Jia, X.L., Malhotra, I., Liu, S.F., Mahmoud, A.A.F. and Oettgen, H.C. (1997). Mice with a targeted deletion of the IgE gene have increased worm burdens and reduced granulomatous inflammation following primary infection with *Schistosoma mansoni*. *J Immunol* 158(1): 294-300.
44. King, C.L., Malhotra, I. and Jia, X.L. (1996). *Schistosoma mansoni*: Protective immunity in IL-4-deficient mice. *Exp Parasitol* 84(2): 245-252.
45. Lewis, F.A. and Wilson, E.M. (1982). *Schistosoma mansoni*: splenic lymphocyte-responses of mice after initial exposure to highly irradiated cercariae. *Exp Parasitol* 54(1): 21-32.
46. Lewis, F.A., Winestock, J., Dingaan, B., Richards, C. and Dean, D.A. (1987). Intraspecific cross-protection in mice immunized with irradiated *Schistosoma mansoni* cercariae. *J Parasitol* 73(4): 787-791.
47. Mangold, B.L. and Dean, D.A. (1984). The migration and survival of gamma-irradiated *Schistosoma mansoni* larvae and the duration of host-parasite contact in relation to the induction of resistance in mice. *Parasitology* 88(Apr): 249-266.
48. Mangold, B.L., Dean, D.A., Coulson, P.S. and Wilson, R.A. (1986). Site requirements and kinetics of immune-dependent elimination of intravascularly administered lung stage schistosomula in mice immunized with highly irradiated cercariae of *Schistosoma mansoni*. *Am J Trop Med Hyg* 35(2): 332-344.
49. Mastin, A.J., Bickle, Q.D. and Wilson, R.A. (1983). *Schistosoma mansoni*: migration and attrition of irradiated and challenge schistosomula in the mouse. *Parasitology*

87(Aug): 87-102.

50. Mclaren, D.J., Pearce, E.J. and Smithers, S.R. (1985). Site potential for challenge attrition in mice, rats and guinea-pigs vaccinated with irradiated cercariae of *Schistosoma mansoni*. *Parasite Immunol* 7(1): 29-44.
51. Mclaren, D.J. and Smithers, S.R. (1988). Serum from CBA/Ca mice vaccinated with irradiated cercariae of *Schistosoma mansoni* protects naive recipients through the recruitment of cutaneous effector-cells. *Parasitology* 97: 287-302.
52. Mclaren, D.J., Strath, M. and Smithers, S.R. (1987). *Schistosoma mansoni*: evidence that immunity in vaccinated and chronically infected CBA/Ca mice is sensitive to treatment with a monoclonal-antibody that depletes cutaneous effector-cells. *Parasite Immunol* 9(6): 667-682.
53. Menson, E.N., Coulson, P.S. and Wilson, R.A. (1989). *Schistosoma mansoni*: circulating and pulmonary leucocyte responses related to the induction of protective immunity in mice by irradiated parasites. *Parasitology* 98 (Pt 1): 43-55.
54. Menson, E.N. and Wilson, R.A. (1989). Lung-phase immunity to *Schistosoma mansoni*. Flow cytometric analysis of macrophage activation states in vaccinated mice. *J Immunol* 143(7): 2342-2348.
55. Menson, E.N. and Wilson, R.A. (1990). Lung-phase immunity to *Schistosoma mansoni*: definition of alveolar macrophage phenotypes after vaccination and challenge of mice. *Parasite Immunol* 12(4): 353-366.
56. Miller, K.L. and Smithers, S.R. (1980). *Schistosoma mansoni*: the attrition of a challenge infection in mice immunized with highly irradiated live cercariae. *Exp Parasitol* 50(2): 212-221.
57. Miller, K.L. and Smithers, S.R. (1982). Localized skin changes at the site of immunization with highly irradiated cercariae of *Schistosoma mansoni* are associated with enhanced resistance to a challenge infection. *Parasitology* 85(Oct): 305-314.
58. Miller, K.L., Smithers, S.R. and Sher, A. (1981). The response of mice immune to *Schistosoma mansoni* to a challenge infection which bypasses the skin: evidence for 2 mechanisms of immunity. *Parasite Immunol* 3(1): 25-31.
59. Minard, P., Dean, D.A., Jacobson, R.H., Vannier, W.E. and Murrell, K.D. (1978).

Immunization of mice with cobalt-60 irradiated *Schistosoma mansoni* cercariae. *Am J Trop Med Hyg* 27(1 Pt 1): 76-86.

60. Minard, P., Dean, D.A., Vannier, W.E. and Murrell, K.D. (1978). Effect of immunization on migration of *Schistosoma mansoni* through lungs. *Am J Trop Med Hyg* 27(1): 87-93.
61. Mitchell, G.F., Davern, K.M., Wood, S.M., Wright, M.D., Argyropoulos, V.P., Mcleod, K.S., Tiu, W.U. and Garcia, E.G. (1990). Attempts to induce resistance in mice to *Schistosoma japonicum* and *Schistosoma mansoni* by exposure to crude schistosome antigens plus cloned glutathione-S-transferases. *Immunol Cell Biol* 68: 377-385.
62. Moloney, N.A., Bickle, Q.D. and Webbe, G. (1985). The induction of specific immunity against *Schistosoma japonicum* by exposure of mice to ultraviolet attenuated cercariae. *Parasitology* 90(Apr): 313-323.
63. Mountford, A.P., Coulson, P.S., Pemberton, R.M., Smythies, L.E. and Wilson, R.A. (1992). The generation of interferon-gamma-producing lymphocytes-t in skin-draining lymph-nodes, and their recruitment to the lungs, is associated with protective immunity to *Schistosoma mansoni*. *Immunology* 75(2): 250-256.
64. Mountford, A.P., Coulson, P.S. and Wilson, R.A. (1988). Antigen localization and the induction of resistance in mice vaccinated with irradiated cercariae of *Schistosoma mansoni*. *Parasitology* 97: 11-25.
65. Mountford, A.P., Hogg, K.G., Coulson, P.S. and Brombacher, F. (2001). Signaling via interleukin-4, receptor alpha chain is required for successful vaccination against schistosomiasis in BALB/c mice. *Infect Immun* 69(1): 228-236.
66. Mountford, A.P. and Wilson, R.A. (1990). *Schistosoma mansoni*: the effect of regional lymphadenectomy on the level of protection induced in mice by radiation-attenuated cercariae. *Exp Parasitol* 71(4): 463-469.
67. Murrell, K.D., Clark, S., Dean, D.A. and Vannier, W.E. (1979). Influence of mouse strain on induction of resistance with irradiated *Schistosoma mansoni* cercariae. *J Parasitol* 65(5): 829-831.
68. Navarrete, S., Rollinson, D. and Agnew, A.M. (1994). Cross-protection between species of the *Schistosoma haematobium* group induced by vaccination with irradiated parasites. *Parasite Immunol* 16(1): 19-25.

69. Noonan, F.P. and Lewis, F.A. (1995). UVB-induced immune suppression and infection with *Schistosoma mansoni*. *Photochem Photobiol* 61(1): 99-105.

70. Oligino, L.D., Percy, A.J. and Harn, D.A. (1988). Purification and immunochemical characterization of a 22 kilodalton surface antigen from *Schistosoma mansoni*. *Mol Biochem Parasitol* 28(2): 95-103.

71. Pemberton, R.M. and Wilson, R.A. (1995). T-helper type-1-dominated lymph node responses induced in C57BL/6 mice by optimally irradiated cercariae of *Schistosoma mansoni* are down-regulated after challenge infection. *Immunology* 84(2): 310-316.

72. Piper, K.P. and McLaren, D.J. (1993). The role of T cells in vaccine immunity in the murine model of *Schistosomiasis mansoni*. *Int J Parasitol* 23(2): 245-256.

73. Pons, H.A., Morgan, J.S., Hutchinson, M.L., Rojkind, M., Groszmann, R.J. and Stadecker, M.J. (1989). Resistance to reinfection in experimental murine schistosomiasis: role of porto-hepatic hemodynamics. *Am J Trop Med Hyg* 41(2): 189-197.

74. Radke, M.G. and Sadun, E.H. (1963). Resistance produced in mice by exposure to irradiated *Schistosoma mansoni* cercariae. *Exp Parasitol* 13: 134-142.

75. Ratcliffe, E.C. and Wilson, R.A. (1991). The magnitude and kinetics of delayed-type hypersensitivity responses in mice vaccinated with irradiated cercariae of *Schistosoma mansoni*. *Parasitology* 103 Pt 1: 65-75.

76. Ratcliffe, E.C. and Wilson, R.A. (1992). The role of mononuclear-cell recruitment to the lungs in the development and expression of immunity to *Schistosoma mansoni*. *Parasitology* 104: 299-307.

77. Reynolds, S.R. and Harn, D.A. (1992). Comparison of irradiated-cercaria schistosome vaccine models that use 15 kilorad and 50 kilorad doses: the 15 kilorad dose gives greater protection, smaller liver sizes, and higher gamma-interferon levels after challenge. *Infect Immun* 60(1): 90-94.

78. Richter, D., Incani, R.N. and Harn, D.A. (1993). Isotype responses to candidate vaccine antigens in protective sera obtained from mice vaccinated with irradiated cercariae of *Schistosoma mansoni*. *Infect Immun* 61(7): 3003-3011.

79. Ruppel, A., Rother, U., Vongerichten, H., Lucius, R. and Diesfeld, H.J. (1985).

Schistosoma mansoni: immunoblot analysis of adult worm proteins. *Exp Parasitol* 60(2): 195-206.

80. Sadun, E.H. (1963). Immunization in schistosomiasis by previous exposure to homologous and heterologous cercariea by inoculation of preparations from schistosomes and by exposure to irradiated cercariae. *Ann N Y Acad Sci* 113: 418-439.
81. Sher, A. and Benno, D. (1982). Decreasing immunogenicity of developing schistosome larvae. *Parasite Immunol* 4(2): 101-107.
82. Sher, A., Coffman, R.L., Hieny, S. and Cheever, A.W. (1990). Ablation of eosinophil and IgE responses with anti-IL-5 or anti-IL-4 antibodies fails to affect immunity against *Schistosoma mansoni* in the mouse. *J Immunol* 145(11): 3911-3916.
83. Sher, A., Correa-Oliveira, R., Hieny, S. and Hussain, R. (1983). Mechanisms of protective immunity against *Schistosoma mansoni* infection in mice vaccinated with irradiated cercariae. IV. Analysis of the role of IgE antibodies and mast cells. *J Immunol* 131(3): 1460-1465.
84. Sher, A., Hieny, S. and James, S. (1984). Mechanisms of protective immunity against *S. mansoni* infection in mice vaccinated with irradiated cercariae. VI. Influence of the major histocompatibility complex. *Parasite Immunol* 6(4): 319-328.
85. Sher, A., Hieny, S., James, S.L. and Asofsky, R. (1982). Mechanisms of protective immunity against *Schistosoma mansoni* infection in mice vaccinated with irradiated cercariae. II. Analysis of immunity in hosts deficient in T lymphocytes, B lymphocytes, or complement. *J Immunol* 128(4): 1880-1884.
86. Simpson, A.J., Hackett, F., Walker, T., de Rossi, R. and Smithers, S.R. (1985). Antibody response against schistosomulum surface antigens and protective immunity following immunization with highly irradiated cercariae of *Schistosoma mansoni*. *Parasite Immunol* 7(2): 133-152.
87. Smith, M.A. and Clegg, J.A. (1984). *Schistosoma mansoni*: decay of resistance induced by gamma irradiated cercariae in the mouse. *Trans R Soc Trop Med Hyg* 78(2): 190-192.
88. Smythies, L.E., Coulson, P.S. and Wilson, R.A. (1992). Monoclonal antibody to IFN-gamma modifies pulmonary inflammatory responses and abrogates immunity to *Schistosoma mansoni* in mice vaccinated with attenuated cercariae. *J Immunol* 149(11):

3654-3658.

89. Smythies, L.E., Pemberton, R.M., Coulson, P.S., Mountford, A.P. and Wilson, R.A. (1992). T cell-derived cytokines associated with pulmonary immune mechanisms in mice vaccinated with irradiated cercariae of *Schistosoma mansoni*. *J Immunol* 148(5): 1512-1518.
90. Stek, M., Jr., Dean, D.A. and Clark, S.S. (1981). Attrition of schistosomes in an irradiation-attenuated cercarial immunization model of *Schistosoma mansoni*. *Am J Trop Med Hyg* 30(5): 1033-1038.
91. Stek, M., Jr., Minard, P. and Cruess, D.F. (1984). Murine immunization by cesium-137 irradiation attenuated *Schistosoma mansoni* cercariae. *J Parasitol* 70(3): 398-402.
92. Stek, M. and Sulaiman, S.M. (1984). Thermal effects on *Schistosoma mansoni* irradiation-attenuated vaccine production and administration. *Proc Helminthol Soc Wash* 51(2): 287-292.
93. Stek, M.F., Minard, P., Dean, D.A. and Hall, J.E. (1981). Immunization of baboons with *Schistosoma mansoni* cercariae attenuated by gamma irradiation. *Science* 212(4502): 1518-1520.
94. Street, M., Coulson, P.S., Sadler, C., Warnock, L.J., McLaughlin, D., Bluethmann, H. and Wilson, R.A. (1999). TNF is essential for the cell-mediated protective immunity induced by the radiation-attenuated schistosome vaccine. *J Immunol* 163(8): 4489-4494.
95. Szumlewicz, A.P. and Olivier, L.J. (1963). *Schistosoma mansoni*: development of challenge infections in mice exposed to irradiated cercariae. *Science* 140(3565): 411-412.
96. Tawfik, A.F. and Colley, D.G. (1986). Effects of anti-schistosomal chemotherapy on immune responses, protection and immunity. II. Concomitant immunity and immunization with irradiated cercariae. *Am J Trop Med Hyg* 35(1): 110-117.
97. Timothy, L.M., Coulson, P.S., Behnke, J.M. and Wilson, R.A. (1992). Cross-reactivity between *Necator americanus* and *Schistosoma mansoni* in mice. *Int J Parasitol* 22(8): 1143-1149.
98. Vignali, D.A., Bickle, Q.D., Taylor, M.G., Tennent, G. and Pepys, M.B. (1988). Comparison of the role of complement in immunity to *Schistosoma mansoni* in rats and

mice. *Immunology* 63(1): 55-61.

99. Vignali, D.A., Crocker, P., Bickle, Q.D., Cobbold, S., Waldmann, H. and Taylor, M.G. (1989). A role for CD4+ but not CD8+ T cells in immunity to *Schistosoma mansoni* induced by 20 krad-irradiated and Ro 11-3128-terminated infections. *Immunology* 67(4): 466-472.

100. Ward, R.E.M. and Mclaren, D.J. (1988). *Schistosoma mansoni*: evidence that eosinophils and or macrophages contribute to skin-phase challenge attrition in vaccinated CBA/Ca mice. *Parasitology* 96: 63-84.

101. Wilson, R.A., Coulson, P.S., Betts, C., Dowling, M.A. and Smythies, L.E. (1996). Impaired immunity and altered pulmonary responses in mice with a disrupted interferon-gamma receptor gene exposed to the irradiated *Schistosoma mansoni* vaccine. *Immunology* 87(2): 275-282.

102. Wilson, R.A., Coulson, P.S. and Dixon, B. (1986). Migration of the schistosomula of *Schistosoma mansoni* in mice vaccinated with radiation-attenuated cercariae, and normal mice: an attempt to identify the timing and site of parasite death. *Parasitology* 92 (Pt 1): 101-116.

103. Wynn, T.A., Jankovic, D., Hieny, S., Cheever, A.W. and Sher, A. (1995). IL-12 enhances vaccine-induced immunity to *Schistosoma mansoni* in mice and decreases T helper 2 cytokine expression, IgE production, and tissue eosinophilia. *J Immunol* 154(9): 4701-4709.

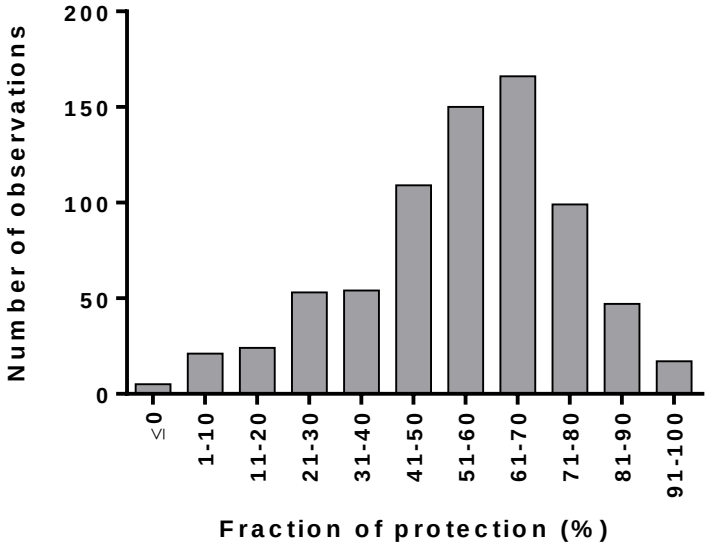
104. Wynn, T.A., Oswald, I.P., Eltoum, I.A., Caspar, P., Lowenstein, C.J., Lewis, F.A., James, S.L. and Sher, A. (1994). Elevated expression of Th1 cytokines and nitric oxide synthase in the lungs of vaccinated mice after challenge infection with *Schistosoma mansoni*. *J Immunol* 153(11): 5200-5209.

105. Wynn, T.A., Reynolds, A., James, S., Cheever, A.W., Caspar, P., Hieny, S., Jankovic, D., Strand, M. and Sher, A. (1996). IL-12 enhances vaccine-induced immunity to schistosomes by augmenting both humoral and cell-mediated immune responses against the parasite. *J Immunol* 157(9): 4068-4078.

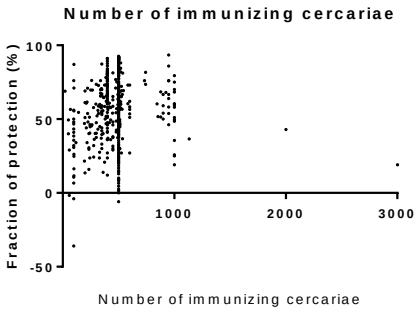
A.2: Potential predictors with reported ranges and codes. N=number of observations which reported the value of predictor.

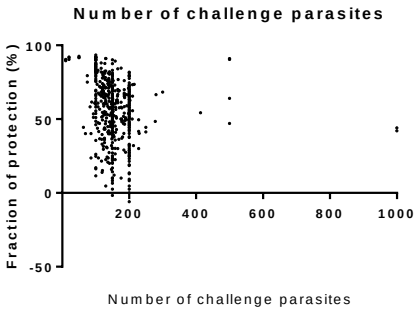
Variable name	Range/Units	N
Number of immunizing parasites (total and number per dose)	50-5000 cercariae	736
Number of challenge parasites	10-1,000 cercariae	621
Number of immunizations	1-5 times	744
Irradiation dose	3-160 krad	745
Host age	6-13 weeks	413
Host sex	Male/Female/mixed	629
Time between the last immunization and challenge	7-230 days	734
Time between challenge and perfusion	14-103 days	728

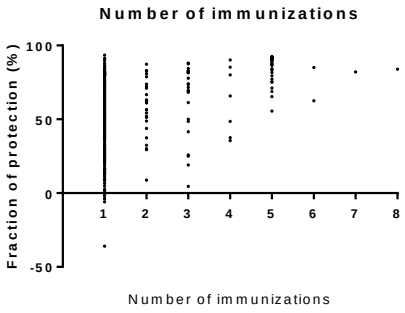
A.3: The distribution of reported fraction of protection for challenge infection after attenuated schistosome cercariae vaccination.

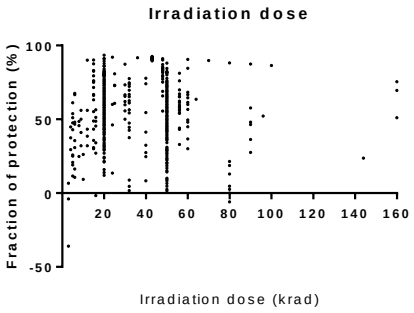


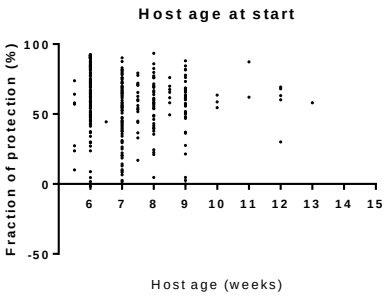
A.4: Graphs of the reported fraction of protection by potential predictors. Data points in scatter plot graphs represent single observations of reported fraction of protection. Bars represent mean and standard error of mean of reported fraction of protection.

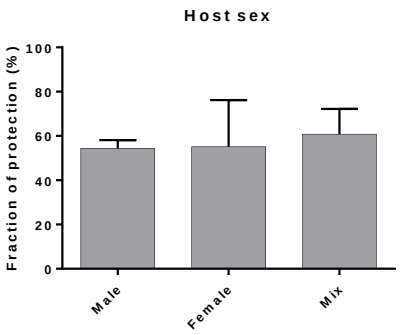


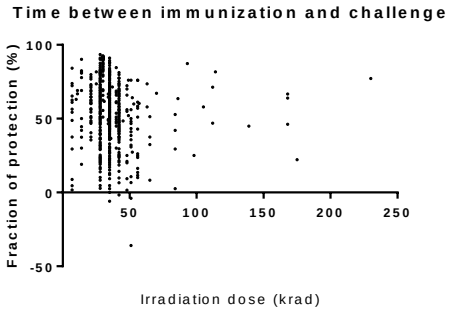


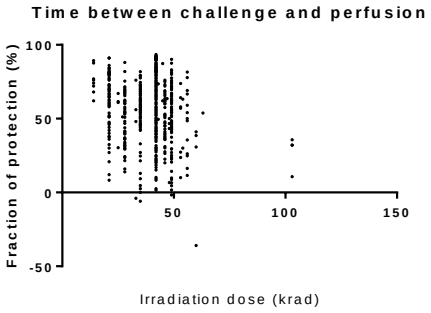












Appendix B: Supplementary materials for Chapter 3.

B.1: The list of mouse host studies where immunization and/or challenge infection used non-*S. mansoni* schistosome species (Group 2 in Figure 3.1).

1. Agnew, A.M., Murare, H.M. and Doenhoff, M.J. (1989). Specific cross-protection between *Schistosoma bovis* and *S.haematobium* induced by highly irradiated infections in mice. *Parasite Immunol* 11(4): 341-349.
2. Agnew, A.M., Murare, H.M. and Doenhoff, M.J. (1993). Immune attrition of adult schistosomes. *Parasite Immunol* 15(5): 261-271.
3. Bickle, Q.D. and Doenhoff, M.J. (1987). Comparison of the live vaccine potential of different geographic isolates of *Schistosoma mansoni*. *J Helminthol* 61(3): 191-195.
4. Cheever, A.W., Hieny, S., Duvall, R.H. and Sher, A. (1983). Lack of resistance to *Schistosoma japonicum* in mice immunized with irradiated *S. mansoni* cercariae. *Trans R Soc Trop Med Hyg* 77(6): 812-814.
5. Hsue, S.Y., Hsue, H.F. and Osborne, J.W. (1965). Immunizing effect of X-irradiated cercariae of *Schistosoma japonicum* in albino mice. *Z Tropenmed Parasitol* 16: 83-89.
6. Laxer, M.J. and Tuazon, C.U. (1992). Migration of 75Se-methionine-labeled *Schistosoma japonicum* in normal and immunized mice. *J Infect Dis* 166(5): 1133-1138.
7. Mitchell, G.F., Davern, K.M., Wood, S.M., Wright, M.D., Argyropoulos, V.P., McLeod, K.S., Tiu, W.U. and Garcia, E.G. (1990). Attempts to induce resistance in mice to *Schistosoma japonicum* and *Schistosoma mansoni* by exposure to crude schistosome antigens plus cloned glutathione-S-transferases. *Immunol Cell Biol* 68 (Pt 6): 377-385.
8. Moloney, N.A., Bickle, Q.D. and Webbe, G. (1985). The induction of specific immunity against *Schistosoma japonicum* by exposure of mice to ultraviolet attenuated cercariae. *Parasitology* 90 (Pt 2): 313-323.
9. Navarrete, S., Rollinson, D. and Agnew, A.M. (1994). Sross-protection between species of the *Schistosoma haematobium* group induced by vaccination with irradiated parasites. *Parasite Immunol* 16(1): 19-25.

10. Zhang, Y., Taylor, M.G., Bickle, Q.D., Wang, H. and Ge, J. (1999). Vaccination of mice with gamma-irradiated *Schistosoma japonicum* cercariae. *Parasite Immunol* 21(2): 111-117.

B.2: The list of *S. mansoni* or *S. haematobium* with baboon host articles used for the analysis (Group 3 in Figure 3.1).

1. Damian, R.T., Powell, M.R., Roberts, M.L., Clark, J.D., Stirewalt, M.A. and Lewis, F.A. (1985). *Schistosoma mansoni*: parasitology and immunology of baboons vaccinated with irradiated cryopreserved schistosomula. *Int J Parasitol* 15(3): 333-344.
2. Farah, I.O. and Nyindo, M. (1996). *Schistosoma mansoni* induces in the Kenyan baboon a novel intestinal pathology that is manifestly modulated by an irradiated cercarial vaccine. *J Parasitol* 82(4): 601-607.
3. Harrison, R.A., Bickle, Q.D., Kiare, S., James, E.R., Andrews, B.J., Sturrock, R.F., Taylor, M.G. and Webbe, G. (1990). Immunization of Baboons with Attenuated Schistosomula of *Schistosoma haematobium* - Levels of Protection Induced by Immunization with Larvae Irradiated with 20 Krad and 60 Krad. *Trans R Soc Trop Med Hyg* 84(1): 89-99.
4. James, E.R., Otieno, M., Harrison, R., Dobinson, A.R., Monorei, J. and Else, J.G. (1986). Partial Protection of Baboons against *Schistosoma mansoni* Using Radiation-Attenuated Cryopreserved Schistosomula. *Trans R Soc Trop Med Hyg* 80(3): 378-384.
5. Kariuki, T.M., Van Dam, G.J., Deelder, A.M., Farah, I.O., Yole, D.S., Wilson, R.A. and Coulson, P.S. (2006). Previous or ongoing schistosome infections do not compromise the efficacy of the attenuated cercaria vaccine. *Infect Immun* 74(7): 3979-3986.
6. Nyindo, M., Borus, P.K., Farah, I.O., Oguya, F.O. and Makawiti, D.W. (1995). *Schistosoma mansoni* in the baboon: modulation of pathology after vaccination with polyclonal anti-idiotypic antibodies. *Scand J Immunol* 42(6): 637-643.
7. Soisson, L.A., Reid, G.D.F., Farah, I.O., Nyindo, M. and Strand, M. (1993). Protective immunity in baboons vaccinated with a recombinant antigen or radiation-attenuated cercariae of *Schistosoma mnsoni* is antibody-dependent. *J Immunol* 151(9): 4782-4789.
8. Stek, M., Minard, P., Dean, D.A. and Hall, J.E. (1981). Immunization of baboons with *Schistosoma mansoni* cercariae attenuated by gamma-irradiation. *Science* 212(4502): 1518-1520.
9. Taylor, M.G., James, E.R., Nelson, G.S., Bickle, Q., Andrews, B.J., Dobinson, A.R. and Webbe, G. (1976). Immunization of baboons against *Schistosoma mansoni* using irradiated *S. mansoni* cercariae and schistosomula and non-irradiated *S. rodhaini* cercariae. *J Helminthol* 50(3): 215-221.

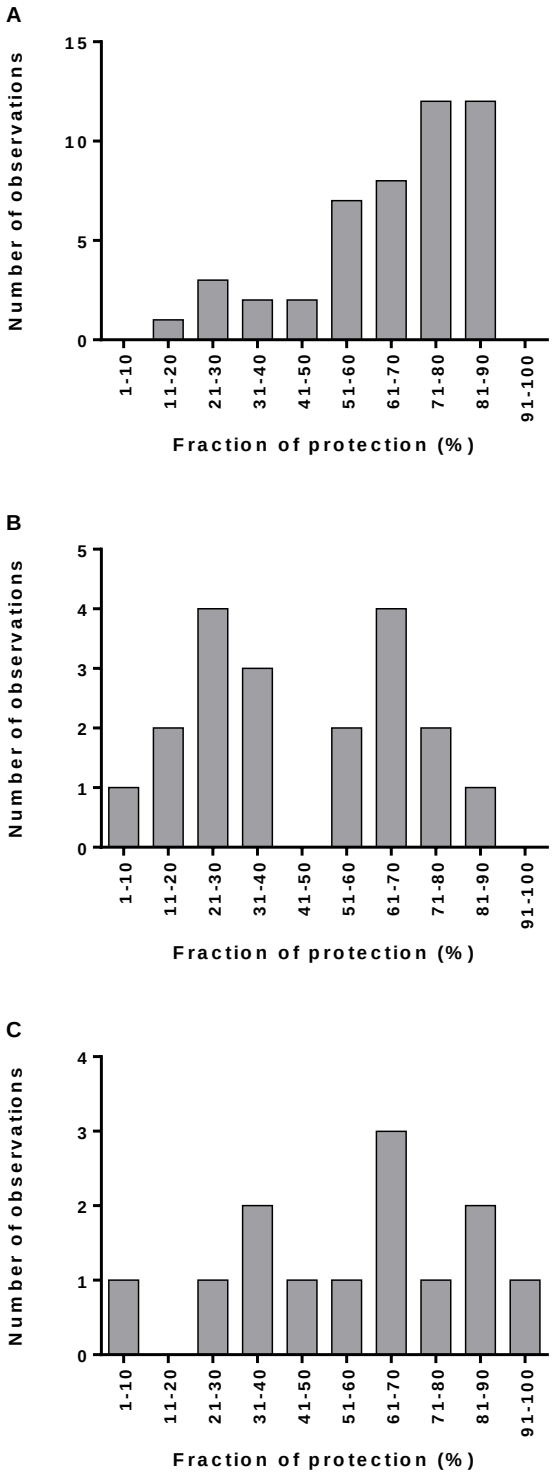
10. Webbe, G., Sturrock, R.F., James, E.R. and James, C. (1982). *Schistosoma haematobium* in the Baboon (*Papio anubis*) - effect of vaccination with irradiated larvae on the subsequent infection with percutaneously applied cercariae. *Trans R Soc Trop Med Hyg* 76(3): 354-361.
11. Yole, D.S., Pemberton, R., Reid, G.D.F. and Wilson, R.A. (1996). Protective immunity to *Schistosoma mansoni* induced in the olive baboon *Papio anubis* by the irradiated cercaria vaccine. *Parasitology* 112: 37-46.

B.3: The list of *S. mansoni* with rat host articles used for the analysis (Group 4 in Figure 3.1).

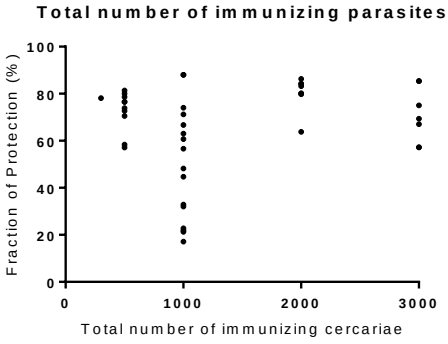
1. Erickson, D.G. and Caldwell, W.L. (1965). Acquired resistance in mice and rats after Exposure to gamma-irradiated cercariae. *Am J Trop Med Hyg* 14(4): 566-573.
2. Ford, M.J., Bickle, Q.D. and Taylor, M.G. (1984). Immunization of rats against *Schistosoma mansoni* using irradiated cercariae, lung schistosomula and liver-stage worms. *Parasitology* 89(Oct): 327-344.
3. Ford, M.J., Bickle, Q.D. and Taylor, M.G. (1987). Immunity to *Schistosoma mansoni* in congenitally athymic, irradiated and mast cell-depleted rats. *Parasitology* 94: 313-326.
4. Ford, M.J., Bickle, Q.D., Taylor, M.G. and Andrews, B.J. (1984). Passive transfer of resistance and the site of immune-dependent elimination of the challenge infection in rats vaccinated with highly irradiated cercariae of *Schistosoma mansoni*. *Parasitology* 89(Dec): 461-482.
5. Ford, M.J., Taylor, M.G., Mchugh, S.M., Wilson, R.A. and Hughes, D.L. (1987). Studies on heterologous resistance between *Schistosoma mansoni* and *Fasciola hepatica* in inbred rats. *Parasitology* 94: 55-67.
6. Mclaren, D.J., Pearce, E.J. and Smithers, S.R. (1985). Site potential for challenge attrition in mice, rats and guinea-pigs vaccinated with irradiated cercariae of *Schistosoma mansoni*. *Parasite Immunol* 7(1): 29-44.
7. Mclaren, D.J. and Smithers, S.R. (1985). *Schistosoma mansoni* - challenge attrition during the lung phase of migration in vaccinated and serum-protected rats. *Exp Parasitol* 60(1): 1-9.
8. Moloney, N.A., Webbe, G. and Hinchcliffe, P. (1987). The induction of species-specific immunity against *Schistosoma japonicum* by exposure of rats to ultra-violet attenuated cercariae. *Parasitology* 94: 49-54.
9. Phillips, S.M. and Reid, W.A. (1980). *Schistosoma mansoni* - immune-response to normal and irradiated cercariae or soluble stage-specific surface immunogens. *Int J Nucl Med Biol* 7(2): 173-186.
10. Smithers, S.R. and Terry, R.J. (1965). Acquired resistance to experimental infections of *Schisntosoma mansoni* in the albino rat. *Parasitology* 55(4): 711-717.

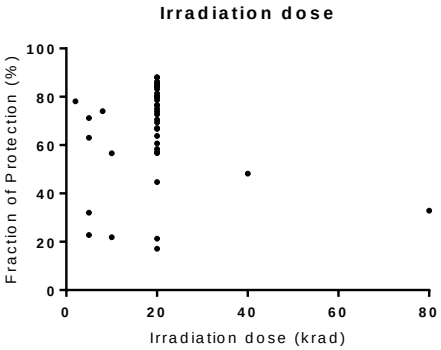
11. Vignali, D.A., Bickle, Q.D., Taylor, M.G., Tennent, G. and Pepys, M.B. (1988). Comparison of the role of complement in immunity to *Schistosoma mansoni* in rats and mice. *Immunology* 63(1): 55-61.

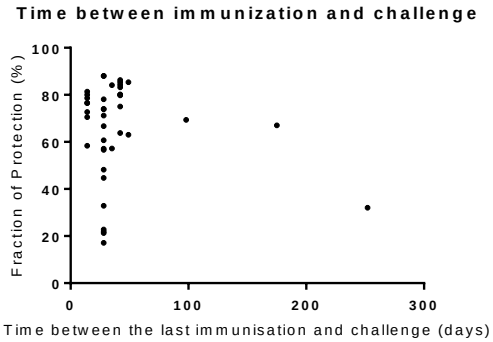
B.4: The distribution of reported fraction of protection for challenge infection after attenuated schistosome cercariae vaccination. (A) rat host with *S. mansoni* studies, (B) baboon host with *S. mansoni* studies, (C) baboon host with *S. haematobium* studies.



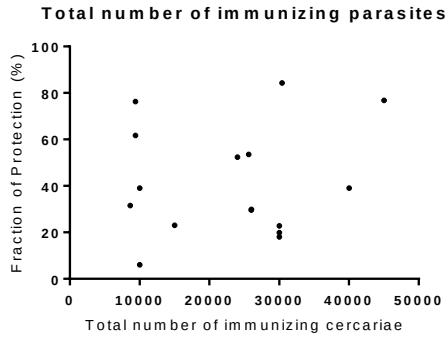
B.5: Graphs of the reported fraction of protection by potential predictors for rat host with *S. mansoni* studies. Data points represent the reported fraction of protection for each observation.

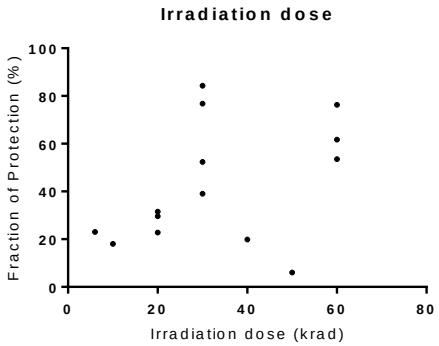


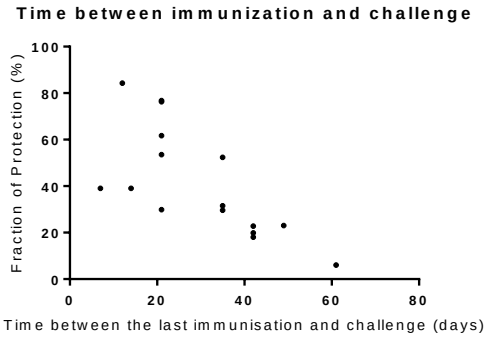




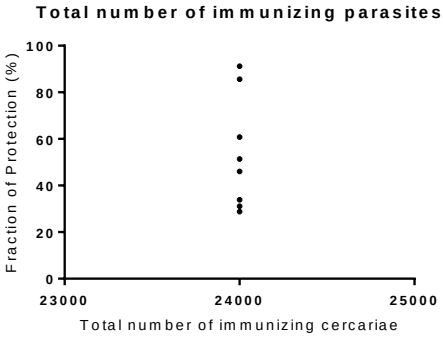
B.6: Graphs of the reported fraction of protection by potential predictors for baboon host with *S. mansoni* studies. Data points represent the reported fraction of protection for each observation.

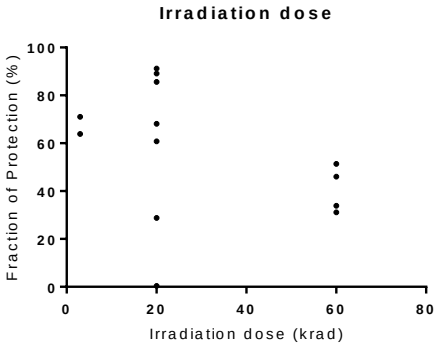


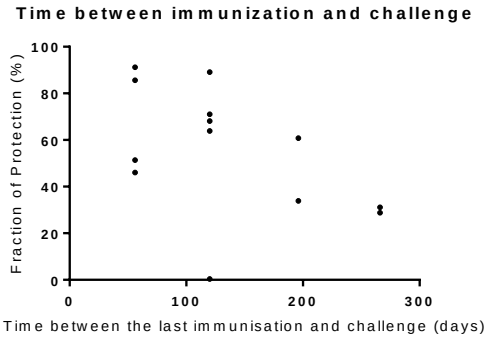




B.7: Graphs of the reported fraction of protection by potential predictors for baboon host with *S. haematobium* studies. Data points represent the reported fraction of protection for each observation.







Appendix C: Supplementary materials for Chapter 4.

C.1: The list of articles used for the analysis.

1. Abebe, F., Gaarder, P.I., Petros, B. and Gundersen, S.G. (2001). Age and sex related differences in antibody responses against *Schistosoma mansoni* soluble egg antigen in a cohort of school children in Ethiopia. *APMIS* 109(12): 816-824.
2. Ali, A.E. and Shaheen, H.I. (1994). Human resistance to reinfection with schistosomes. II. Specific IgA titres before & 3 months after praziquantel treatment. *J Egypt Soc Parasitol* 24(3): 505-512.
3. Feldmeier, H., Gastl, G.A., Poggensee, U., Daffalla, A.A., Nogueira-Queiroz, J.A., Capron, A. and Peter, H.H. (1988). Immune response in chronic *Schistosomiasis haematobium* and *mansoni*. Reversibility of alterations after anti-parasitic treatment with praziquantel. *Scand J Immunol* 28(2): 147-155.
4. Fouda, E.E., Ali, A.I., Emam, M. and Khalek, A.S.A. (2007). IgE and skin test reactivity in relation to anti-parasitic treatment. *J Allergy Clin Immun* 119(1): S23-S23.
5. Grogan, J.L., Kremsner, P.G., vanDam, G.J., Metzger, W., Mordmuller, B., Deelder, A.M. and Yazdanbakhsh, M. (1996). Antischistosome IgG4 and IgE responses are affected differentially by chemotherapy in children versus adults. *J Infect Dis* 173(5): 1242-1247.
6. Hamadto, H.H., Rashed, S.M., el Said, A. and Elhayawan, I.A. (1990). Humoral and cellular immune response in schistosomiasis pre and post praziquantel therapy. *J Egypt Soc Parasitol* 20(2): 667-672.
7. Hussein, H.M., Kaddah, M.A., el-Borai, Y.A., el Batanony, G.A., Abdel Ghafar, F.A. and Zakaria, S. (1996). Host and parasite determinants of morbidity in Egyptian children with schistosomiasis. *J Egypt Soc Parasitol* 26(3): 755-772.
8. Ismail, M.M., Bruce, J.I., Attia, M.M., Farghaly, A.M., Ali, A.E. and El Laithi, S. (1992). Different immunoglobulin classes and eosinophils as a monitor of cure in schistosomal cases using praziquantel. *J Trop Med* 2(2): 99-108.
9. Joseph, S., Jones, F.M., Laidlaw, M.E., Mohamed, G., Mawa, P.A., Namujju, P.B., Kizza, M., Watera, C., Whitworth, J.A., Dunne, D.W. and Elliott, A.M. (2004). Impairment of the *Schistosoma mansoni*-specific immune responses elicited by

treatment with praziquantel in Ugandans with HIV-1 coinfection. *J Infect Dis* 190(3): 613-618.

10. Mutapi, F., Ndhlovu, P.D., Hagan, P., Spicer, J.T., Mduluza, T., Turner, C.M., Chandiwana, S.K. and Woolhouse, M.E. (1998). Chemotherapy accelerates the development of acquired immune responses to Schistosoma haematobium infection. *J Infect Dis* 178(1): 289-293.
11. Mutapi, F., Ndhlovu, P.D., Hagan, P. and Woolhouse, M.E. (1998). Changes in specific anti-egg antibody levels following treatment with praziquantel for *Schistosoma haematobium* infection in children. *Parasite Immunol* 20(12): 595-600.
12. Nagaty, I.M., el Hayawan, I.A., Nasr, M.E. and el Hamshery, A.H. (1996). Observations on possible immunity to reinfection among school children after schistosomiasis treatment. *J Egypt Soc Parasitol* 26(2): 443-452.
13. Nassr, A., Hassan, M.M., Abdel Salam, F.M., Lashin, A.H., Shahin, W.A. and Amin, H. (2002). IgG isotypes in schistosomiasis patients before and after praziquantel. *J Egypt Soc Parasitol* 32(3): 931-952.
14. Naus, C.W.A., van Dam, G.J., Kremsner, P.G., Krijger, F.W. and Deelder, A.M. (1998). Human IgE, IgG subclass, and IgM Responses to worm and egg antigens in schistosomiasis haematobium: A 12-month study of reinfection in Cameroonian children. *Clin Infect Dis* 26(5): 1142-1147.
15. Reilly, L., Magkrioti, C., Mduluza, T., Cavanagh, D.R. and Mutapi, F. (2008). Effect of treating *Schistosoma haematobium* infection on *Plasmodium falciparum*-specific antibody responses. *Bmc Infect Dis* 8(158).
16. Satti, M.Z., Cahen, P., Skov, P.S., Joseph, S., Jones, F.M., Fitzsimmons, C., Hoffmann, K.F., Reimert, C., Kariuki, H.C., Kazibwe, F., Mwatha, J.K., Kimani, G., Vennervald, B.J., Ouma, J.H., Kabatereine, N.B. and Dunne, D.W. (2004). Changes in IgE- and antigen-dependent histamine-release in peripheral blood of *Schistosoma mansoni*-infected Ugandan fishermen after treatment with praziquantel. *BMC Immunol* 5(6).
17. Satti, M.Z., Lind, P., Vennervald, B.J., Sulaiman, S.M., Daffalla, A.A. and Ghalib, H.W. (1996). Specific immunoglobulin measurements related to exposure and resistance to *Schistosoma mansoni* infection in Sudanese canal cleaners. *Clin Exp Immunol* 106(1): 45-54.

18. Satti, M.Z., Sulaiman, S.M., Homeida, M.M.A., Younis, S.A. and Ghalib, H.W. (1996). Clinical, parasitological and immunological features of canal cleaners hyper-exposed-to *Schistosoma mansoni* in the Sudan. *Clin Exp Immunol* 104(3): 426-431.
19. Snyman, J.R., de Sommers, K., Steinmann, M.A. and Lizamore, D.J. (1997). Effects of calcitriol on eosinophil activity and antibody responses in patients with schistosomiasis. *Eur J Clin Pharmacol* 52(4): 277-280.
20. Snyman, J.R. and Sommers de, K. (1998). Effect of levamisole on the immune response of patients with schistosomiasis after treatment with praziquantel. *Clin Drug Investig* 15(6): 483-489.
21. Tweyongyere, R., Mawa, P.A., Emojong, N.O., Mpairwe, H., Jones, F.M., Duong, T., Dunne, D.W., Vennervald, B.J., Katunguka-Rwakishaya, E. and Elliott, A.M. (2009). Effect of praziquantel treatment of *Schistosoma mansoni* during pregnancy on intensity of infection and antibody responses to schistosome antigens: results of a randomised, placebo-controlled trial. *BMC Infect Dis* 9(32).
22. van Lieshout, L., Stelma, F.F., Guisse, F., Falcao Ferreira, S.T., Polman, K., van Dam, G.J., Diakhate, M., Sow, S., Deelder, A. and Gryseels, B. (1999). The contribution of host-related factors to low cure rates of praziquantel for the treatment of *Schistosoma mansoni* in Senegal. *Am J Trop Med Hyg* 61(5): 760-765.
23. Vereecken, K., Naus, C.W.A., Polman, K., Scott, J.T., Diop, M., Gryseels, B. and Kestens, L. (2007). Associations between specific antibody responses and resistance to reinfection in a Senegalese population recently exposed to *Schistosoma mansoni*. *Trop Med Int Health* 12(3): 431-444.
24. Walter, K., Fulford, A.J.C., McBeath, R., Joseph, S., Jones, F.M., Kariuki, H.C., Mwatha, J.K., Kimani, G., Kabatereine, N.B., Vennervald, B.J., Ouma, J.H. and Dunne, D.W. (2006). Increased human IgE induced by killing *Schistosoma mansoni* in vivo is associated with pretreatment Th2 cytokine responsiveness to worm antigens. *J Immunol* 177(8): 5490-5498.
25. Wilson, S., Jones, F.M., Fofana, H.K.M., Doucoure, A., Landoure, A., Kimani, G., Mwatha, J.K., Sacko, M., Vennervald, B.J. and Dunne, D.W. (2013). Rapidly boosted plasma il-5 induced by treatment of human *Schistosomiasis haematobium* is dependent on antigen dose, ige and eosinophils. *PLoS Negl Trop Dis* 7(3): e2149.
26. Zinyowera, S., Muchaneta-Kubara, C.E., Mutapi, F., Midzi, N., Ndlovu, P.D. and Mduluza, T. (2006). Changes in the humoral immune responses after chemotherapy in

single and co-infected individuals with *Schisosoma haematobium* and *Plasmodium falciparum*. *Cent Afr J Med* 52(9-12): 104-111.

C.2: Scientific articles or governmental reports which used to obtain schistosome infection intensity or infection prevalence of the target areas.

1) Studies failed to report pre-treatment infection intensity

Reference	Reference used to obtain infection intensity
Ali *et al.* 1994 Fouda *et al.* 2007 Ismail *et al.* 1992 Nagaty *et al.* 1996	El-Khoby, T., *et al.* 2000."The epidemiology of schistosomiasis in Egypt: summary findings in nine governorates." Am J Trop Med Hyg 62(2 Suppl): 88-99.

Reference	Reference used to obtain prevalence
Nassr *et al.* 2002	Barakat, R. M. 2013."Epidemiology of Schistosomiasis in Egypt: Travel through Time: Review." J Adv Res 4(5): 425-432.
Zinyowera *et al.* 2006	Chimbari, M. J. 2012."Enhancing schistosomiasis control strategy for zimbabwe: building on past experiences." J Parasitol Res 2012: 353768.

2) Studies failed to report infection prevalence of the study area

C.3: Frequencies of antibody isotypes with range of days after chemotherapy. *Number of observations.

anti-SEA	N*	days after chemotherapy	anti-WWA	N*	days after chemotherapy
IgA	19	35-180	IgA	16	35-180
IgE	23	21-180	IgE	35	21-180
IgG1	17	30-126	IgG1	22	30-90
IgG2	15	30-126	IgG2	14	30-42
IgG3	15	30-126	IgG3	20	30-63
IgG4	20	21-126	IgG4	21	21-90
IgG	15	35-180	IgG	23	21-180
IgM	27	30-180	IgM	27	30-180

C.4: Tables of data distribution of anti-SEA antibodies: A) IgA, B) IgE, C)IgG1, D) IgG2, E)IgG3, F)IgG4, G)IgG, H)IgM. The number in the cell shows the number of observations being used for the analyses after data imputations.

A) IgA

Predictor name	Antibody levels change	
Age/infection intensity	Increase	Decrease
child/light	1	0
child/heavy	1	0
adolescent/light	3	4
adolescent/heavy	1	4
adult/light	3	1
adult/heavy	1	0
Prevalence	Increase	Decrease
Low/Moderate	4	5
High	6	4
Schistosome species	Increase	Decrease
S. mansoni	6	6
S. haematobium	0	2
co-infection of *S. mansoni* and *S. haematobium*	4	1

B) IgE

Predictor name	Antibody levels change	
Age/infection intensity	Increase	Decrease
child/light	0	1
child/heavy	3	0
adolescent/light	1	3
adolescent/heavy	4	1
adult/light	6	1
adult/heavy	2	1
Prevalence	Increase	Decrease
Low/Moderate	5	5
High	11	2
Schistosome species	Increase	Decrease
S. mansoni	12	5
S. haematobium	4	0
co-infection of *S. mansoni* and *S. haematobium*	0	2

C) IgG1

Predictor name	Antibody levels change	
Age/infection intensity	Increase	Decrease
child/light	1	0
child/heavy	2	0
adolescent/light	2	0
adolescent/heavy	1	3
adult/light	3	3
adult/heavy	0	2
Prevalence	Increase	Decrease
Low/Moderate	4	1
High	5	7
Schistosome species	Increase	Decrease
S. mansoni	6	8
S. haematobium	2	0
co-infection of *S. mansoni* and *S. haematobium*	1	0

D) IgG2

Predictor name	Antibody levels change	
Age/infection intensity	Increase	Decrease
child/light	0	0
child/heavy	2	0
adolescent/light	0	1
adolescent/heavy	2	2
adult/light	5	1
adult/heavy	0	2
Prevalence	Increase	Decrease
Low/Moderate	2	2
High	7	4
Schistosome species	Increase	Decrease
S. mansoni	8	5
S. haematobium	1	1
co-infection of *S. mansoni* and *S. haematobium*	9	6

E) IgG3

Predictor name	Antibody levels change	
Age/infection intensity	Increase	Decrease
child/light	0	0
child/heavy	0	2
adolescent/light	1	0
adolescent/heavy	1	3
adult/light	3	3
adult/heavy	1	1
Prevalence	Increase	Decrease
Low/Moderate	2	2
High	4	7
Schistosome species	Increase	Decrease
S. mansoni	5	8
S. haematobium	1	1
co-infection of *S. mansoni* and *S. haematobium*	0	0

F) IgG4

Predictor name	Antibody levels change	
Age/infection intensity	Increase	Decrease
child/light	0	2
child/heavy	1	2
adolescent/light	1	0
adolescent/heavy	1	3
adult/light	1	6
adult/heavy	2	1
Prevalence	Increase	Decrease
Low/Moderate	1	4
High	5	10
Schistosome species	Increase	Decrease
S. mansoni	4	13
S. haematobium	2	1
co-infection of *S. mansoni* and *S. haematobium*	0	0

G) IgG

Predictor name	Antibody levels change	
Age/infection intensity	Increase	Decrease
child/light	0	1
child/heavy	0	2
adolescent/light	1	10
adolescent/heavy	1	0
adult/light	0	0
adult/heavy	0	0
Prevalence	Increase	Decrease
Low/Moderate	1	11
High	1	2
Schistosome species	Increase	Decrease
S. mansoni	1	4
S. haematobium	0	0
co-infection of *S. mansoni* and *S. haematobium*	1	9

H) IgM

Predictor name	Antibody levels change	
Age/infection intensity	Increase	Decrease
child/light	1	1
child/heavy	2	2
adolescent/light	1	11
adolescent/heavy	0	4
adult/light	3	0
adult/heavy	1	1
Prevalence	Increase	Decrease
Low/Moderate	1	12
High	7	7
Schistosome species	Increase	Decrease
S. mansoni	6	9
S. haematobium	1	1
co-infection of *S. mansoni* and *S. haematobium*	1	9

C.5: Tables of data distribution of anti-WWA antibodies: A) IgA, B) IgE, C)IgG1, D) IgG2, E)IgG3, F)IgG4, G)IgG, H)IgM. The number in the cell shows the number of observations being used for the analyses after data imputations.

A) IgA

Predictor name	Antibody levels change	
Age/infection intensity	Increase	Decrease
child/light	2	0
child/heavy	1	0
adolescent/light	3	1
adolescent/heavy	3	0
adult/light	4	0
adult/heavy	2	0
Prevalence	Increase	Decrease
Low/Moderate	6	1
High	9	0
Schistosome species	Increase	Decrease
S. mansoni	11	1
S. haematobium	0	0
co-infection of *S. mansoni* and *S. haematobium*	4	0

B) IgE

Predictor name	Antibody levels change	
Age/infection intensity	Increase	Decrease
child/light	4	2
child/heavy	3	3
adolescent/light	3	0
adolescent/heavy	5	2
adult/light	9	1
adult/heavy	2	1
Prevalence	Increase	Decrease
Low/Moderate	7	4
High	19	5
Schistosome species	Increase	Decrease
S. mansoni	15	5
S. haematobium	5	1
co-infection of *S. mansoni* and *S. haematobium*	6	3

C) IgG1

Predictor name	Antibody levels change	
Age/infection intensity	Increase	Decrease
child/light	2	0
child/heavy	2	2
adolescent/light	1	0
adolescent/heavy	3	0
adult/light	8	2
adult/heavy	1	1
Prevalence	Increase	Decrease
Low/Moderate	3	2
High	14	3
Schistosome species	Increase	Decrease
S. mansoni	15	5
S. haematobium	2	0
co-infection of *S. mansoni* and *S. haematobium*	0	0

D) IgG2

Predictor name	Antibody levels change	
Age/infection intensity	Increase	Decrease
child/light	1	0
child/heavy	2	0
adolescent/light	0	0
adolescent/heavy	3	0
adult/light	5	2
adult/heavy	1	0
Prevalence	Increase	Decrease
Low/Moderate	2	1
High	10	1
Schistosome species	Increase	Decrease
S. mansoni	11	2
S. haematobium	1	0
co-infection of *S. mansoni* and *S. haematobium*	0	0

E) IgG3

Predictor name	Antibody levels change	
Age/infection intensity	Increase	Decrease
child/light	1	1
child/heavy	2	2
adolescent/light	1	0
adolescent/heavy	1	2
adult/light	6	3
adult/heavy	1	0
Prevalence	Increase	Decrease
Low/Moderate	3	1
High	9	7
Schistosome species	Increase	Decrease
S. mansoni	10	8
S. haematobium	2	0
co-infection of *S. mansoni* and *S. haematobium*	0	0

F) IgG4

Predictor name	Antibody levels change	
Age/infection intensity	Increase	Decrease
child/light	2	0
child/heavy	4	1
adolescent/light	0	0
adolescent/heavy	3	0
adult/light	7	1
adult/heavy	2	1
Prevalence	Increase	Decrease
Low/Moderate	2	1
High	16	2
Schistosome species	Increase	Decrease
S. mansoni	16	3
S. haematobium	2	0
co-infection of *S. mansoni* and *S. haematobium*	0	0

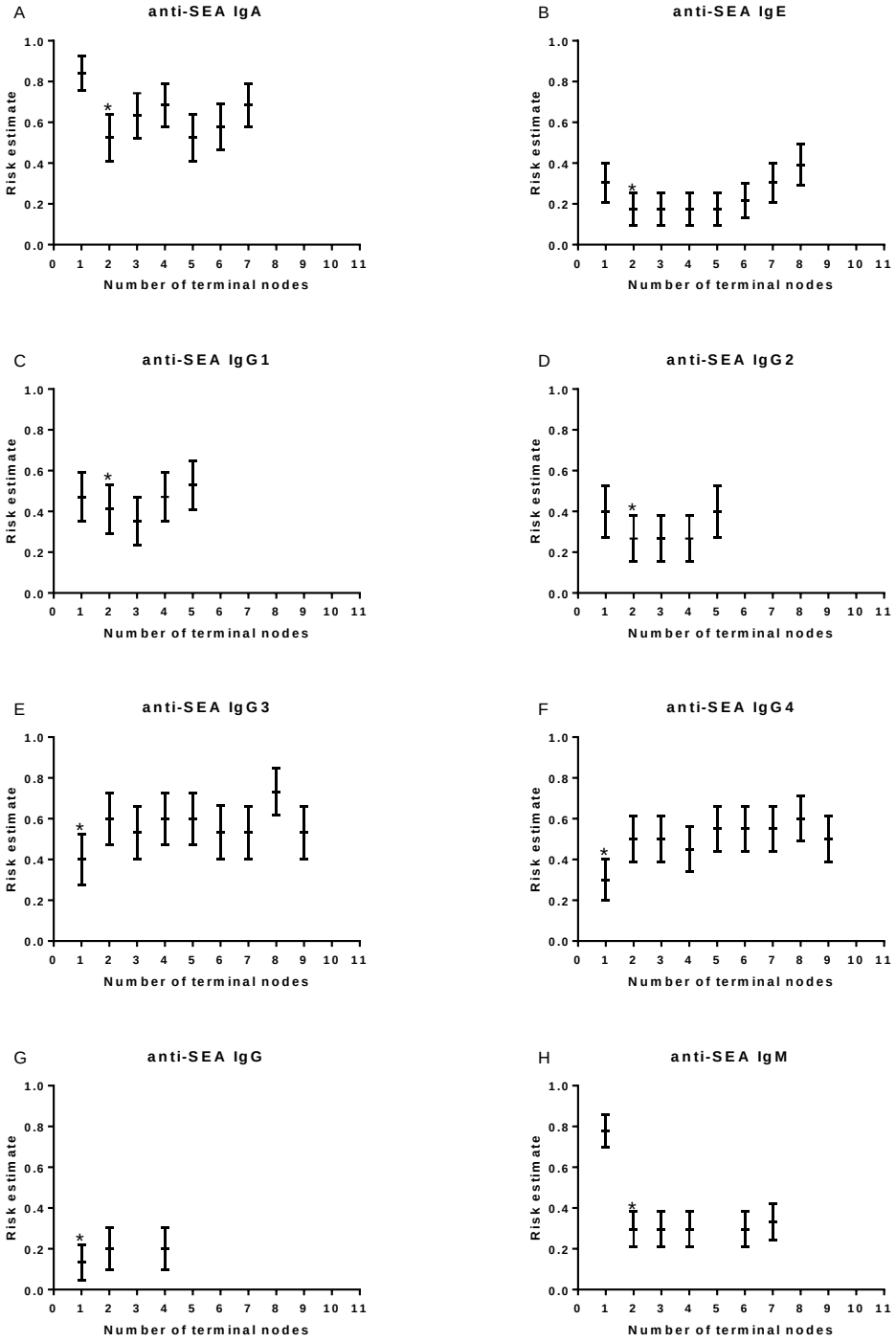
G) IgG

Predictor name	Antibody levels change	
Age/infection intensity	Increase	Decrease
child/light	1	1
child/heavy	4	1
adolescent/light	3	7
adolescent/heavy	4	0
adult/light	2	0
adult/heavy	0	0
Prevalence	Increase	Decrease
Low/Moderate	8	8
High	6	1
Schistosome species	Increase	Decrease
S. mansoni	6	2
S. haematobium	4	0
co-infection of *S. mansoni* and *S. haematobium*	4	7

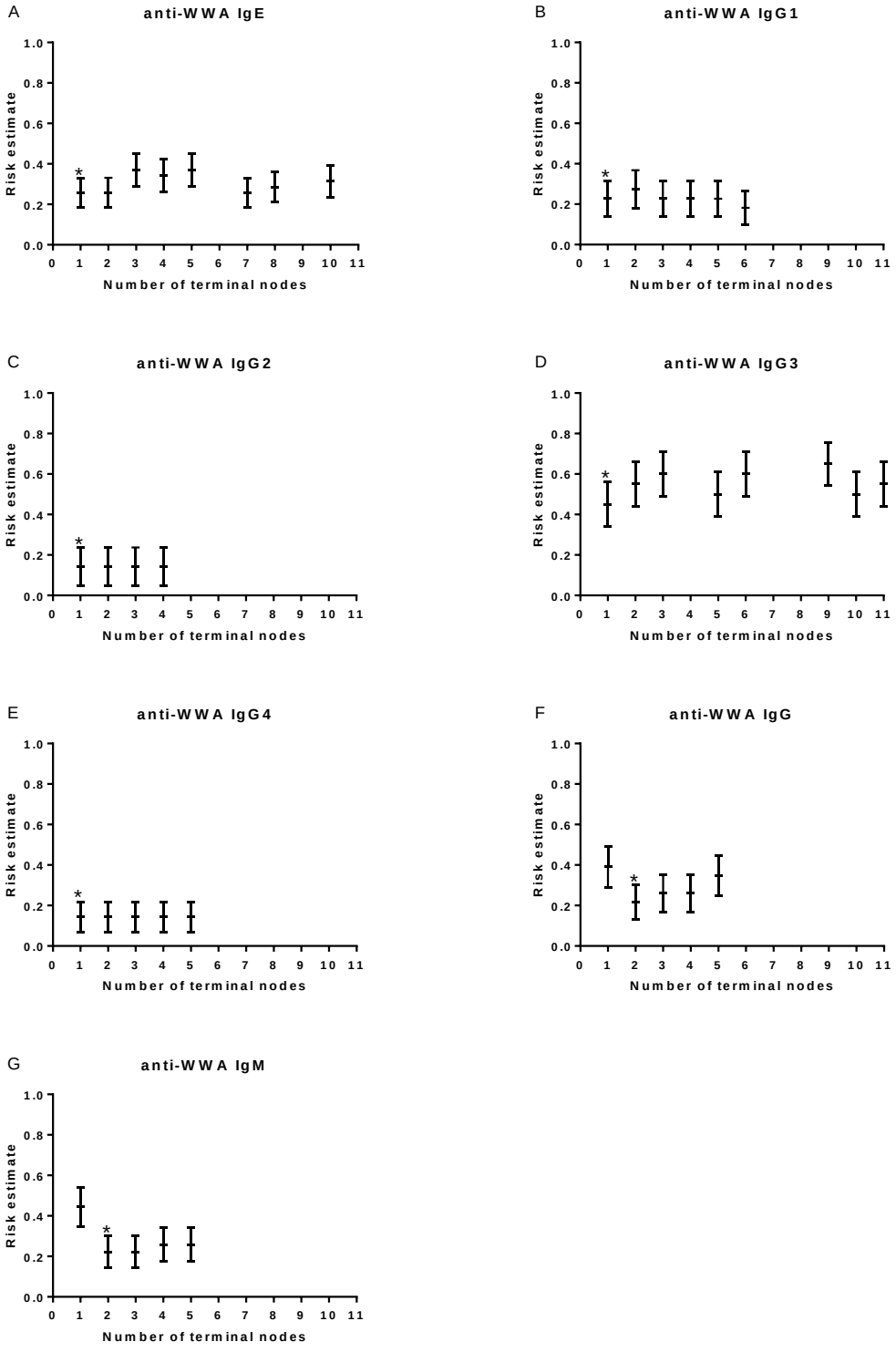
H) IgM

Predictor name	Antibody levels change	
Age/infection intensity	Increase	Decrease
child/light	2	1
child/heavy	6	0
adolescent/light	1	9
adolescent/heavy	1	2
adult/light	4	0
adult/heavy	1	0
Prevalence	Increase	Decrease
Low/Moderate	2	10
High	13	2
Schistosome species	Increase	Decrease
S. mansoni	12	4
S. haematobium	1	0
co-infection of *S. mansoni* and *S. haematobium*	2	8

C.6: Cross-validation analysis results of anti-SEA (A) IgA, (B) IgE, (C) IgG1, (D) IgG2, (E) IgG3, (F) IgG4, (D) IgG, and (H) IgM. Each plot represents the mean risk estimate and standard error. Asterisks indicate the optimum tree sizes that is identified by the cross validation analysis.



C.7: Cross-validation analysis results of anti-WWA (A) IgE, (B) IgG1, (C) IgG2, (D) IgG3, (E) IgG4, (F) IgG, and (G) IgM. Each plot represents the mean risk estimate and standard error. Asterisks indicate the optimum tree sizes which were identified by the cross validation analyses.



Appendix D: Supplementary materials for Chapter 5.

D.1: the list of articles used for the meta-analysis

1. Abu-Elyazeed, R.R., Mansour, N.S., Habib, M. and Podgore, J.K. (1993). *Schistosoma mansoni* infection 3 months after praziquantel therapy among farmers in Qalyub, Egypt. *J Trop Med* 2(4): 3-7.
2. Abu-Elyazeed, R.R., Mansour, N.S., Youssef, F.G., Boghdadi, A.M., el Khoby, T.A., Hassanein, Y.A. and el Gamal, R.R. (1998). Seasonality as a determinant of the efficacy of praziquantel in population-based chemotherapy: lessons from the practice. *J Egypt Soc Parasitol* 28(1): 1-7.
3. Adoubryn, K.D., Kouadio-Yapo, C.G., Ouhon, J., Aka, N.A.D., Bintto, F. and Assoumou, A. (2012). Intestinal parasites in children in Biankouma, Ivory Coast (mountaineous western region): efficacy and safety of praziquantel and albendazole. [French]. *Medecine et Sante Tropicales* 22(2): 170-176.
4. Augusto, G., Magnussen, P., Kristensen, T.K., Appleton, C.C. and Vennervald, B.J. (2009). The influence of transmission season on parasitological cure rates and intensity of infection after praziquantel treatment of *Schistosoma haematobium*-infected schoolchildren in Mozambique. *Parasitology* 136(13): 1771-1779.
5. Banwat, M.E., Daboer, J.C., Envuladu, E.A., Lar, L.A. and Ogbonna, C.C. (2011). Effect of mass chemotherapy with Praziquantel on the prevalence of schistosomiasis in school children in Langai community of Plateau State. *J Med Trop* 13(2): 119-123.
6. Barakat, R. and El Morshedy, H. (2011). Efficacy of two praziquantel treatments among primary school children in an area of high *Schistosoma mansoni* endemicity, Nile Delta, Egypt. *Parasitology* 138(4): 440-446.
7. Black, C.L., Steinauer, M.L., Mwinzi, P.N.M., Secor, W.E., Karanja, D.M.S. and 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. *Trop Med Int Health* 14(4): 450-457.
8. Borrmann, S., Szlezak, N., Faucher, J.F., Matsiegui, P.B., Neubauer, R., Binder, R.K., Lell, B. and Kremsner, P.G. (2001). Artesunate and praziquantel for the treatment of *Schistosoma haematobium* infections: A double-blind, randomized, placebo-controlled study. *J Infect Dis* 184(10): 1363-1366.

9. Botros, S., Sayed, H., El-Dusoki, H., Sabry, H., Rabie, I., El-Ghannam, M., Hassanein, M., El-Wahab, Y.A. and Engels, D. (2005). Efficacy of mirazid in comparison with praziquantel in Egyptian *Schistosoma mansoni*-infected school children and households. *Am J Trop Med Hyg* 72(2): 119-123.
10. Burchard, G.D., Kern, P., Baltes, R. and Dietrich, M. (1984). Comparative trial of oltipraz versus praziquantel in the treatment of urinary schistosomiasis in the gabon. *Tropenmed Parasitol* 35(2): 91-94.
11. Bustinduy, A.L., Sousa-Figueiredo, J.C., Adriko, M., Betson, M., Fenwick, A., Kabatereine, N. and Stothard, J.R. (2013). Fecal occult blood and fecal calprotectin as point-of-care markers of intestinal morbidity in ugandan children with *Schistosoma mansoni* infection. *PLoS Negl Trop Dis* 7(11).
12. Butterworth, A.E., Sturrock, R.F., Ouma, J.H., Mbugua, G.G., Fulford, A.J.C., Kariuki, H.C. and Koech, D. (1991). Comparison of different chemotherapy strategies against *Schistosoma mansoni* in Machakos District, Kenya: effects on human infection and morbidity. *Parasitology* 103(3): 339-355.
13. Campagne, G., Garba, A., Barkire, H., Vera, C., Sidiki, A. and Chippaux, J.P. (2001). Long-term echographic monitoring of children with Schistosomiasis haematobia after praziquantel. *Trop Med Int Health* 6(1): 24-30.
14. Chuks Ejezie, G. and Okeke, G.C. (1987). Chemotherapy in the control of urinary schistosomiasis in Nigeria. *J Trop Med Hyg* 90(3): 149-151.
15. Clercq, D.d., Hanne, C. and Vercruysse, J. (2000). Selected chemotherapy and seasonally transmitted *Schistosoma haematobium* infections in the middle valley of the Senegal River Basin. *Trans R Soc Trop Med Hyg* 94(2): 198-199.
16. Clercq, D.d., Vercruysse, J., Kongs, A., Verle, P., Dompnier, J.P. and Faye, P.C. (2002). Efficacy of artesunate and praziquantel in *Schistosoma haematobium* infected school children. *Acta Trop* 82(1): 61-66.
17. Davis, A., Biles, J.E., Ulrich, A.M. and Dixon, H. (1981). Tolerance and efficacy of praziquantel in phase II A and II B therapeutic trials in Zambian patients. *Arzneimittelforschung* 31(3a): 568-574.
18. Degu, G., Mengistu, G. and Jones, J. (2002). Praziquantel efficacy against *Schistosomiasis mansoni* in schoolchildren in north-west Ethiopia. *Trans R Soc Trop Med Hyg* 96(4): 444-445.

19. Doehring, E., Ehrich, J.H.H. and Reider, F. (1986). Daily urinary protein loss in *Schistosoma haematobium* infection. *Am J Trop Med Hyg* 35(5): 954-958.
20. El-Ghandour, S., El-Ayat, A. and Saleh, M. (1989). Optimizing praziquantel dose for mass treatment of children with *Schistosoma mansoni* in Egypt: A trial to cut down the cost for developing countries. *Pediatric Reviews and Communications* 4(1-2): 61-66.
21. El-Hawey, A.M., Abou-Taleb, S.A., El-Gammal, H.A., Abdel-Rahman, M.M. and Sabry, A.A. (1992). Profile of portal hypertension in hepatosplenic schistosomiasis. *J Egypt Soc Parasitol* 22(2): 305-309.
22. El-Morshedy, H., Kinosien, B., Barakat, R., Omer, E., Khamis, N., Deelder, A.M. and Phillips, M. (1996). Circulating anodic antigen for detection of *Schistosoma mansoni* infection in Egyptian patients. *Am J Trop Med Hyg* 54(2): 149-153.
23. Erko, B., Degarege, A., Tadesse, K., Mathiwos, A. and 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(3): 235-239.
24. Farid, L., el-Masry, N.A., Bassily, S., Trabolsi, B. and Wallace, C.K. (1984). Treatment of bilharzial obstructive uropathy with praziquantel. *J Infect Dis* 150(2): 307-308.
25. Friis, H. and Byskov, J. (1989). The effect of praziquantel against *Schistosoma mansoni*-infections in Botswana. *Trop Geogr Med* 41(1): 49-51.
26. Garba, A., Tohon, Z., Sidiki, A., Chippaux, J.P. and Chabalier, F.d. (2001). Efficacy and tolerance of praziquantel in school-aged children in a *Schistosoma haematobium* hyperendemic area (Niger, 1999). *Bull Soc Pathol Exot* 94(1): 42-45.
27. Groning, E., Bakathir, H., Salem, A., Albert, L. and Fernandez, R. (1985). Effectiveness of and tolerance to praziquantel in schistosomiasis. *Rev Cubana Med Trop* 37(2): 215-219.
28. Gryseels, B., Nkulikyinka, L. and Coosemans, M.H. (1987). Field trials of praziquantel and oxamniquine for the treatment of *Schistosomiasis mansoni* in Burundi. *Trans R Soc Trop Med Hyg* 81(4): 641-644.
29. Gryseels, B., Nkulikyinka, L. and Engels, D. (1991). Repeated community-based chemotherapy for the control of *Schistosoma mansoni*: effect of screening and selective treatment on prevalences and intensities of infection. *Am J Trop Med Hyg* 45(4): 509-517.

30. Guidi, A., Andolina, C., Makame Ame, S., Albonico, M., Cioli, D. and Juma Haji, H. (2010). Praziquantel efficacy and long-term appraisal of schistosomiasis control in Pemba Island. *Trop Med Int Health* 15(5): 614-618.
31. Guisse, F., Polman, K., Stelma, F.F., Mbaye, A., Talla, I., Niang, M., Deelder, A.M., Ndir, O. and Gryseels, B. (1997). Therapeutic evaluation of two different dose regimens of praziquantel in a recent *Schistosoma mansoni* focus in northern Senegal. *Am J Trop Med Hyg* 56(5): 511-514.
32. Gyoten, J., Kimura, E. and Muhoho, N.D. (1992). *Schistosoma haematobium* reinfection occurring shortly after treatment with praziquantel. *Jpn J Trop Med Hyg* 20(2): 157-164.
33. Hamm, D.M., Agossou, A., Gantin, R.G., Kocherscheidt, L., Banla, M., Dietz, K. and Soboslay, P.T. (2009). Coinfections with *Schistosoma haematobium*, Necator americanus and Entamoeba histolytica/Entamoeba dispar in children: Chemokine and cytokine responses and changes after antiparasite treatment. *J Infect Dis* 199(11): 1583-1591.
34. Hassanein, H.I., Zoheiry, M.M.K., Voss, B., El-Attar, G.M., Hassan, S.I., Demerdash, Z.A., El-Baz, H.M.G. and Yousif, O.A. (1997). Effect of early treatment with praziquantel on serum connective tissue metabolite markers in children and adolescents with intestinal *Schistosomiasis mansoni*. *Arzneimittelforschung* 47(1): 84-87.
35. Hatz, C.F., Vennervald, B.J., Nkulila, T., Vounatsou, P., Kombe, Y., Mayombana, C., Mshinda, H. and Tanner, M. (1998). Evolution of *Schistosoma haematobium*-related pathology over 24 months after treatment with praziquantel among school children in southeastern Tanzania. *Am J Trop Med Hyg* 59(5): 775-781.
36. Ismail, M.M., Attia, M.M., El-Badawy, A.A., Farghaly, A.M., Husein, M.H. and Metwally, A. (1994). Treatment of schistosomiasis with praziquantel among school children. *J Egypt Soc Parasitol* 24(3): 487-494.
37. Ismail, M.M., Bruce, J.I., Attia, M., Husein, M.H., Sabah, A.A. and Tayel, S.E. (1988). The status of schistosomiasis before and after treatment among school pupils in a village in the nile delta egypt. *J Egypt Soc Parasitol* 18(1): 287-296.
38. Jonge, N.d., De Caluwe, P., Hilberath, G.W., Krijger, F.W., Polderman, A.M. and Deelder, A.M. (1989). Circulating anodic antigen levels in serum before and after chemotherapy with praziquantel in *Schistosomiasis mansoni*. *Trans R Soc Trop Med Hyg* 83(3): 368-372.
39. Jonge, N.d., Schommer, G., Feldmeier, H., Krijger, F.W., Dafalla, A.A., Bienzle, U. and Deelder, A.M. (1990). Mixed Schistosoma haematobium and S. mansoni infection:

effect of different treatments on the serum level of circulating anodic antigen (CAA). *Acta Trop* 48(1): 25-35.

40. Kabatereine, N.B., Kemijumbi, J., Ouma, J.H., Sturrock, R.F., Butterworth, A.E., Madsen, H., Ornbjerg, N., Dunne, D.W. and Vennnervald, B.J. (2003). Efficacy and side effects of praziquantel treatment in a highly endemic *Schistosoma mansoni* focus at Lake Albert, Uganda. *Trans R Soc Trop Med Hyg* 97(5): 599-603.
41. Kahama, A.I., Odek, A.E., Kihara, R.W., Vennervald, B.J., Kombe, Y., Nkulila, T., Hatz, C.F., Ouma, J.H. and Deelder, A.M. (1999). Urine circulating soluble egg antigen in relation to egg counts, hematuria, and urinary tract pathology before and after treatment in children infected with *Schistosoma haematobium* in Kenya. *Am J Trop Med Hyg* 61(2): 215-219.
42. Kahama, A.I., Vennervald, B.J., Kombe, Y., Kihara, R.W., Ndzovu, M., Mungai, P. and Ouma, J.H. (1999). Parameters associated with *Schistosoma haematobium* infection before and after chemotherapy in school children from two villages in the Coast province of Kenya. *Trop Med Int Health* 4(5): 335-340.
43. Karanja, D.H.S., Boyer, A.E., Strand, M., Colley, D.G., Nahlen, B.L., Ouma, J.H. and Secor, W.E. (1998). Studies on schistosomiasis in western Kenya: II. Efficacy of praziquantel for treatment of schistosomiasis in persons coinfected with human immunodeficiency VIRUS-1. *Am J Trop Med Hyg* 59(2): 307-311.
44. Katz, N., Rocha, R.S. and De Souza, C.P. (1991). Efficacy of alternating therapy with oxamniquine and praziquantel to treat *Schistosoma mansoni* in children following failure of first treatment. *Am J Trop Med Hyg* 44(5): 509-512.
45. Keiser, J., N'Guessan, N.A., Adoubryn, K.D., Silue, K.D., Vounatsou, P., Hatz, C., Utzinger, J. and N'Goran, E.K. (2010). Efficacy and safety of mefloquine, artesunate, mefloquine artesunate, and praziquantel against *Schistosoma haematobium*: randomized, exploratory open label trial. *Clin Infect Dis* 50(9): 1205-1213.
46. Keiser, J., Silue, K.D., Adiossan, L.K., N'Guessan, N.A., Monsan, N.C., Utzinger, J. and N'Goran, E.K. (2014). Praziquantel, mefloquine-praziquantel, and mefloquine-artesunate-praziquantel against *Schistosoma haematobium*: A randomized, exploratory, open-label trial. *PLoS Negl Trop Dis* 8(7).
47. Kern, P., Burchard, G.D. and Dietrich, M. (1984). Comparative study of oltipraz versus praziquantel for treatment of schistosomiasis with intestinal manifestation in the Gabon (*Schistosoma intercalatum* and *S. haematobium*). *Tropenmed Parasitol* 35(2): 95-99.

48. Khalil, H.M., El-Hawey, A.M., Baki, M.H.A., El-Hadi, H.M. and Sharara, M.O.A. (1986). Evaluation of praziquantel efficiency in the treatment of schistosomiasis. *J Egypt Soc Parasitol* 16(2): 403-412.
49. Kihara, J.H., Muhoho, N., Njomo, D., Mwobobia, I.K., Josyline, K., Mitsui, Y., Awazawa, T., Amano, T. and Mwandawiro, C. (2007). Drug efficacy of praziquantel and albendazole in school children in Mwea Division, Central Province, Kenya. *Acta Trop* 102(3): 165-171.
50. Kiliku, F.M., Kimura, E., Muhoho, N., Migwi, D.K. and Katsumata, T. (1991). The usefulness of urinalysis reagent strips in selecting *Schistosoma haematobium* egg positives before and after treatment with praziquantel. *J Trop Med Hyg* 94(6): 401-406.
51. Kimura, E., Moji, K., Uga, S., Kiliku, F.M., Migwi, D.K., Mutua, W.R., Muhoho, N.D. and Aoki, Y. (1992). Effects of *Schistosoma haematobium* infection on mental test-scores of kenyan school-children. *Trop Med Parasitol* 43(3): 155-158.
52. Kjetland, E.F., Mduluza, T., Ndhlovu, P.D., Gomo, E., Gwanzura, L., Midzi, N., Mason, P.R., Friis, H. and Gundersen, S.G. (2006). Genital schistosomiasis in women: a clinical 12-month in vivo study following treatment with praziquantel. *Trans R Soc Trop Med Hyg* 100(8): 740-752.
53. Latham, M.C., Stephenson, L.S., Kurz, K.M. and Kinoti, S.N. (1990). Metrifonate or praziquantel treatment improves physical fitness and appetite of Kenyan schoolboys with *Schistosoma haematobium* and hookworm infections. *Am J Trop Med Hyg* 43(2): 170-179.
54. Massoud, A.A.E., El Kholy, A.M. and Anwar, W.A. (1984). Assessment on efficacy of praziquantel against *Schistosoma mansoni* infection. *J Trop Med Hyg* 87(3): 119-121.
55. McMahon, J.E. (1981). Observations on praziquantel against *Schistosoma haematobium*. *Arzneimittelforschung*: 579-580.
56. McMahon, J.E. (1983). A comparative trial of praziquantel, metrifonate and niridazole against *Schistosoma haematobium*. *Ann Trop Med Parasit* 77(2): 139-142.
57. Metwally, A., Bennett, J., Botros, S., Ebeid, F. and Elattar, G. (1995). Impact of drug-dosage and brand on bioavailability and efficacy of praziquantel. *Pharmacol Res* 31(1): 53-59.
58. Midzi, N., Sangweme, D., Zinyowera, S., Mapingure, M.P., Brouwer, K.C., Kumar, N., Mutapi, F., Woelk, G. and Mduluza, T. (2008). Efficacy and side effects of praziquantel

treatment against *Schistosoma haematobium* infection among primary school children in Zimbabwe. *Trans R Soc Trop Med Hyg* 102(8): 759-766.

59. Mohamed, A.A., Mahgoub, H.M., Magzoub, M., Gasim, G.I., Eldein, W.N., Ahmed, A.e.A.A. and Adam, I. (2009). Artesunate plus sulfadoxine/pyrimethamine versus praziquantel in the treatment of *Schistosoma mansoni* in eastern Sudan. *Trans R Soc Trop Med Hyg* 103(10): 1062-1064.
60. Mohammed, E.H., Eltayeb, M. and Ibrahim, H. (2006). Haematological and biochemical morbidity of *Schistosoma haematobium* in school children in Sudan. *Sultan Qaboos Univ Med J* 6(2): 59-64.
61. Muhumuza, S., Olsen, A., Katahoire, A., Kiragga, A.N. and Nuwaha, F. (2014). Effectiveness of a pre-treatment snack on the uptake of mass treatment for schistosomiasis in uganda: A cluster randomized trial. *PLoS Med* 11(5).
62. Mutapi, F., Hagan, P., Woolhouse, M.E.J., Mduluza, T. and Ndhlovu, P.D. (2003). Chemotherapy-induced, age-related changes in antischistosome antibody responses. *Parasite Immunol* 25(2): 87-97.
63. Mutapi, F., Ndhlovu, P.D., Hagan, P., Spicer, J.T., Mduluza, T., Turner, C.M.R., Chandiwana, S.K. and Woolhouse, M.E.J. (1998). Chemotherapy accelerates the development of acquired immune responses to *Schistosoma haematobium* infection. *J Infect Dis* 178(1): 289-293.
64. Mutapi, F., Ndhlovu, P.D., Hagan, P. and Woolhouse, M.E.J. (1998). Changes in specific anti-egg antibody levels following treatment with praziquantel for *Schistosoma haematobium* infection in children. *Parasite Immunol* 20(12): 595-600.
65. Mutapi, F., Rujeni, N., Bourke, C., Mitchell, K., Appleby, L., Nausch, N., Midzi, N. and 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(5).
66. N'Goran, E.K., Utzinger, J., N'Guessan, A.N., Muller, I., Zamble, K., Lohourignon, K.L., Traore, M., Sosthene, B.A., Lengeler, C. and Tanner, M. (2001). Reinfection with *Schistosoma haematobium* following school-based chemotherapy with praziquantel in four highly endemic villages in Cote d'Ivoire. *Trop Med Int Health* 6(10): 817-825.
67. Navaratnam, A.M.D., Sousa-Figueiredo, J.C., Stothard, J.R., Kabatereine, N.B., Fenwick, A. and 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(7): 400-407.

68. Nega, B., Gundersen, S.G., Fekadu, A., Hailu, B., Girmay, M. and Teferi, G. (1999). Praziquantel side effects and efficacy related to *Schistosoma mansoni* egg loads and morbidity in primary school children in north-east Ethiopia. *Acta Trop* 72(1): 53-63.
69. Nkengazong, L., Njiokou, F., Teukeng, F., Enyong, P. and Wanji, S. (2009). Reassessment of endemicity level of urinary schistosomiasis in the Kotto-Barombi focus (South West Cameroon) and impact of mass drug administration (MDA) on the parasitic indices. *J Cell Anim Biol* 3(9): 159-164.
70. Obonyo, C.O., Muok, E.M.O. and Mwinzi, P.N.M. (2010). Efficacy of artesunate with sulfalene plus pyrimethamine versus praziquantel for treatment of *Schistosoma mansoni* in Kenyan children: an open-label randomised controlled trial. *Lancet Infect Dis* 10(9): 603-611.
71. Ofoezie, I.E. (2000). Patterns of reinfection following praziquantel treatment of urinary schistosomiasis at a period of low transmission. *Acta Trop* 75(1): 123-126.
72. Olds, G.R., King, C., Hewlett, J., Olveda, R., Wu, G., Ouma, J., Peters, P., McGarvey, S., Odhiambo, O., Koech, D., Liu, C.Y., Aligui, G., Gachihi, G., Kombe, Y., Parraga, I., Ramirez, B., Whalen, C., Horton, R.J. and Reeve, P. (1999). Double-blind placebo-controlled study of concurrent administration of albendazole and praziquantel in schoolchildren with schistosomiasis and geohelminths. *J Infect Dis* 179(4): 996-1003.
73. Olliaro, P.L., Vaillant, M.T., Belizario, V.J., Lwambo, N.J.S., Ouldabdallahi, M., Pieri, O.S., Amarillo, M.L., Kaatano, G.M., Diaw, M., Domingues, A.C., Favre, T.C., Lapujade, O., Alves, F. and Chitsulo, L. (2011). A multicentre randomized controlled trial of the efficacy and safety of single-dose praziquantel at 40 mg/kg vs. 60 mg/kg for treating intestinal schistosomiasis in the philippines, mauritania, tanzania and brazil. *PLoS Negl Trop Dis* 5(6).
74. Olsen, A., Nawiri, J. and Friis, H. (2000). The impact of iron supplementation on reinfection with intestinal helminths and *Schistosoma mansoni* in western Kenya. *Trans R Soc Trop Med Hyg* 94(5): 493-499.
75. Olsen, A., Thiong'o, F.W., Ouma, J.H., Mwaniki, D., Magnussen, P., Michaelsen, K.F., Friis, H. and Geissler, P.W. (2003). Effects of multimicronutrient supplementation on helminth reinfection: a randomized, controlled trial in Kenyan schoolchildren. *Trans R Soc Trop Med Hyg* 97(1): 109-114.

76. Opara, K.N., Mbagwu, H.C. and Ekpo, U.F. (2003). Urinary schistosomiasis in two endemic communities of Cross River Basin: prevalence, intensity, and treatment. *MSJM* 3(1): 28-34.
77. Ouldabdallahi, M., Ousmane, B., Ouldbezeid, M., Mamadou, D., Konate, L. and Chitsulo, L. (2013). Comparison of the efficacy and safety of praziquantel administered in single dose of 40 versus 60 mg/kg for treating urinary schistosomiasis in Mauritania. *Bull Soc Pathol Exot* 106(3): 167-169.
78. Oyediran, A.B., Kofie, B.A., Bammeke, A.O. and Bamgboye, E.A. (1981). Clinical experience with praziquantel in the treatment of Nigerian patients infected with *S. haematobium*. *Arzneimittelforschung* 31(3a): 581-584.
79. Polderman, A.M., Gryseels, B. and De Caluwe, P. (1988). Cure rates and egg reduction in treatment of intestinal schistosomiasis with oxamniquine and praziquantel in Maniema, Zaire. *Trans R Soc Trop Med Hyg* 82(1): 115-116.
80. Reimert, C.M., Mshinda, H.M., Hatz, C.F., Kombe, Y., Nkulila, T., Poulsen, L.K., Christensen, N.O. and Vennervald, B.J. (2000). Quantitative assessment of eosinophiluria in *Schistosoma haematobium* infections: A new marker of infection and bladder morbidity. *Am J Trop Med Hyg* 62(1): 19-28.
81. Reimert, C.M., Ouma, J.H., Mwanje, M.T., Magak, P., Poulsen, L.K., Vennervald, B.J., Christensen, N.O., Kharazmi, A. and Bendtzen, K. (1993). Indirect assessment of eosinophiluria in urinary schistosomiasis using eosinophil Cationic Protein (ECP) and Eosinophil Protein-X (EPX). *Acta Trop* 54(1): 1-12.
82. Reta, B. and Erko, B. (2013). Efficacy and side effects of praziquantel in the treatment for *Schistosoma mansoni* infection in school children in Senbete Town, northeastern Ethiopia. *Trop Med Int Health* 18(11): 1338-1343.
83. Saathoff, E., Olsen, A., Magnussen, P., Kvalsvig, J.D., Becker, W. and Appleton, C.C. (2004). Patterns of *Schistosoma haematobium* infection, impact of praziquantel treatment and re-infection after treatment in a cohort of schoolchildren from rural KwaZulu-Natal/South Africa. *BMC Infect Dis* 4.
84. Sacko, M., Magnussen, P., Traore, M., Landoure, A., Doucoure, A., Reimert, C.M. and Vennervald, B.J. (2009). The effect of single dose versus two doses of praziquantel on *Schistosoma haematobium* infection and pathology among school-aged children in Mali. *Parasitology* 136(13): 1851-1857.

85. Satti, M.Z., Lind, P., Vennervald, B.J., Sulaiman, S.M., Daffalla, A.A. and Ghalib, H.W. (1996). Specific immunoglobulin measurements related to exposure and resistance to *Schistosoma mansoni* infection in Sudanese canal cleaners. *Clin Exp Immunol* 106(1): 45-54.
86. Scherrer, A.U., Sjoeberg, M.K., Allangba, A., Traore, M., Lohourignon, L.K., Tschannen, A.B., N'Goran, E.K. and Utzinger, J. (2009). Sequential analysis of helminth egg output in human stool samples following albendazole and praziquantel administration. *Acta Trop* 109(3): 226-231.
87. Schutte, C.H., Osman, Y., Van Deventer, J.M. and Mosese, G. (1983). Effectiveness of praziquantel against the South African strains of *Schistosoma haematobium* and *S. mansoni*. *S Afr Med J* 64(1): 7-10.
88. Scott, J.T., Turner, C.M.R., Mutapi, F., Woolhouse, M.E.J., Ndhlovu, P.D. and Hagan, P. (2001). Cytokine responses to mitogen and *Schistosoma haematobium* antigens are different in children with distinct infection histories. *Parasite Immunol* 23(10): 519-526.
89. Simonsen, P.E., Nega, A. and Furu, P. (1990). Intestinal schistosomiasis among children in a labour village of Wonji Sugar Estate, Ethiopia. *East Afr Med J* 67(8): 532-538.
90. Sissoko, M.S., Dabo, A., Traore, H., Diallo, M., Traore, B., Konate, D., Niare, B., Diakite, M., Kamate, B., Traore, A., Bathily, A., Tapily, A., Toure, O.B., Cauwenbergh, S., Jansen, H.F. and Doumbo, O.K. (2009). Efficacy of artesunate plus sulfamethoxypyrazine/pyrimethamine versus praziquantel in the treatment of *Schistosoma haematobium* in children. *PLoS One* 4(10).
91. Snyman, J.R., Sommers, K.D., Steinmann, M.A. and Lizamore, D.J. (1997). Effects of calcitriol on eosinophil activity and antibody responses in patients with schistosomiasis. *Eur J Clin Pharmacol* 52(4): 277-280.
92. Sousa-Figueiredo, J.C., Betson, M., Atuhaire, A., Arinaitwe, M., Navaratnam, A.M., Kabatereine, N.B., Bickle, Q. and Stothard, J.R. (2012). Performance and safety of praziquantel for treatment of intestinal schistosomiasis in infants and preschool children. *PLoS Negl Trop Dis* 6(10): e1864.
93. Sousa-Figueiredo, J.C., Pleasant, J., Day, M., Betson, M., Rollinson, D., Montresor, A., Kazibwe, F., Kabatereine, N.B. and Stothard, J.R. (2010). Treatment of intestinal schistosomiasis in Ugandan preschool children: best diagnosis, treatment efficacy and side-effects, and an extended praziquantel dosing pole. *Int Health* 2(2): 103-113.

94. Stete, K., Krauth, S.J., Coulibaly, J.T., Knopp, S., Hattendorf, J., Muller, I., Lohourignon, L.K., Kern, W.V., N'Goran, E.K. and Utzinger, J. (2012). Dynamics of *Schistosoma haematobium* egg output and associated infection parameters following treatment with praziquantel in school-aged children. *Parasite Vector* 5(298).
95. Taddese, K. and Zein, Z.A. (1988). Comparison between the efficacy of oxamniquine and praziquantel in the treatment of *Schistosoma mansoni* infections on a sugar estate in Ethiopia. *Ann Trop Med Parasit* 82(2): 175-180.
96. Tchuente, L.A., Shaw, D.J., Polla, L., Cioli, D. and Vercruysse, J. (2004). Efficacy of praziquantel against *Schistosoma haematobium* infection in children. *Am J Trop Med Hyg* 71(6): 778-782.
97. Thiong'o, F.W., Mbugua, G.G., Ouma, J.H. and Sturrock, R.K. (2002). Efficacy of oxamniquine and praziquantel in school children from two *Schistosoma mansoni* endemic areas. *East Afr Med J* 79(1): 29-33.
98. Tweyongyere, R., Mawa, P.A., Emojong, N.O., Mpairwe, H., Jones, F.M., Duong, T., Dunne, D.W., Vennervald, B.J., Katunguka-Rwakishaya, E. and Elliott, A.M. (2009). Effect of praziquantel treatment of *Schistosoma mansoni* during pregnancy on intensity of infection and antibody responses to schistosome antigens: results of a randomised, placebo-controlled trial. *BMC Infect Dis* 9.
99. Utzinger, J., Booth, M., N'Goran, E.K., Muller, I., Tanner, M. and Lengeler, C. (2001). Relative contribution of day-to-day and intra-specimen variation in faecal egg counts of *Schistosoma mansoni* before and after treatment with praziquantel. *Parasitology* 122: 537-544.
100. Utzinger, J., N'Goran, E.K., N'Dri, A., Lengeler, C. and Tanner, M. (2000). Efficacy of praziquantel against *Schistosoma mansoni* with particular consideration for intensity of infection. *Trop Med Int Health* 5(11): 771-778.
101. Webster, B.L., Diaw, O.T., Seye, M.M., Faye, D.S., Stothard, J.R., Sousa-Figueiredo, J.C. and Rollinson, D. (2013). Praziquantel treatment of school children from single and mixed infection foci of intestinal and urogenital schistosomiasis along the Senegal River Basin: monitoring treatment success and re-infection patterns. *Acta Trop* 128(2): 292-302.
102. Webster, M., Fallon, P.G., Fulford, A.J.C., Butterworth, A.E., Ouma, J.H., Kimani, G. and Dunne, D.W. (1997). Effect of praziquantel and oxamniquine treatment on

human isotype responses to *Schistosoma mansoni*: Elevated IgE to adult worm. *Parasite Immunol* 19(7): 333-335.

103. Wilkins, H.A., Blumenthal, U.J., Hagan, P., Hayes, R.J. and Tulloch, S. (1987). Resistance to reinfection after treatment of urinary schistosomiasis. *Trans R Soc Trop Med Hyg* 81(1): 29-35.

104. Wilkins, H.A. and Moore, P.J. (1987). Comparative trials of regimes for the treatment of urinary schistosomiasis in The Gambia. *J Trop Med Hyg* 90(2): 83-92.

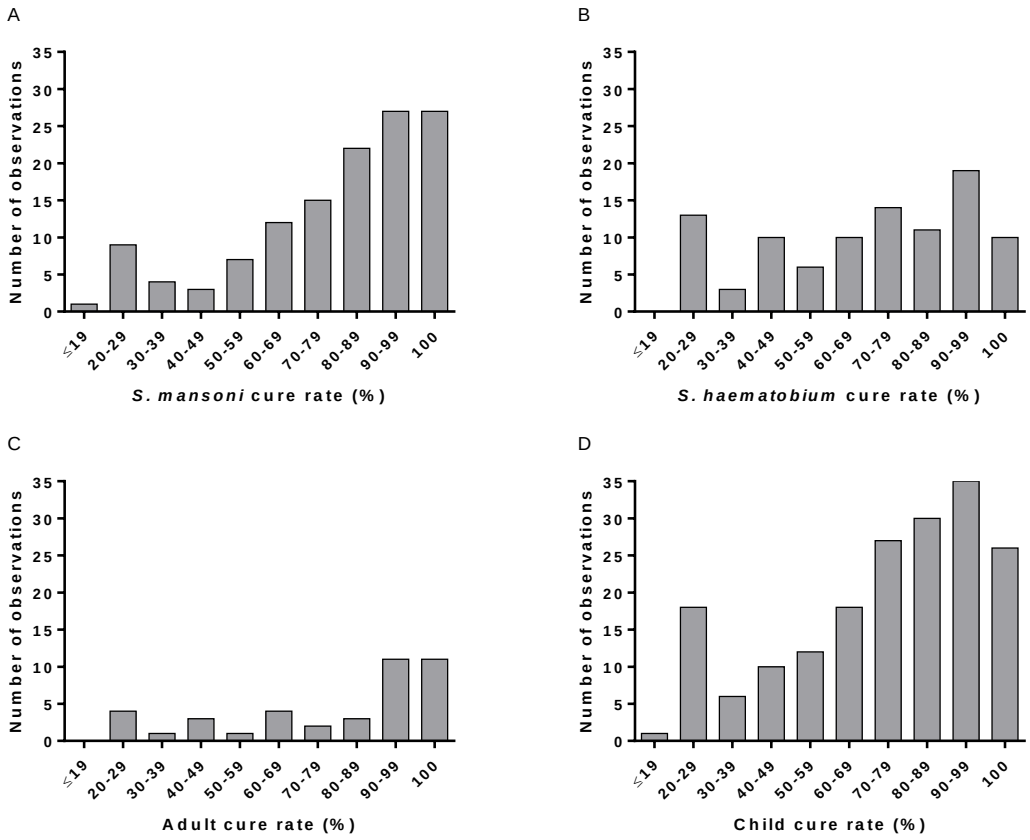
105. Wilson, S., Jones, F.M., Kenty, L.C., Mwatha, J.K., Kimani, G., Kariuki, H.C. and Dunne, D.W. (2014). Posttreatment changes in cytokines induced by *Schistosoma mansoni* egg and worm antigens: dissociation of immunity- and morbidity-associated type 2 responses. *J Infect Dis* 209(11): 1792-1800.

106. Wu, W.X. (1994). Clinical observation on the treatment of 1627 cases of schistosomiasis haematobia with praziquantel of different dosages. *Chinese Journal of Parasitology & Parasitic Diseases* 12(4): 288-290.

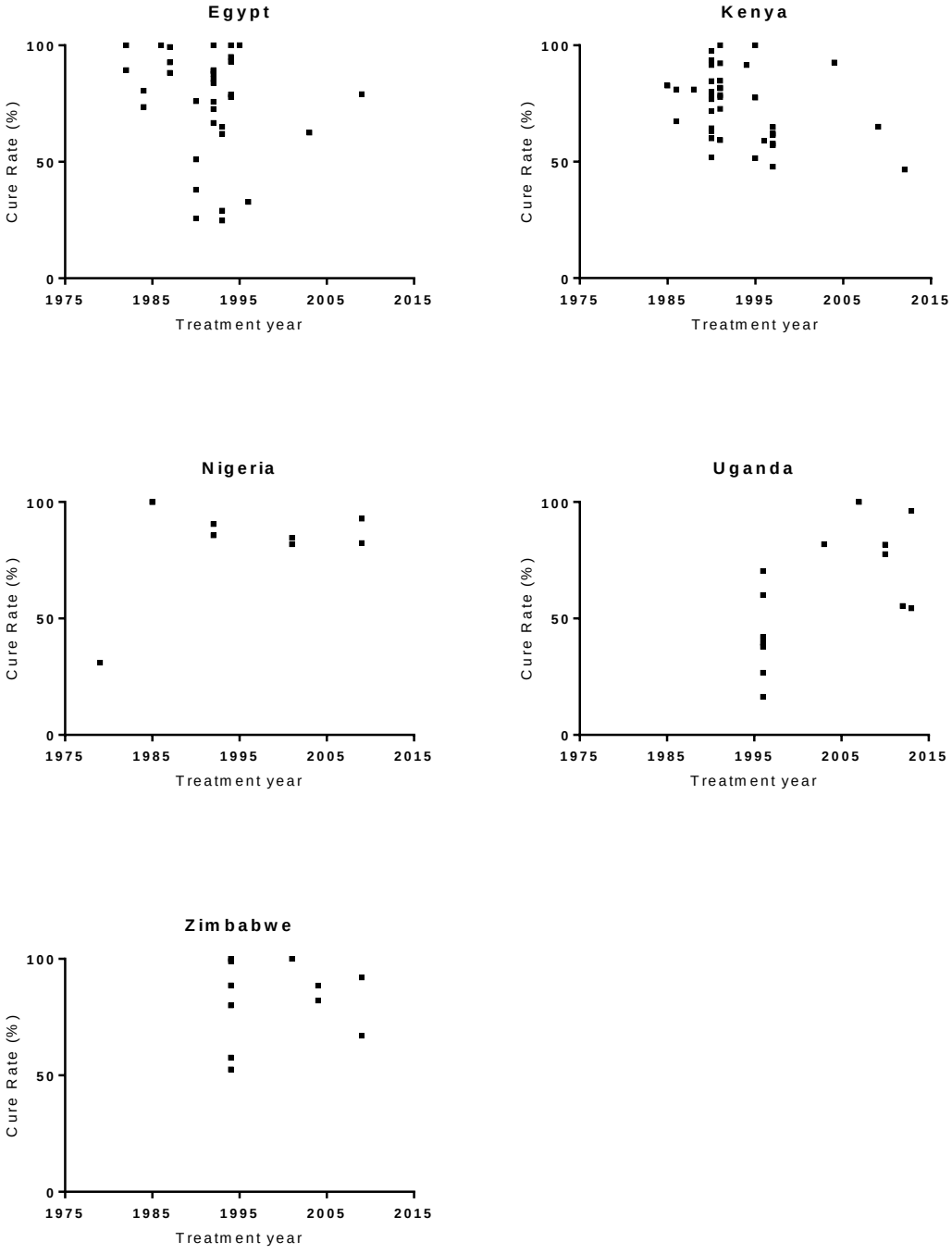
D.2: Table of a treatment year range reported by each country.

Country	Treatment years	No. of observations
Brazil	1989-2006	3
Burundi	1984-1985	14
Cameroon	2002-2007	5
Congo	1986-1987	5
Cote D'Ivoire	1997-2011	8
Egypt	1982-2009	35
Ethiopia	1983-2011	10
Gabon	1982	4
Gambia	1983-1985	6
Kenya	1985-2012	41
Mali	1992-2007	7
Mauritania	2005-2006	6
Niger	1995-1999	2
Nigeria	1979-2009	11
Senegal	1993-2007	9
South Africa	1981-1998	6
Sudan	1987-2008	7
Tanzania	1979-2007	10
Uganda	1996-2013	14
Zambia	1979	3
Zimbabwe	1994-2009	11
Others	1983-2007	6

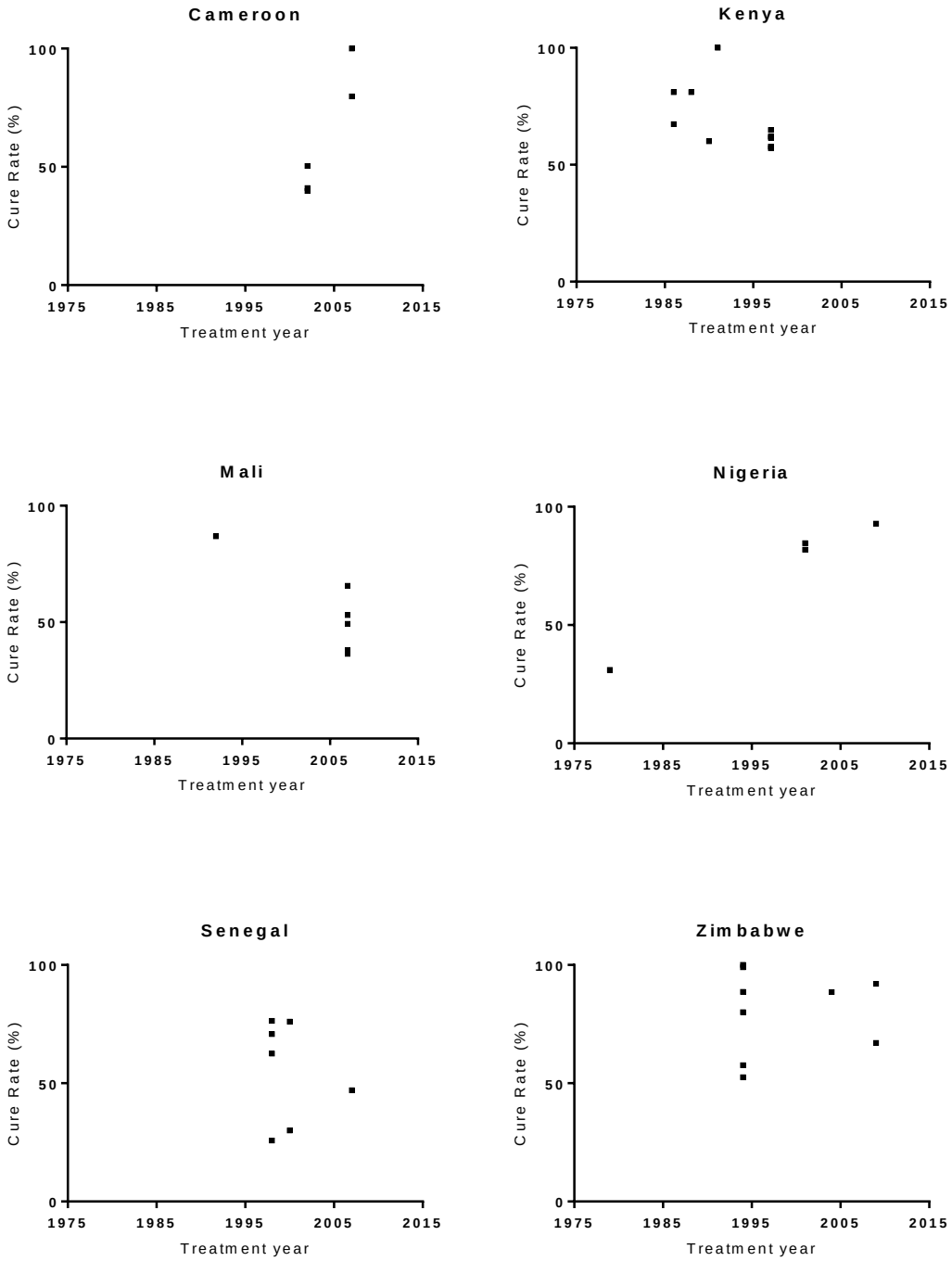
D.3: The distribution of reported parasitological cure rates following praziquantel treatment.(A) *S. mansoni* infection, (B) *S. haematobium* infection, (C) schistosome infection among adults, and (D) schistosome infection among children.



D.4: Figure illustrating the reported cure rates by treatment years from 1979 to 2013 for Egypt, Kenya, Nigeria, Uganda, and Zimbabwe. Countries with 11 or more observations were selected for these subgroups analyses. The results of random-effects meta-regressions using article ID as a random effect, weighting with number of participants treatment year effect on cure rate were not significant [Egypt: F(1, 12) =1.048,p=0.326, Kenya: F(1, 15) =1.931, p=0.185, Nigeria: F(1, 3) =1.045, p=0.382, Uganda: F(1, 12) =3.921, p=0.071, Zimbabwe: F(1, 9) =0.046, p=0.835].



D. 5: Figure illustrating the reported cure rates among *S. haematobium* infected children after 40 mg/kg body weight praziquantel treatment by treatment year for Cameroon, Kenya, Mali, Nigeria, Senegal and Zimbabwe. Countries with five or more observations were selected.



Appendix E: A paper arising from this thesis

frontiers in
IMMUNOLOGY

ORIGINAL RESEARCH ARTICLE
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A meta-analysis of experimental studies of attenuated *Schistosoma mansoni* vaccines in the mouse model

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Schistosomiasis is a water-borne, parasitic disease of major public health importance. There has been considerable effort for several decades toward the development of a vaccine against the disease. Numerous mouse experimental studies using attenuated *Schistosoma mansoni* parasites for vaccination have been published since 1960s. However, to date, there has been no systematic review or meta-analysis of these data. The aim of this study is to identify measurable experimental conditions that affect the level of protection against re-infection with *S. mansoni* in mice vaccinated with radiation attenuated cercariae. Following a systematic review, a total of 755 observations were extracted from 105 articles (published 1963–2007) meeting the searching criteria. Random effects meta-regression models were used to identify the influential predictors. Three predictors were found to have statistically significant effects on the level of protection from vaccination: increasing numbers of immunizing parasites had a positive effect on fraction of protection whereas increasing radiation dose and time to challenge infection had negative effects. Models showed that the irradiated cercariae vaccine has the potential to achieve protection as high as 78% with a single dose vaccination. This declines slowly over time but remains high for at least 8 months after the last immunization. These findings provide insights into the optimal delivery of attenuated parasite vaccination and into the nature and development of protective vaccine induced immunity against schistosomiasis, which may inform the formulation of human vaccines and the predicted duration of protection and thus frequency of booster vaccines.

Keywords: schistosomiasis, attenuated cercariae, protective immunity, random effects meta-regression, animal model, systematic review

INTRODUCTION

Schistosomiasis is a water-borne parasitic disease of major public health importance. More than 4.5 million disability adjusted life years (DALYs) are lost each year worldwide due to schistosome infection (1–4). Human schistosomiasis is mainly caused by three species: *Schistosoma mansoni*, *Schistosoma haematobium*, and *Schistosoma japonicum* (5). More than 90% of reported cases are from sub-Saharan Africa where both *S. mansoni* and *S. haematobium* infections are endemic (6). The vast majority of control programs use the antihelminthic drug praziquantel for mass drug administration. This low-cost and efficacious drug has achieved a significant reduction in disease prevalence and infection intensity in many endemic areas (7–10). However, there are multiple reports of re-infection after chemotherapy (11–13). In addition, praziquantel can clear only adult worms and has little or no effect on existing eggs and immature worms (14). This means that there is need for additional complementary interventions, one of which is vaccination.

Slowly developing acquired immunity plays a crucial role in the reduction of infection prevalence and intensity in older age groups in endemic areas (15, 16). This suggests that exposure to schistosome antigens can promote protective immunity in humans; however, to date, there is no licensed schistosome vaccine (17, 18). Currently, the leading vaccine candidate is the 28 kDa *S. haematobium* GST (Sh28GST, Brand name: Bilhvax), which is now in phase 3 clinical trials (19–21). Alongside recombinant antigen vaccine studies, the attenuated live cercariae vaccine has been studied extensively in mouse models (22, 23). Properly prepared attenuated cercariae live long enough to invade the host skin and stimulate protective acquired immunity against subsequent challenge infection but die in the host's body before they mature into adult worms (24). Attenuated schistosome cercariae vaccination experiments in animals use cercariae, which are weakened by ionizing radiation (X-ray or gamma ray), ultraviolet, heat, or chemical treatment. Host animals are immunized with attenuated parasites either once or several times before challenge infection with non-attenuated pathogenic cercariae. A certain number of days after the challenge infection, immunized animals and control animals are perfused to quantify the level of protection due to immunization by comparing the number of adult worms recovered from both groups.

A large number of mouse experimental studies using attenuated *S. mansoni* cercariae for vaccination have been published since 1960s (25); however, such studies have never been systematically analyzed. The aim of this study was to conduct a meta-analysis to identify measurable experimental conditions (predictors) that affect the level of protection against challenge infection of vaccinated animals. In addition, levels of each predictor associated with maximum levels of protection were estimated.

MATERIALS AND METHODS

SYSTEMATIC REVIEW

An electronic literature search was performed using Science Citation Index Expanded, Conference Proceedings Citation Index and BIOSIS Citation Index, all of which were provided through Web of Knowledge[1]. Alongside these, EMBASE[2], OVID MEDICINE[3], and CAB abstracts, were searched simultaneously though OvidSP[4]. Reference lists of all articles identified by the electronic search were searched manually for additional relevant reference. In addition, ProQuest Dissertations and Thesis Full Text[5] was searched as a source of pre-published and gray literature. The search terms were chosen to be as inclusive as possible and were "cercaria*" AND ("irradiat*" OR "attenuat*") AND ("vaccin*OR schistosom*"). Also, we searched by "Attenuate*" AND "schistosome*" AND "vaccin*." This search was completed in July 2013. After duplicated articles were removed a total of 1,013 articles were identified. Titles and abstracts were screened by at least two independent reviewers to exclude those that were not relevant to an attenuated schistosome vaccine animal model. Full texts of potentially relevant articles were reviewed by two independent reviewers for further selection. Non-English articles were included, and several Chinese and German articles were identified and translated into English by a native Chinese speaker and German speaker, respectively, for the analysis.

A study was considered eligible if it met all of the following inclusion criteria: (1) vaccination with attenuated cercariae; (2) use of ionizing radiation for attenuation; (3) use of percutaneous immunization and challenge (i.e., the natural transmission route for schistosome infection); (4) challenge infection using normal (non-attenuated) cercariae; (5) worm burden measured after the challenge infection via perfusion; (6) outcome (fraction of protection) reported or could be calculated. In this study, fraction of protection means the proportion of reduction in worm burden in vaccinated mice compared to that of control mice group. For articles, which reported worm count after challenge infection, the following equation was used to calculate the outcome: fraction of protection = [(average number of worms per mouse retrieved from control group − average number of worms per mouse retrieved from vaccinated group)/average number of worms per mouse retrieved from control group]. In the case of articles, which failed to report worm counts (allowing calculation of this quantity), only those that stated that they used the same equation as above were included.

Studies were excluded if they met any of the following exclusion criteria: (1) immunizing attenuated cercariae developed to adulthood; (2) hosts were transgenic or genetically engineered; (3) hosts had an *in vivo* depletion of immune cells; (4) attenuated cercariae were prepared by any means other than ionizing irradiation; (5) a non-cercarial vaccine was used (e.g., adult worm, schistosomula, subunit); (6) an artificial infection was conducted prior to vaccination; or (7) hosts were treated with anthelmintic drugs.

Articles often reported results from multiple separate experiments such as use of different doses of attenuated parasite. In these cases, results from each experiment were recorded as an observation. A total of 755 observations from 105 articles (articles are listed in Supplementary Material) meeting searching criteria and also using mouse as a host and *S. mansoni* for vaccination and challenge infection. Although the mouse is not a natural host for schistosome infection, it is the most commonly used animal for attenuated schistosome parasite vaccine animal model. A list of potential predictors (given in **Table 1**) was drawn up and these quantities were extracted from each article. These potential predictors have been suggested their importance by review articles and also their quantities been reported by many experimental studies (26). When an article reported a dose range rather than an exact dose the mid-value was used for the analysis.

STATISTICAL ANALYSIS

Random effects meta-regression

Random effects meta-regression was used to identify the influential predictors and effect of dose on protection. Multiple observations (1–56) were recorded from single articles and therefore article was included as a random effect in the models. The models were built using a backwards stepwise procedure with eight potential predictors (listed in **Table 1**). The effect of the number of immunizing parasites was explored in two ways in the two separate models: as an average number of immunizing parasites per dose or as a total number of immunizing parasites. Correlations between variables were examined visually by scatter plot graphs for all possible predictor combinations (data not shown). Then,

Table 1 | Possible predictors investigated and their units/codes.

Variable name	Units/code
Number of immunizing parasites (total and number per dose)	Number of parasites log10 transformed
Number of challenge parasites	Number of parasites log10 transformed
Number of immunizations	Count
Irradiation dose	Krad
Host age	Weeks
Host sex	Male, female, mixed
Time between the last immunization and challenge	Days
Time between challenge and perfusion	Days

[1]http://www.webofknowledge.com
[2]http://www.elsevier.com
[3]http://www.ovid.com
[4]http://ovidsp.tx.ovid.com
[5]http://www.proquest.com

all the possible combinations of two-way interactions of potential predictors were examined using a random effects meta-regression model with two-way interactions. The outcome variable (fraction of protection) was transformed as −ln(1 − fraction of protection) to reduce the skewness of residuals (27). Although using confidence intervals and SE is the most common weighting method for meta-regression (28), many studies in our dataset failed to report either confidence intervals or SD and there were no comparable studies, which enabled us to justify imputing them. Two kinds of information were available on the size of the studies: the number of control animals and the number of vaccinated animals. The majority of studies used similar numbers of control and vaccinated animals; however, there were several articles, which used a higher number of vaccinated animals than control animals. To account for the impact of these unbalanced studies, the number of control animals was used as the more conservative weighting option.

Missing values and outliers

Several outliers were excluded from the analysis. They were six observations with animals kept longer than 300 days or <7 days after the last immunization and four observations that used more than 10,000 cercariae for immunization. After excluding outliers 745 observations were kept for further selection.

When the numbers of control animals were not reported in an article and only the numbers of vaccinated animals were given, numbers of control animals were then imputed by a linear regression imputation method between numbers of vaccinated and control animals for all studies (29). When the observation was missing for both the number of control animals and vaccinated animals (4 observations from 4 articles), the average number of control animals of the remaining data set was used for imputation, which was 10 control animals. Out of 745 observations, 725 observations from 100 articles reported all predictors and were used for the analysis.

Statistical software

Papers identified by systematic review were recorded by Thomson Reuters EndNote and the extracted data were entered on a Microsoft Excel 2010 spread sheet for further analysis. IBM SPSS Statistics Version 19.0 and Minitab. Inc., MINITAB 16 were used for statistical analysis. GraphPad Software GraphPad Prism version 6.03 was used for graphical expression.

RESULTS

Among eight potential predictors (**Table 1**), three predictors were found to have statistically significant effects ($P < 0.05$) on the outcome value −ln(1 − fraction of protection) following the backwards stepwise selection: the log10 transformed total number of immunizing parasites ($P < 0.001$), the irradiation dose ($P < 0.001$), and the time between the last immunization and challenge ($P = 0.04$) (**Table 2**). The reported ranges of each predictor were the total number of immunizing parasites (50–5,000 cercariae), the irradiation dose (3–160 krad), and the time between the last immunization and challenge (7–230 days). All identified predictors were significant ($P < 0.05$) in the model no matter with or without outliers in the model. The number of immunizing parasites was significant in the model regardless of the version of this variable used, i.e., the average number of immunizing parasites per dose or total number of immunizing parasites. In both cases, the models were initially considered with the number of immunizations. When the total number of immunizing parasites was used as a predictor, the number of immunizations was not significant. Therefore, for the final model, the total number of immunizing parasite was used as a predictor with number of immunizations excluded from the model.

Table 2 | Results from random effects meta-regression models.

Predictors	Coefficient	SE	*P*-value
Number of immunizing parasites per dose (log10 transformed)	0.4338	0.0661	<0.001
Irradiation dose	−0.0047	0.0008	0.04
Time between the last immunization and challenge	−0.0015	0.0007	<0.001

Positive coefficients indicate the predictor's positive dose effect on fraction of protection whereas negative coefficients indicate predictor's negative influence on fraction of protection.

The interaction between log10 transformed total number of immunizing parasites and the time between the last immunization and challenge was statistically significant ($P = 0.04$). However, this interaction was excluded from the final model for the following reasons: (1) the model with the interaction showed biologically implausible fitted values of fraction of protection for some predictors, (2) the model with/without interaction showed similar fitted values for the fraction of protection around the most frequent values of predictors.

Fitted graphs for each predictor are shown in **Figure 1** with the outcome variable back-transformed to fraction of protection. Fitted graphs for each predictor were generated by fixing other predictor values at their modes: 500 immunizing parasites, 28 days for the time between the last immunization and challenge, and 20 krad for irradiation dose (solid line in **Figure 1**). The fitted graph of total number of immunizing parasites and fraction of protection showed the lowest level of predicted protection was 41% with 50 cercariae, which increased up to 75% with 5,000 cercariae (solid line in **Figure 1A**). Similarly, the minimum level of protection predicted for 160 krad irradiation was 26% protection, which increased to 65% with 3 krad irradiation (solid line in **Figure 1B**). The estimated level of protection 7 days after the last immunization was 63%, which reduced to 49% by 230 days after the last immunization (solid line in **Figure 1C**). Fitted graphs showed that the total number of immunizing parasites had a positive impact on the fraction of protection whereas irradiation dose and the time between the last immunization and challenge had negative impacts (**Figure 1**). Besides this, to estimate the highest protection, fitted graphs for each predictor were generated with other predictor values at their optimal level: 5,000 immunizing parasites, 7 days for the time between the last immunization and challenge, and 3 krad for irradiation dose (dashed line in **Figure 1**). The models suggested that highest achievable protection was 78% at 7 days after the last immunization, with the mouse immunized with 5,000 cercariae, which were attenuated with 3 krad (dashed

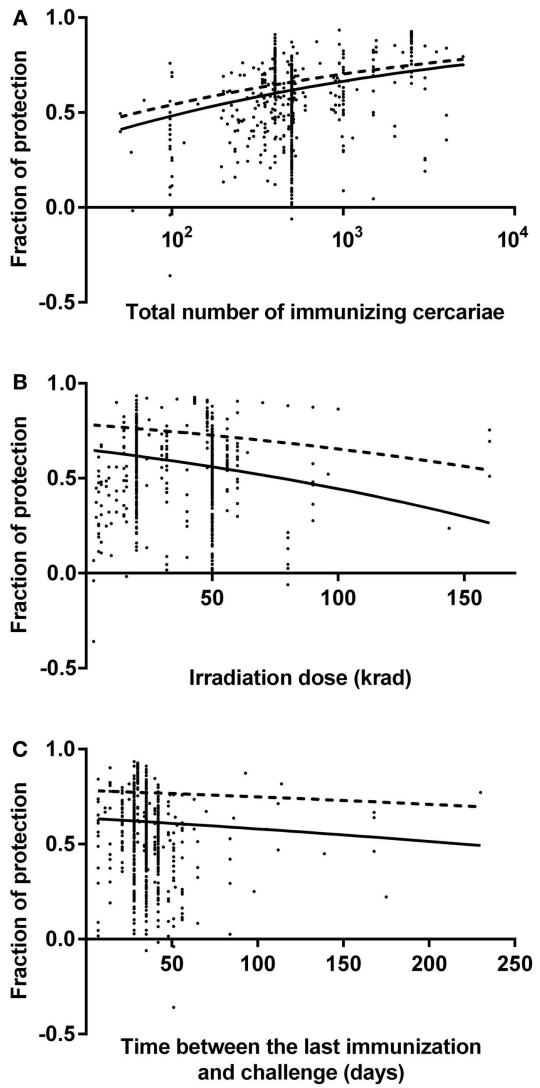

FIGURE 1 | Fitted graphs for identified predictors from a random effects meta-regression model. Identified predictors effects on fraction of protection in mouse model: **(A)** the number of immunizing cercariae over the range 50–5,000 cercariae, **(B)** the irradiation dose over the range 3–160 krad, **(C)** the time between the last immunization and challenge over the range 7–230 days. Data points indicate reported fraction of protection for each observation. Negative fractions indicate that vaccination was associated with increase of schistosome worm burden. Lines are fitted graphs generated from random effects meta-regression model (see text). Dashed lines in the graphs show the highest level of protection over range that could be achieved.

line in **Figure 1**). This 78% protection will decrease over time but would stay as high as 70% by 230 days after the last immunization (dashed line in **Figure 1C**).

DISCUSSION

Irradiated *S. mansoni* cercariae vaccines have been tested experimentally against schistosome infection for decades, with important insights obtained from the individual experiments (25). Although the translation of the irradiated parasites vaccine in humans has not been pursued for schistosomiasis, a precedent for this type of approach for human vaccination has been set by malaria vaccine, which uses live attenuated sporozoites (Sanaria® PfSPZ Vaccine) and has now completed phase 1/2a clinical trial (30). This study represents a meta-analysis of the experimental irradiated cercariae vaccine studies to identify robust variables that affect the levels of protection to inform human vaccine research and development.

The random effect meta-regression models identified three predictors of a reduction in worm burden: these were the total number of irradiated cercariae per immunization, the time between the last immunization and challenge, and the irradiation dose for parasite attenuation. We identified a positive correlation between the number of irradiated cercariae per immunization and the level of protection within the range of 50–5,000 cercariae used in the original studies. The models suggested that the optimally prepared irradiated cercariae vaccine could achieve a protection as high as 78% against challenge infection. As fitted graphs have shown, this is predicted for a single vaccination with 5,000 cercariae attenuated with 3 krad irradiation. This protection declines over time, but remains high for at least 8 months after the last immunization. Approximately 70% protection against challenge infection could be achieved after 8 months.

The number of immunizing cercariae represents the antigen dose, our results show a positive dose dependency of schistosome attenuated vaccine for higher protection. Studies of live attenuated vaccine for malaria infection also reported a similar positive correlation between the dose of immunizing parasites and the level of protection against future infection. Recently, as part of the phase 1 clinical trial of the human malaria vaccine using live attenuated sporozoites (Sanaria® PfSPZ Vaccine), a dose-escalation trial was conducted using 7,500–135,000 irradiated *Plasmodium falciparum* sporozoites per immunization. The participants group that received the highest dose per immunization achieved the highest levels of protection against challenge infection (31–33). Although the adequate number of immunizing schistosome parasites are needed to protect baboon hosts has not been well quantified yet, experimental studies have been conducted with up to 45,000 schistosome parasites and reported positive protections (34–36). These reports suggest that a large number of attenuated cercariae would be required for vaccination in humans. The intermediate host snails have been reported to shed a large number of cercariae that is approximately 3,600–6,000 cercariae per snail over the first 50 days of shedding (37). Schistosome infected snails and cercariae are commercially available from organizations such as the NIH-NIAID Schistosomiasis Resource Center (38) and Schistosomiasis Collection at the Museum at National History Museum, London for laboratory use (39). Clearly producing an adequate number of cercariae of a satisfactory quality to use in vaccinations is still highly challenging (18). Although we cannot directly translate animal vaccine study results into human use, their value is in highlighting the nature and development of vaccine induced protective immunity against schistosomiasis. For example, the dynamic relationships between vaccination dose and level of protection are informative for human studies, as has been alluded to by drug induced resistance against re-infection (40, 41). It is also worth mentioning that human

vaccination trials using infection or irradiated parasite vaccination have recently been conducted in human *P. falciparum* studies (42–44).

The result from the random effects meta-regression model showed a decrease in the fraction of protection with an increased time between the last immunization and challenge. This period between immunization and challenge represents the time to secondary encounter with the same antigen. When the initial encounter with the antigen takes place via infection or vaccination, the number of B and T cell produced against the antigen increases dramatically (45–48). Only a small fraction of those cells will survive as antigen-specific memory T and B cells or as long-lived plasma cells and they will be maintained for a long time (45–48). The duration of immune memory in humans after the vaccination is still controversial (49). However, there are several reports estimates for the length of immune memory after the last booster vaccination; 15 years for combined hepatitis A and B vaccine (50), 22 years for hepatitis B vaccine (51), over 30 years for poliovirus vaccine (49, 52), and over 60 years for small pox vaccine (49, 53). A longitudinal immuno-epidemiological study of schistosomiasis has been conducted by Butterworth et al., which reported that the protection induced by chemotherapy can remain for as long as 21 months after the treatment (54). However, other studies reported treated participants' re-infection within 1 year (12, 55). One of the difficulties in evaluating the length of protective immunity in humans is that, in contrast to experimental animals, humans encounter a variety of antigens that could stimulate their immune systems through their daily life. In addition, people infected and being treated for schistosomiasis normally live in schistosomiasis endemic areas. Regarding the influence of schistosome infection on vaccine efficacy, Kariuki et al. have shown that the protection levels induced by attenuated cercariae vaccination were high in baboon hosts in a group chronically infected and then treated after vaccination, as well as in a group that was infected and previously treated before vaccination (36). In addition, encounters with infectious cercariae by people in endemic areas may work as a "natural booster" to stimulate protective immunity. In our study, the times between the last immunization and challenge (7–230 days) were relatively short compared with the life span of humans and schistosome parasites. This reflects that the average lifespan of a mouse is much shorter than that of the schistosome parasite (56, 57). The decrease in the fraction of protection over time was captured with our models even within this relatively short time range. This result would suggest that boosting vaccines may be necessary for long lasting protection against schistosomes.

There are several different cercariae attenuation methods as we described in the introduction. Within these, ionizing radiation (X-ray and gamma ray) is the most commonly used attenuation method for attenuated schistosome cercariae preparation. Two relatively high irradiation doses around 20 or 50 krad have been reported as the optimal doses for parasite attenuation (58, 59) and, in fact, many past studies have applied these irradiation doses. However, our results suggest that a lower irradiation dose could improve protection. The lower irradiation doses enable attenuated parasites to live longer in the vaccinated host. After vaccination, irradiated cercariae have been reported to be present around the skin exposure site for approximately 4 days and then gradually moved to the lungs where they transformed from cercariae into lung-stage schistosomula (60). It has been reported that the immunizing parasite has to reach the lungs and transform to lung-stage schistosomula to elicit protective immunity against challenge infection (60, 61), which may not be the case for cercariae attenuated with high doses of ionizing radiation. Several studies have reported that non-attenuated challenge cercariae in vaccinated mice slowly move to the lungs and then gradually disappear (61, 62). Several studies report that cercariae exposed to extremely high irradiation doses will die in the host skin before they start to migrate inside the host body (60, 63). Mountford et al. reported that hosts needed to be exposed to both highly irradiated cercariae, which die in the host skin, and lung-stage schistosome parasites to elicit protective immunity (64). One of the possible reasons for the high levels of protection observed when using irradiated cercariae vaccine is that hosts are exposed to a wide variety of antigens, which are expressed by different parasite life stages. Parasites, which were attenuated with lower irradiation dose, can survive long enough to express a variety of antigens from different life stages (65). However, in practice, allowing parasites to live longer inside vaccinated people may not be well accepted or ethically approved. The results of our meta-analysis suggests that for recombinant vaccine development the combination of antigens, which are unique to the different schistosome life stages may be an important factor in achieving a better protection.

CONCLUSION

In this study, we identified three predictors for effective immunization against schistosome infection using attenuated cercariae: the total number of immunizing parasites, the irradiation dose, and the time between the last immunization and challenge. The study results suggested that the optimally prepared irradiated cercariae vaccine could achieve a protection as high as 78% against challenge infection. Within the reported dose range, the maximum protection could be achieved with the highest number of immunizing cercariae (5,000 cercariae) and the lowest irradiation dose (3 krad). This protection slowly declines but remains high for at least 8 months after the last immunization. This achievable protection is much higher than the partial protection reported by the *S. mansoni* purified antigens that failed to achieve consistent protection above 40% in mice (21, 66, 67). Although none of the radiation attenuated cercariae vaccine studies achieved complete protection against challenge infection, it is thought that partial protection induced by immunization can significantly reduce both schistosome related morbidity and parasite transmission (68, 69). This meta-analysis shows there is the high potential for an attenuated cercarial vaccine, while also providing insights helpful for schistosome vaccine development more generally.

AUTHOR CONTRIBUTIONS

The initial conception and design of the work: KM, CB, and FM. Performed the systematic review: MF, KM, and CB. Contributed to draft manuscript editing/reviewing: MF, KM, CB, MW, and FM. Statistical analyses of the data: MF, with inputs from MW, and FM. All authors contributed to the revisions and approved the final version of the manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at http://www.frontiersin.org/Journal/10.3389/fimmu.2015.00085/abstract

REFERENCES

1. King CH, Dangerfield-Cha M. The unacknowledged impact of chronic schistosomiasis. *Chronic Illn* (2008) **4**(1):65–79. doi:10.1177/1742395307084407
2. 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):733–6. doi:10.1016/S1473-3099(10)70099-2
3. King CH, Dickman K, Tisch DJ. Reassessment of the cost of chronic helmintic infection: a meta-analysis of disability-related outcomes in endemic schistosomiasis. *Lancet* (2005) **365**(9470):1561–9. doi:10.1016/S0140-6736(05)66457-4
4. World Health Organization. *Prevention and Control of Schistosomiasis and Soil-Transmitted Helminthiasis*. Geneve: World Health Organization (2002). Available from: http://whqlibdoc.who.int/trs/WHO_TRS_912.pdf
5. Centers for Disease Control and Prevention. Chapter 3: infectious diseases related to travel, schistosomiasis. *Traveler's Health* (2013). Available from: http://wwwnc.cdc.gov/travel/yellowbook/2012/chapter-3-infectious-diseases-related-to-travel/schistosomiasis.htm
6. Hotez PJ, Kamath A. Neglected tropical diseases in sub-Saharan Africa: review of their prevalence, distribution, and disease burden. *PLoS Negl Trop Dis* (2009) **3**(8):e412. doi:10.1371/journal.pntd.0000412
7. World Health Organization. *Schistosomiasis Fact Sheet No 115* (2014). Available from: http://www.who.int/mediacentre/factsheets/fs115/en/index.html
8. Evans D, McFarland D, Adamani W, Eigege A, Miri E, Schulz J, et al. Cost-effectiveness of triple drug administration (TDA) with praziquantel, ivermectin and albendazole for the prevention of neglected tropical diseases in Nigeria. *Ann Trop Med Parasitol* (2011) **105**(8):537–47. doi:10.1179/2047773211Y.0000000010
9. Midzi N, Sangweme D, Zinyowera S, Mapingure MP, Brouwer KC, Kumar N, et al. Efficacy and side effects of praziquantel treatment against *Schistosoma haematobium* infection among primary school children in Zimbabwe. *Trans R Soc Trop Med Hyg* (2008) **102**(8):759–66. doi:10.1016/j.trstmh.2008.03.010
10. Liu R, Dong HF, Guo Y, Zhao QP, Jiang MS. Efficacy of praziquantel and artemisinin derivatives for the treatment and prevention of human schistosomiasis: a systematic review and meta-analysis. *Parasit Vectors* (2011) **4**:201. doi:10.1186/1756-3305-4-201
11. Webster BL, Diaw OT, Seye MM, Faye DS, Stothard JR, Sousa-Figueiredo JC, et al. Praziquantel treatment of school children from single and mixed infection foci of intestinal and urogenital schistosomiasis along the Senegal River Basin: monitoring treatment success and re-infection patterns. *Acta Trop* (2013) **128**(2):292–302. doi:10.1016/j.actatropica.2012.09.010
12. Tukahebwa EM, Vennervald BJ, Nuwaha F, Kabatereine NB, Magnussen P. Comparative efficacy of one versus two doses of praziquantel on cure rate of *Schistosoma mansoni* infection and re-infection in Mayuge District, Uganda. *Trans R Soc Trop Med Hyg* (2013) **107**(6):397–404. doi:10.1093/trstmh/trt024
13. Leenstra T, Coutinho HM, Acosta LP, Langdon GC, Su L, Olveda RM, et al. *Schistosoma japonicum* reinfection after praziquantel treatment causes anemia associated with inflammation. *Infect Immun* (2006) **74**(11):6398–407. doi:10.1128/IAI.00757-06
14. Xiao SH, Mei JY, Jiao PY. The in vitro effect of mefloquine and praziquantel against juvenile and adult *Schistosoma japonicum*. *Parasitol Res* (2009) **106**(1):237–46. doi:10.1007/s00436-009-1656-x
15. Woolhouse ME, Hagan P. Seeking the ghost of worms past. *Nat Med* (1999) **5**(11):1225–7. doi:10.1038/15169
16. Mitchell KM, Mutapi F, Savill NJ, Woolhouse ME. Explaining observed infection and antibody age-profiles in populations with urogenital schistosomiasis. *PLoS Comput Biol* (2011) **7**(10):e1002237. doi:10.1371/journal.pcbi.1002237
17. McWilliam HE, Driguez P, Piedrafita D, McManus DP, Meeusen EN. Novel immunomic technologies for schistosome vaccine development. *Parasite Immunol* (2012) **34**(5):276–84. doi:10.1111/j.1365-3024.2011.01330.x
18. Mutapi F, Billingsley PF, Secor WE. Infection and treatment immunizations for successful parasite vaccines. *Trends Parasitol* (2013) **29**(3):135–41. doi:10.1016/j.pt.2013.01.003
19. Riveau G, Deplanque D, Remoue F, Schacht AM, Vodougnon H, Capron M, et al. Safety and immunogenicity of rSh28GST antigen in humans: phase 1 randomized clinical study of a vaccine candidate against urinary schistosomiasis. *PLoS Negl Trop Dis* (2012) **6**(7):e1704. doi:10.1371/journal.pntd.0001704
20. ClinicalTrials.gov. *Efficacy of Vaccine Sh28GST in Association with Praziquantel (PZQ) for Prevention of Clinical Recurrences of Schistosoma Haematobium Pathology (Bilhvax)* (2012). Available from: http://clinicaltrials.gov/ct2/show/study/NCT00870649
21. Mountford AP, Jenkins SJ. Vaccine development lessons from the study of initial immune responses to invading larvae. In: Colley DG, Secor WE, editors. *Schistosomiasis*. New York, NY: Springer (2005). p. 65–79.
22. Wilson RA, Coulson PS. Strategies for a schistosome vaccine: can we manipulate the immune response effectively? *Microbes Infect* (1999) **1**(7):535–43. doi:10.1016/S1286-4579(99)80093-8
23. Hewitson JP, Hamblin PA, Mountford AP. Immunity induced by the radiation-attenuated schistosome vaccine. *Parasite Immunol* (2005) **27**(7–8):271–80. doi:10.1111/j.1365-3024.2005.00764.x
24. Smithers SR. Immunizing effect of irradiated cercariae of *Schistosoma mansoni* in rhesus monkeys. *Nature* (1962) **194**:1146–7. doi:10.1038/1941146a0
25. Bickle QD. Radiation attenuated schistosome vaccination: a brief historical perspective. *Parasitology* (2009) **136**(12):1621–32. doi:10.1017/S0031182009005848
26. Dean DA. Schistosoma and related genera: acquired resistance in mice. *Exp Parasitol* (1983) **55**(1):1–104. doi:10.1016/0014-4894(83)90002-4
27. Vittinghoff E. *Regression Methods in Biostatistics: Linear, Logistic, Survival, and Repeated Measures Models*. 2nd ed. New York, NY: Springer (2012). p. 108–29.
28. Borenstein M, Hedges LV, Higgins JPT, Rothstein HR. *Introduction to Meta-Analysis*. Chichester: John Wiley & Sons (2009). p. xxviii, 421.
29. Little RJA, Rubin DB. *Statistical Analysis with Missing Data*. 2nd ed. Hoboken, NJ: Wiley (2002). p. 59–74.
30. Malaria Vaccine Initiative. *Fact Sheet, Sanaria PfSPZ Malaria Vaccine Candidate* (2010). Available from: http://www.malariavaccine.org/files/MVIfactsheet_Sanaria_110106_000.pdf
31. Seder RA, Chang LJ, Enama ME, Zephir KL, Sarwar UN, Gordon IJ, et al. Protection against malaria by intravenous immunization with a nonreplicating sporozoite vaccine. *Science* (2013) **341**(6152):1359–65. doi:10.1126/science.1241800
32. Epstein JE, Tewari K, Lyke KE, Sim BKL, Billingsley PF, Laurens MB, et al. Live attenuated malaria vaccine designed to protect through hepatic CD8(+) T cell immunity. *Science* (2011) **334**(6055):475–80. doi:10.1126/science.1211548
33. Epstein JE, Richie TL. The whole parasite, pre-erythrocytic stage approach to malaria vaccine development: a review. *Curr Opin Infect Dis* (2013) **26**(5):420–8. doi:10.1097/Qco.0000000000000002
34. Harrison RA, Bickle QD, Kiare S, James ER, Andrews BJ, Sturrock RF, et al. Immunization of baboons with attenuated schistosomula of *Schistosoma haematobium*: levels of protection induced by immunization with larvae irradiated with 20 and 60 krad. *Trans R Soc Trop Med Hyg* (1990) **84**(1):89–99. doi:10.1016/0035-9203(90)90393-S
35. Yole DS, Pemberton R, Reid GD, Wilson RA. Protective immunity to *Schistosoma mansoni* induced in the olive baboon *Papio anubis* by the irradiated cercaria vaccine. *Parasitology* (1996) **112**(Pt 1):37–46. doi:10.1017/S0031182000065057
36. Kariuki TM, Van Dam GJ, Deelder AM, Farah IO, Yole DS, Wilson RA, et al. Previous or ongoing schistosome infections do not compromise the efficacy of the attenuated cercaria vaccine. *Infect Immun* (2006) **74**(7):3979–86. doi:10.1128/IAI.01657-05
37. Chu KY, Sabbaghian H, Massoud J. Host-parasite relationship of *Bulinus truncatus* and *Schistosoma haematobium* in Iran. 2. Effect of exposure dosage of miracidia on the biology of the snail host and the development of the parasites. *Bull World Health Organ* (1966) **34**(1):121–30.

38. Lewis FA, Liang YS, Raghavan N, Knight M. The NIH-NIAID schistosomiasis resource center. *PLoS Negl Trop Dis* (2008) **2**(7):e267. doi:10.1371/journal.pntd.0000267
39. The Natural History Museum. *Schistosomiasis Collection at the Natural History Museum* (2013). Available from: http://www.nhm.ac.uk/research-curation/collections/curation-groups/scan/index.html
40. Mutapi F, Burchmore R, Mduluza T, Foucher A, Harcus Y, Nicoll G, et al. Praziquantel treatment of individuals exposed to *Schistosoma haematobium* enhances serological recognition of defined parasite antigens. *J Infect Dis* (2005) **192**(6):1108–18. doi:10.1086/432553
41. Bourke CD, Nausch N, Rujeni N, Appleby LJ, Mitchell KM, Midzi N, et al. Integrated analysis of innate, Th1, Th2, Th17, and regulatory cytokines identifies changes in immune polarisation following treatment of human schistosomiasis. *J Infect Dis* (2013) **208**(1):159–69. doi:10.1093/infdis/jis524
42. Roestenberg M, McCall M, Hopman J, Wiersma J, Luty AJ, van Gemert GJ, et al. Protection against a malaria challenge by sporozoite inoculation. *N Engl J Med* (2009) **361**(5):468–77. doi:10.1056/NEJMoa0805832
43. Roestenberg M, Teirlinck AC, McCall MB, Teelen K, Makamdop KN, Wiersma J, et al. Long-term protection against malaria after experimental sporozoite inoculation: an open-label follow-up study. *Lancet* (2011) **377**(9779):1770–6. doi:10.1016/S0140-6736(11)60360-7
44. Good MF. The ability to inoculate purified malaria sporozoites will accelerate vaccine and drug discovery. *Am J Trop Med Hyg* (2014) **91**(3):437–8. doi:10.4269/ajtmh.14-0395
45. Sallusto F, Lanzavecchia A, Araki K, Ahmed R. From vaccines to memory and back. *Immunity* (2010) **33**(4):451–63. doi:10.1016/j.immuni.2010.10.008
46. Harty JT, Badovinac VP. Shaping and reshaping CD8+ T-cell memory. *Nat Rev Immunol* (2008) **8**(2):107–19. doi:10.1038/nri2251
47. Kaech SM, Wherry EJ. Heterogeneity and cell-fate decisions in effector and memory CD8+ T cell differentiation during viral infection. *Immunity* (2007) **27**(3):393–405. doi:10.1016/j.immuni.2007.08.007
48. Farber DL, Yudanin NA, Restifo NP. Human memory T cells: generation, compartmentalization and homeostasis. *Nat Rev Immunol* (2014) **14**(1):24–35. doi:10.1038/nri3567
49. Crotty S, Ahmed R. Immunological memory in humans. *Semin Immunol* (2004) **16**(3):197–203. doi:10.1016/j.smim.2004.02.008
50. Van Damme P, Leroux-Roels G, Crasta P, Messier M, Jacquet JM, Van Herck K. Antibody persistence and immune memory in adults, 15 years after a three-dose schedule of a combined hepatitis A and B vaccine. *J Med Virol* (2012) **84**(1):11–7. doi:10.1002/jmv.22264
51. McMahon BJ, Dentinger CM, Bruden D, Zanis C, Peters H, Hurlburt D, et al. Antibody levels and protection after hepatitis B vaccine: results of a 22-year follow-up study and response to a booster dose. *J Infect Dis* (2009) **200**(9):1390–6. doi:10.1086/606119
52. Bottiger M, Gustavsson O, Svensson A. Immunity to tetanus, diphtheria and poliomyelitis in the adult population of Sweden in 1991. *Int J Epidemiol* (1998) **27**(5):916–25. doi:10.1093/ije/27.5.916
53. Crotty S, Felgner P, Davies H, Glidewell J, Villarreal L, Ahmed R. Cutting edge: long-term B cell memory in humans after smallpox vaccination. *J Immunol* (2003) **171**(10):4969–73. doi:10.4049/jimmunol.171.10.4969
54. Butterworth AE, Capron M, Cordingley JS, Dalton PR, Dunne DW, Kariuki HC, et al. Immunity after treatment of human *Schistosomiasis mansoni*. II. Identification of resistant individuals, and analysis of their immune responses. *Trans R Soc Trop Med Hyg* (1985) **79**(3):393–408. doi:10.1016/0035-9203(85)90391-8
55. Garba A, Lamine MS, Barkire N, Djibo A, Sofo B, Gouvras AN, et al. Efficacy and safety of two closely spaced doses of praziquantel against *Schistosoma haematobium* and *S. mansoni* and re-infection patterns in school-aged children in Niger. *Acta Trop* (2013) **128**(2):334–44. doi:10.1016/j.actatropica.2012.08.008
56. Kohn RR. Effect of antioxidants on life-span of C57bl mice. *J Gerontol* (1971) **26**(3):378–80. doi:10.1093/geronj/26.3.378
57. Fulford AJC, Butterworth AE, Ouma JH, Sturrock RF. A statistical approach to schistosome population-dynamics and estimation of the life-span of *Schistosoma mansoni* in man. *Parasitology* (1995) **110**:307–16. doi:10.1017/S0031182000080896
58. Minard P, Dean DA, Vannier WE, Murrell KD. Effect of immunization on migration of *Schistosoma mansoni* through lungs. *Am J Trop Med Hyg* (1978) **27**(1 Pt 1):87–93.
59. Bickle QD, Taylor MG, Doenhoff MJ, Nelson GS. Immunization of mice with gamma-irradiated intramuscularly injected schistosomula of *Schistosoma mansoni*. *Parasitology* (1979) **79**(2):209–22. doi:10.1017/S0031182000053300
60. Mangold BL, Dean DA. The migration and survival of gamma-irradiated *Schistosoma mansoni* larvae and the duration of host-parasite contact in relation to the induction of resistance in mice. *Parasitology* (1984) **88**(Pt 2):249–66.
61. Dean DA, Mangold BL. Evidence that both normal and immune elimination of *Schistosoma mansoni* take place at the lung stage of migration prior to parasite death. *Am J Trop Med Hyg* (1992) **47**(2):238–48.
62. Wilson RA, Coulson PS, Dixon B. Migration of the schistosomula of *Schistosoma mansoni* in mice vaccinated with radiation-attenuated cercariae, and normal mice – an attempt to identify the timing and site of parasite death. *Parasitology* (1986) **92**:101–16. doi:10.1017/S0031182000063484
63. Hsu SY, Hsu HF, Burmeister LF. *Schistosoma mansoni*: vaccination of mice with highly X-irradiated cercariae. *Exp Parasitol* (1981) **52**(1):91–104. doi:10.1016/0014-4894(81)90065-5
64. Mountford AP, Coulson PS, Pemberton RM, Smythies LE, Wilson RA. The generation of interferon-gamma-producing T lymphocytes in skin-draining lymph nodes, and their recruitment to the lungs, is associated with protective immunity to *Schistosoma mansoni*. *Immunology* (1992) **75**(2):250–6.
65. Curwen RS, Ashton PD, Johnston DA, Wilson RA. The *Schistosoma mansoni* soluble proteome: a comparison across four life-cycle stages. *Mol Biochem Parasitol* (2004) **138**(1):57–66. doi:10.1016/j.molbiopara.2004.06.016
66. Bergquist NR, Colley DG. Schistosomiasis vaccine:research to development. *Parasitol Today* (1998) **14**(3):99–104. doi:10.1016/S0169-4758(97)01207-6
67. Wilson RA, Coulson PS. The development of a schistosome vaccine. In: Maule AG, Marks NJ, editors. *Parasitic Flatworms Molecular Biology, Biochemistry, Immunology and Physiology*. Cambridge: CABI Pub (2006). p. 303–26.
68. Burke ML, Jones MK, Gobert GN, Li YS, Ellis MK, McManus DP. Immunopathogenesis of human schistosomiasis. *Parasite Immunol* (2009) **31**(4):163–76. doi:10.1111/j.1365-3024.2009.01098.x
69. Mitchell KM, Mutapi F, Savill NJ, Woolhouse MEJ. Protective immunity to *Schistosoma haematobium* infection is primarily an anti-fecundity response stimulated by the death of adult worms. *Proc Natl Acad Sci U S A* (2012) **109**(33):13347–52. doi:10.1073/pnas.1121051109

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References

Abd El-Aal, A.A., El-Arousy, M.H., El-Gendy, A.M., Tunkul Ael, K., Ismail, S.A. and El-Badry, A.A. (2005). Early post-treatment immunoglobulin profile in human schistosomiasis. *J Egypt Soc Parasitol* 35(1): 167-180.

Abdul-Ghani, R.A. and Hassan, A.A. (2010). Murine schistosomiasis as a model for human schistosomiasis mansoni: similarities and discrepancies. *Parasitol Res* 107(1): 1-8.

Abebe, F., Gaarder, P.I., Petros, B. and Gundersen, S.G. (2001). Age- and sex-related differences in antibody responses against *Schistosoma mansoni* soluble egg antigen in a cohort of school children in Ethiopia. *Apmis* 109(12): 816-824.

Alemu, A., Atnafu, A., Addis, Z., Shiferaw, Y., Teklu, T., Mathewos, B., Birhan, W., Gebretsadik, S. and Gelaw, B. (2011). Soil transmitted helminths and *Schistosoma mansoni* infections among school children in zarima town, northwest Ethiopia. *BMC Infect Dis* 11: 189.

Altman, D.G., Egger, M. and Smith, G.D. (2001). Systematic reviews in health care meta-analysis in context. London, UK, Wiley InterScience.

Andrade, Z.A. (2009). Schistosomiasis and liver fibrosis. *Parasite Immunol* 31(11): 656-663.

Aryeetey, M.E., Aholu, C., Wagatsuma, Y., Bentil, G., Nkrumah, F.K. and Kojima, S. (1999). Health education and community participation in the control of urinary schistosomiasis in Ghana. *East Afr Med J* 76(6): 324-329.

Asaolu, S.O. and Ofoezie, I.E. (2003). The role of health education and sanitation in the control of helminth infections. *Acta Trop* 86(2-3): 283-294.

Bax, L. and Moons, K.G. (2011). Beyond publication bias. *J Clin Epidemiol* 64(5): 459-462.

Ben-Ami, F. and Heller, J. (2001). Biological control of aquatic pest snails by the black carp *Mylopharyngodon piceus*. *BioControl* 22(2): 131-138.

Bergquist, N.R. and Colley, D.G. (1998). Schistosomiasis vaccine:research to development. *Parasitol Today* 14(3): 99-104.

Berhe, N., Gundersen, S.G., Abebe, F., Birrie, H., Medhin, G. and 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 Trop* 72(1): 53-63.

Bickle, Q.D. (2009). Radiation attenuated schistosome vaccination: a brief historical perspective.

Parasitology 136(12): 1621-1632.

Bickle, Q.D., Andrews, B.J., Doenhoff, M.J., Ford, M.J. and Taylor, M.G. (1985). Resistance against *Schistosoma mansoni* induced by highly irradiated infections: studies on species specificity of immunization and attempts to transfer resistance. *Parasitology* 90(2): 301-312.

Bickle, Q.D., Bogh, H.O., Johansen, M.V. and Zhang, Y.B. (2001). Comparison of the vaccine efficacy of gamma-irradiated *Schistosoma japonicum* cercariae with the defined antigen Sj62(IrV-5) in pigs. *Vet Parasitol* 100(1-2): 51-62.

Bickle, Q.D., Dobinson, T. and James, E.R. (1979). The effects of gamma-irradiation on migration and survival of *Schistosoma mansoni* schistosomula in mice. *Parasitology* 79(2): 223-230.

Bickle, Q.D., Taylor, M.G., Doenhoff, M.J. and Nelson, G.S. (1979). Immunization of mice with gamma-irradiated intramuscularly injected schistosomula of *Schistosoma mansoni*. *Parasitology* 79(2): 209-222.

Biolchini Cde, L., Neves, R.H., Hulstijn, M., Gomes, D.C. and Machado-Silva, J.R. (2006). Development of *Schistosoma mansoni* worms in mice analyzed by bright field and confocal microscopy. *Mem Inst Oswaldo Cruz* 101 Suppl 1: 261-265.

Black, C.L., Steinauer, M.L., Mwinzi, P.N.M., Secor, W.E., Karanja, D.M.S. and 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. *Trop Med Int Health* 14(4): 450-457.

Boctor, F.N. and Peter, J.B. (1990). IgG subclasses in human chronic schistosomiasis: over-production of schistosome-specific and non-specific IgG4. *Clin Exp Immunol* 82(3): 574-578.

Borenstein, M. (2009). Factors that affect precision. Introduction to meta-analysis. Chichester, U.K., John Wiley & Sons: 51-55.

Borenstein, M. (2009). Fixed-effect versus random-effects models. Introduction to meta-analysis. Chichester, U.K., John Wiley & Sons: 61-102.

Borenstein, M. (2009). Introduction to meta-analysis. Chichester, U.K., John Wiley & Sons.

Botros, S., Sayed, H., Amer, N., El-Ghannam, M., Bennett, J.L. and Day, T.A. (2005). Current status of sensitivity to praziquantel in a focus of potential drug resistance in Egypt. *Int J Parasitol* 35(7): 787-791.

Bottiger, M., Gustavsson, O. and Svensson, A. (1998). Immunity to tetanus, diphtheria and poliomyelitis in the adult population of Sweden in 1991. *Int J Epidemiol* 27(5): 916-925.

Brannick, M.T., Yang, L.Q. and Cafri, G. (2011). Comparison of Weights for Meta-Analysis of r and d Under Realistic Conditions. *Organizational Research Methods* 14(4): 587-607.

Breiman, L. (1984). Classification and regression trees. Belmont,Calif, Wadsworth International.

Burchard, G.D., Kern, P., Baltes, R. and Dietrich, M. (1984). Comparative trial of oltipraz versus praziquantel in the treatment of urinary schistosomiasis in the Gabon. *Tropenmed Parasitol* 35(2): 91-94.

Burke, M.L., Jones, M.K., Gobert, G.N., Li, Y.S., Ellis, M.K. and McManus, D.P. (2009). Immunopathogenesis of human schistosomiasis. *Parasite Immunol* 31(4): 163-176.

Butterworth, A.E., Benstedsmith, R., Capron, A., Capron, M., Dalton, P.R., Dunne, D.W., Grzych, J.M., Kariuki, H.C., Khalife, J., Koech, D., Mugambi, M., Ouma, J.H., Siongok, T.K.A. and Sturrock, R.F. (1987). Immunity in human Schistosomiasis *mansoni*: prevention by blocking antibodies of the expression of immunity in young children. *Parasitology* 94(2): 281-300.

Butterworth, A.E., Capron, M., Cordingley, J.S., Dalton, P.R., Dunne, D.W., Kariuki, H.C., Kimani, G., Koech, D., Mugambi, M., Ouma, J.H. and et al. (1985). Immunity after treatment of human Schistosomiasis *mansoni*. II. Identification of resistant individuals, and analysis of their immune responses. *Trans R Soc Trop Med Hyg* 79(3): 393-408.

Caldas, I.R., Correa-Oliveira, R., Colosimo, E., Carvalho, O.S., Massara, C.L., Colley, D.G. and Gazzinelli, G. (2000). Susceptibility and resistance to *Schistosoma mansoni* reinfection: parallel cellular and isotypic immunologic assessment. *Am J Trop Med Hyg* 62(1): 57-64.

Canales, M., Enriquez, A., Ramos, E., Cabrera, D., Dandie, H., Soto, A., Falcon, V., Rodriguez, M. and delaFuente, J. (1997). Large-scale production in *Pichia pastoris* of the recombinant vaccine Gavac(TM) against cattle tick. *Vaccine* 15(4): 414-422.

CDC (2016). Schistosomiasis. New York, Oxford University Press.

Cesari, I.M., Ballen, D.E., Mendoza, L., Ferrer, A., Pointier, J.P., Kombila, M., Richard-Lenoble, D. and Theron, A. (2010). Immunoblot analysis of membrane antigens of *Schistosoma mansoni*, *Schistosoma intercalatum*, and *Schistosoma haematobium* against Schistosoma-infected patient sera. *Parasitol Res* 106(5): 1225-1231.

Chan, M.S., Mutapi, F., Woolhouse, M.E.J. and Isham, V.S. (2000). Stochastic simulation and the detection of immunity to schistosome infections. *Parasitology* 120: 161-169.

Chandiwana, S.K. and Woolhouse, M.E. (1991). Heterogeneities in water contact patterns and the epidemiology of *Schistosoma haematobium*. *Parasitology* 103(3): 363-370.

Cheever, A.W. (1969). Quantitative comparison of the intensity of *Schistosoma mansoni* infections in man and experimental animals. *Trans R Soc Trop Med Hyg* 63(6): 781-795.

Cheever, A.W., Macedonia, J.G., Mosimann, J.E. and Cheever, E.A. (1994). Kinetics of egg production and egg excretion by *Schistosoma mansoni* and *S. japonicum* in mice infected

with a single pair of worms. *Am J Trop Med Hyg* 50(3): 281-295.

Cheever, A.W., Mosimann, J.E., Deb, S., Cheever, E.A. and Duvall, R.H. (1994). Natural history of *Schistosoma mansoni* infection in mice: egg production, egg passage in the feces, and contribution of host and parasite death to changes in worm numbers. *Am J Trop Med Hyg* 50(3): 269-280.

Chiappelli, F. (2010). Evidence-based practice toward optimizing clinical outcomes. Berlin ; London, Springer,.

Christie, J.D., Crouse, D., Smith, J.H., Pineda, J., Ishak, E.A. and Kamel, I.A. (1986). Patterns of *Schistosoma haematobium* egg distribution in the human lower urinary tract. II. Obstructive uropathy. *Am J Trop Med Hyg* 35(4): 752-758.

Cleland, C.R., Tukahebwa, E.M., Fenwick, A. and Blair, L. (2014). Mass drug administration with praziquantel reduces the prevalence of *Schistosoma mansoni* and improves liver morbidity in untreated preschool children. *Trans R Soc Trop Med Hyg* 108(9): 575-581.

Clennon, J.A., Mungai, P.L., Muchiri, E.M., King, C.H. and Kitron, U. (2006). Spatial and temporal variations in local transmission of *Schistosoma haematobium* in Msambweni, Kenya. *Am J Trop Med Hyg* 75(6): 1034-1041.

Cline, B.L., Rymzo, W.T., Hiatt, R.A., Knight, W.B. and Berrios-Duran, L.A. (1977). Morbidity from *Schistosoma mansoni* in a Puerto Rican community: a population-based study. *Am J Trop Med Hyg* 26(1): 109-117.

ClinicalTrials.gov. (2012) Efficacy of vaccine Sh28GST in association with praziquantel (PZQ) for prevention of clinical recurrences of *Schistosoma haematobium* pathology (Bilhvax). Retrieved Aug 18, 2014, from http://clinicaltrials.gov/ct2/show/study/NCT00870649.

clinicaltrials.gov. (2012) Efficacy of vaccine Sh28GST in association with praziquantel (PZQ) for prevention of clinical recurrences of *Schistosoma haematobium* pathology (Bilhvax). from http://clinicaltrials.gov/ct2/show/study/NCT00870649.

Colley, D.G., Bustinduy, A.L., Secor, W.E. and King, C.H. (2014). Human schistosomiasis. *Lancet* 383(9936): 2253-2264.

Colley, D.G. and Secor, W.E. (2014). Immunology of human schistosomiasis. *Parasite Immunol* 36(8): 347-357.

Coulibaly, J.T., N'Gbesso Y, K., Knopp, S., Keiser, J., N'Goran, E.K. and Utzinger, J. (2012). Efficacy and safety of praziquantel in preschool-aged children in an area co-endemic for *Schistosoma mansoni* and *S. haematobium*. *PLoS Negl Trop Dis* 6(12): e1917.

Crotty, S. and Ahmed, R. (2004). Immunological memory in humans. *Semin Immunol* 16(3): 197-203.

Crotty, S., Felgner, P., Davies, H., Glidewell, J., Villarreal, L. and Ahmed, R. (2003). Cutting edge: long-term B cell memory in humans after smallpox vaccination. *J Immunol* 171(10): 4969-4973.

Curwen, R.S., Ashton, P.D., Johnston, D.A. and Wilson, R.A. (2004). The *Schistosoma mansoni* soluble proteome: a comparison across four life-cycle stages. *Mol Biochem Parasitol* 138(1): 57-66.

Dai, J.R., Wang, W., Liang, Y.S., Li, H.J., Guan, X.H. and Zhu, Y.C. (2008). A novel molluscicidal formulation of niclosamide. *Parasitol Res* 103(2): 405-412.

Danso-Appiah, A., Olliaro, P.L., Donegan, S., Sinclair, D. and Utzinger, J. (2013). Drugs for treating *Schistosoma mansoni* infection. *Cochrane Database Syst Rev* 28(2): CD000528.

de Veer, M. and Meeusen, E. (2011). New developments in vaccine research - unveiling the secret of vaccine adjuvants. *Discov Med* 64: 195-204.

Dean, D.A. (1983). *Schistosoma* and related genera: acquired resistance in mice. *Exp Parasitol* 55(1): 1-104.

Dean, D.A. and Mangold, B.L. (1992). Evidence that both normal and immune elimination of *Schistosoma mansoni* take place at the lung stage of migration prior to parasite death. *Am J Trop Med Hyg* 47(2): 238-248.

Deeks, J.J. and Higgins, J.P. (2010). Statistical algorithms in Review Manager 5. Statistical Methods Group of The Cochrane Collaboration.

Degarege, A., Legesse, M., Medhin, G., Teklehaymanot, T. and Erko, B. (2014). Day-to-day fluctuation of point-of-care circulating cathodic antigen test scores and faecal egg counts in children infected with *Schistosoma mansoni* in Ethiopia. *BMC Infect Dis* 14: 210.

Demeure, C.E., Rihet, P., Abel, L., Ouattara, M., Bourgois, A. and Dessein, A.J. (1993). Resistance to *Schistosoma mansoni* in humans - influence of the IgE/IgG4 balance and IgG2 in immunity to reinfection after chemotherapy. *J Infect Dis* 168(4): 1000-1008.

Doenhoff, M.J., Cioli, D. and Utzinger, J. (2008). Praziquantel: mechanisms of action, resistance and new derivatives for schistosomiasis. *Curr Opin Infect Dis* 21(6): 659-667.

Doenhoff, M.J., Sabah, A.A., Fletcher, C., Webbe, G. and Bain, J. (1987). Evidence for an immune-dependent action of praziquantel on *Schistosoma mansoni* in mice. *Trans R Soc Trop Med Hyg* 81(6): 947-951.

Dunne, D.W., Butterworth, A.E., Fulford, A.J., Kariuki, H.C., Langley, J.G., Ouma, J.H., Capron, A., Pierce, R.J. and Sturrock, R.F. (1992). Immunity after treatment of human schistosomiasis: association between IgE antibodies to adult worm antigens and resistance to reinfection. *Eur*

J Immunol 22(6): 1483-1494.

Dusseldorp, E., van Genugten, L., van Buuren, S., Verheijden, M.W. and van Empelen, P. (2014). Combinations of techniques that effectively change health behavior: Evidence from Meta-CART analysis. *Health Psychol* 33(12): 1530-1540.

Duval, S. and Tweedie, R. (2000). Trim and fill: A simple funnel-plot-based method of testing and adjusting for publication bias in meta-analysis. *Biometrics* 56(2): 455-463.

El Ridi, R. and Tallima, H. (2015). Why the radiation-attenuated cercarial immunization studies failed to guide the road for an effective schistosomiasis vaccine: A review. *J Adv Res* 6(3): 255-267.

Epstein, J.E. and Richie, T.L. (2013). The whole parasite, pre-erythrocytic stage approach to malaria vaccine development: a review. *Curr Opin Infect Dis* 26(5): 420-428.

Epstein, J.E., Tewari, K., Lyke, K.E., Sim, B.K.L., Billingsley, P.F., Laurens, M.B., Gunasekera, A., Chakravarty, S., James, E.R., Sedegah, M., Richman, A., Velmurugan, S., Reyes, S., Li, M., Tucker, K., Ahumada, A., Ruben, A.J., Li, T., Stafford, R., Eappen, A.G., Tamminga, C., Bennett, J.W., Ockenhouse, C.F., Murphy, J.R., Komisar, J., Thomas, N., Loyevsky, M., Birkett, A., Plowe, C.V., Loucq, C., Edelman, R., Richie, T.L., Seder, R.A. and Hoffman, S.L. (2011). Live attenuated malaria vaccine designed to protect through hepatic CD8(+) T cell immunity. *Science* 334(6055): 475-480.

Evans, D., McFarland, D., Adamani, W., Eigege, A., Miri, E., Schulz, J., Pede, E., Umbugadu, C., Ogbu-Pearse, P. and Richards, F.O. (2011). Cost-effectiveness of triple drug administration (TDA) with praziquantel, ivermectin and albendazole for the prevention of neglected tropical diseases in Nigeria. *Ann Trop Med Parasitol* 105(8): 537-547.

Fallon, P.G., Cooper, R.O., Probert, A.J. and Doenhoff, M.J. (1992). Immune-dependent chemotherapy of schistosomiasis. *Parasitology* 105(Suppl): S41-48.

Farber, D.L., Yudanin, N.A. and Restifo, N.P. (2014). Human memory T cells: generation, compartmentalization and homeostasis. *Nat Rev Immunol* 14(1): 24-35.

Farid, L., el-Masry, N.A., Bassily, S., Trabolsi, B. and Wallace, C.K. (1984). Treatment of bilharzial obstructive uropathy with praziquantel. *J Infect Dis* 150(2): 307-308.

Feldmeier, H., Stevens, W.J., Bridts, C.H., Daffalla, A.A. and Buttner, D.W. (1983). Effect of chemotherapy and reinfection on IgE containing and IgG-containing circulating immune complexes, serum IgE and IgE antibodies in patients chronically infected with *Schistosoma mansoni* and *Schistosoma haematobium*. *Int Arch Allergy Appl Immunol* 72(3): 211-218.

Fenwick, A. (1969). Baboons as reservoir hosts of *Schistosoma mansoni*. *Trans R Soc Trop Med Hyg* 63(5): 557-567.

Fenwick, A. (2015). Praziquantel: do we need another antischistosoma treatment? *Future Med Chem* 7(6): 677-680.

Ford, M.J., Bickle, Q.D. and Taylor, M.G. (1984). Immunization of rats against *Schistosoma mansoni* using irradiated cercariae, lung schistosomula and liver-stage worms. *Parasitology* 89(Oct): 327-344.

Ford, M.J., Bickle, Q.D., Taylor, M.G. and Andrews, B.J. (1984). Passive transfer of resistance and the site of immune-dependent elimination of the challenge infection in rats vaccinated with highly irradiated cercariae of *Schistosoma mansoni*. *Parasitology* 89(Dec): 461-482.

Fujian, S., Lee, H. and Yoon, K.L. (2015). Publication bias: what is it? How do we measure it? How do we avoid it? *Open Access J Clin Trials* 5: 71-81.

Fukushige, M., Mitchell, K.M., Bourke, C.D., Woolhouse, M.E. and Mutapi, F. (2015). A meta-analysis of experimental studies of attenuated *Schistosoma mansoni* vaccines in the mouse model. *Front Immunol* 6: 85.

Fulford, A.J.C., Butterworth, A.E., Ouma, J.H. and Sturrock, R.F. (1995). A statistical approach to schistosome population-dynamics and estimation of the life-span of *Schistosoma mansoni* in man. *Parasitology* 110(3): 307-316.

Fulford, A.J.C., Butterworth, A.E., Sturrock, R.F. and Ouma, J.H. (1992). On the use of age-intensity data to detect immunity to parasitic infections, with special reference to *Schistosoma mansoni* in Kenya. *Parasitology* 105(2): 219-227.

Garba, A., Barkiré, N., Djibo, A., Lamine, M.S., Sofo, B., Gouvras, A.N., Bosqué-Oliva, E., Webster, J.P., Stothard, J.R., Utzinger, J. and 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(3): 212-219.

Garba, A., Lamine, M.S., Barkire, N., Djibo, A., Sofo, B., Gouvras, A.N., Labbo, R., Sebangou, H., Webster, J.P., Fenwick, A. and Utzinger, J. (2013). Efficacy and safety of two closely spaced doses of praziquantel against *Schistosoma haematobium* and *S. mansoni* and re-infection patterns in school-aged children in Niger. *Acta Trop* 128(2): 334-344.

Garba, A., Tohon, Z., Sidiki, A., Chippaux, J.P. and de Chabalier, F. (2001). Efficacity of praziquantel in school-aged children in a hyperendemic zone for *Schistoma haematobium* (Niger, 1999). *Bull Soc Pathol Exot* 94(1): 42-45.

Gomes, Y.M., Pereira, V.R.A., Nakazawa, M., Montarroyos, U., Souza, W.V. and Abath, F.G.C. (2002). Antibody isotype responses to egg antigens in human chronic Schistosomiasis *mansoni* before and after treatment. *Mem Inst Oswaldo Cruz* 97: 111-112.

Gönnert, R. and Andrews, P. (1977). Praziquantel, a new board-spectrum antischistosomal agent. *Z Parasitenkd* 52(2): 129-150.

Gouvras, A.N., Kariuki, C., Koukounari, A., Norton, A.J., Lange, C.N., Ireri, E., Fenwick, A., Mkoji, G.M. and Webster, J.P. (2013). The impact of single versus mixed *Schistosoma haematobium* and *S. mansoni* infections on morbidity profiles amongst school-children in Taveta, Kenya. *Acta Trop.* 128(2): 309-317.

Gower, C.M., Gouvras, A.N., Lamberton, P.H., Deol, A., Shrivastava, J., Mutombo, P.N., Mbuh, J.V., Norton, A.J., Webster, B.L., Stothard, J.R., Garba, A., Lamine, M.S., Kariuki, C., Lange, C.N., Mkoji, G.M., Kabatereine, N.B., Gabrielli, A.F., Rudge, J.W., Fenwick, A., Sacko, M., Dembele, R., Lwambo, N.J., Tchuem Tchuente, L.A., Rollinson, D. and Webster, J.P. (2013). Population genetic structure of *Schistosoma mansoni* and *Schistosoma haematobium* from across six sub-Saharan African countries: implications for epidemiology, evolution and control. *Acta Trop* 128(2): 261-274.

Gray, D.J., McManus, D.P., Li, Y., Williams, G.M., Bergquist, R. and Ross, A.G. (2010). Schistosomiasis elimination: lessons from the past guide the future. *Lancet Infect Dis* 10(10): 733-736.

Gray, D.J., Williams, G.M., Li, Y., Chen, H., Forsyth, S.J., Li, R.S., Barnett, A.G., Guo, J., Ross, A.G., Feng, Z. and McManus, D.P. (2009). A cluster-randomised intervention trial against *Schistosoma japonicum* in the Peoples' Republic of China: bovine and human transmission. *PLoS One* 4(6): e5900.

Grogan, J.L., Kremsner, P.G., van Dam, G.J., Deelder, A.M. and Yazdanbakhsh, M. (1997). Anti-schistosome IgG4 and IgE at 2 years after chemotherapy: infected versus uninfected individuals. *J Infect Dis* 176(5): 1344-1350.

Grogan, J.L., Kremsner, P.G., van Dam, G.J., Metzger, W., Mordmuller, B., Deelder, A.M. and Yazdanbakhsh, M. (1996). Antischistosome IgG4 and IgE responses are affected differentially by chemotherapy in children versus adults. *J Infect Dis* 173(5): 1242-1247.

Groning, E., Bakathir, H., Salem, A., Albert, L. and Fernandez, R. (1985). Effectiveness and tolerance of praziquantel in schistosomiasis. *Rev Cubana Med Trop* 37(2): 215-219.

Gryseels, B., Nkulikyinka, L. and Coosemans, M.H. (1987). Field trials of praziquantel and oxamniquine for the treatment of Schistosomiasis *mansoni* in Burundi. *Trans R Soc Trop Med Hyg* 81(4): 641-644.

Gryseels, B., Polman, K., Clerinx, J. and Kestens, L. (2006). Human schistosomiasis. *Lancet* 368(9541): 1106-1118.

Grzych, J.M., Grezel, D., Xu, C.B., Neyrinck, J.L., Capron, M., Ouma, J.H., Butterworth, A.E. and Capron, A. (1993). IgA antibodies to a protective antigen in human Schistosomiasis *mansoni*. *J Immunol* 150(2): 527-535.

Guidi, A., Andolina, C., Makame Ame, S., Albonico, M., Cioli, D. and Juma Haji, H. (2010). Praziquantel efficacy and long-term appraisal of schistosomiasis control in Pemba Island. *Trop Med Int Health* 15(5): 614-618.

Guisse, F., Polman, K., Stelma, F.F., Mbaye, A., Talla, I., Niang, M., Deelder, A.M., Ndir, O. and Gryseels, B. (1997). Therapeutic evaluation of two different dose regimens of praziquantel in a recent *Schistosoma mansoni* focus in northern Senegal. *Am J Trop Med Hyg* 56(5): 511-514.

Hagan, P., Blumenthal, U.J., Dunn, D., Simpson, A.J. and Wilkins, H.A. (1991). Human IgE, IgG4 and resistance to reinfection with *Schistosoma haematobium*. *Nature* 349(6306): 243-245.

Hamadto, H.H., Rashed, S.M., el Said, A. and Elhayawan, I.A. (1990). Humoral and cellular immune response in schistosomiasis pre and post praziquantel therapy. *J Egypt Soc Parasitol* 20(2): 667-672.

Harnett, W. and Kusel, J.R. (1986). Increased exposure of parasite antigens at the surface of adult male *Schistosoma mansoni* exposed to praziquantel invitro. *Parasitology* 93(2): 401-405.

Harris, A.R., Russell, R.J. and Charters, A.D. (1984). A review of schistosomiasis in immigrants in Western Australia, demonstrating the unusual longevity of *Schistosoma mansoni*. *Trans R Soc Trop Med Hyg* 78(3): 385-388.

Harrison, R.A., Bickle, Q.D., Kiare, S., James, E.R., Andrews, B.J., Sturrock, R.F., Taylor, M.G. and Webbe, G. (1990). Immunization of Baboons with Attenuated Schistosomula of *Schistosoma haematobium* - Levels of Protection Induced by Immunization with Larvae Irradiated with 20 Krad and 60 Krad. *Trans R Soc Trop Med Hyg* 84(1): 89-99.

Harty, J.T. and Badovinac, V.P. (2008). Shaping and reshaping CD8+ T-cell memory. *Nat Rev Immunol* 8(2): 107-119.

Hewitson, J.P., Hamblin, P.A. and Mountford, A.P. (2005). Immunity induced by the radiation-attenuated schistosome vaccine. *Parasite Immunol* 27(7-8): 271-280.

Higgins, J.P., Deeks, J.J. and Altman, D.G. (2011). Chapter 16: Special topics in statistics. Cochrane Handbook for Systematic Reviews of Interventions Version 5.1.0. Higgins JPT, G. S., The Cochrane Collaboration.

Higgins, J.P., Thompson, S.G., Deeks, J.J. and Altman, D.G. (2003). Measuring inconsistency in meta-analyses. *BMJ* 327(7414): 557-560.

Higgins, J.P.T. and Green, S. (2011) Cochrane handbook for systematic reviews of interventions version 5.1.0. Retrieved 27/07/2016, 2016, from www.handbook.cochrane.org. .

Higon, M., Cowan, G., Nausch, N., Cavanagh, D., Oleaga, A., Toledo, R., Stothard, J.R., Antunez, O., Marcilla, A., Burchmore, R. and Mutapi, F. (2011). Screening trematodes for novel intervention targets: a proteomic and immunological comparison of *Schistosoma haematobium*, *Schistosoma bovis* and *Echinostoma caproni*. *Parasitology* 138(12): 1607-1619.

Horak, P., Dvorak, J., Kolarova, L. and Trefil, L. (1999). Trichobilharzia regenti, a pathogen of the avian and mammalian central nervous systems. *Parasitology* 119(6): 577-581.

Hsu, S.Y., Hsu, H.F. and Burmeister, L.F. (1981). *Schistosoma mansoni*: Vaccination of mice with highly X-irradiated cercariae. *Exp Parasitol* 52(1): 91-104.

Hu, G.H., Hu, J., Song, K.Y., Lin, D.D., Zhang, J., Cao, C.L., Xu, J., Li, D. and Jiang, W.S. (2005). The role of health education and health promotion in the control of schistosomiasis: experiences from a 12-year intervention study in the Poyang Lake area. *Acta Trop* 96(2-3): 232-241.

Humphries, D., Nguyen, S., Boakye, D., Wilson, M. and Cappello, M. (2012). The promise and pitfalls of mass drug administration to control intestinal helminth infections. *Curr Opin Infect Dis* 25(5): 584-589.

Hunter, J.E. and Schmidt, F.L. (2004). Methods of meta-analysis : correcting error and bias in research findings. Thousand Oaks, Calif., Sage.

Huyse, T., Van den Broeck, F., Jombart, T., Webster, B.L., Diaw, O., Volckaert, F.A., Balloux, F., Rollinson, D. and Polman, K. (2013). Regular treatments of praziquantel do not impact on the genetic make-up of *Schistosoma mansoni* in Northern Senegal. *Infect Genet Evol* 18: 100-105.

Jarrett, W.F., Jennings, F.W., Mc, I.W., Mulligan, W. and Urquhart, G.M. (1960). Immunological studies on *Dictyocaulus viviparus* infection; immunity produced by the administration of irradiated larvae. *Immunology* 3: 145-151.

Jonge, N.d., Schommer, G., Feldmeier, H., Krijger, F.W., Dafalla, A.A., Bienzle, U. and Deelder, A.M. (1990). Mixed *Schistosoma haematobium* and *S. mansoni* infection: effect of different treatments on the serum level of circulating anodic antigen (CAA). *Acta Trop* 48(1): 25-35.

Jordan, P. and Webbe, G. (1969). Human schistosomiasis. London, Heinemann Medical.

Joseph, S., Jones, F.M., Laidlaw, M.E., Mohamed, G., Mawa, P.A., Namujju, P.B., Kizza, M., Watera, C., Whitworth, J.A.G., Dunne, D.W. and Elliott, A.M. (2004). Impairment of the

Schistosoma mansoni-specific immune responses elicited by treatment with praziquantel in Ugandans with HIV-1 coinfection. *J Infect Dis* 190(3): 613-618.

Juni, P., Holenstein, F., Sterne, J., Bartlett, C. and Egger, M. (2002). Direction and impact of language bias in meta-analyses of controlled trials: empirical study. *Int J Epidemiol* 31(1): 115-123.

Kabatereine, N.B., Kemijumbi, J., Ouma, J.H., Sturrock, R.F., Butterworth, A.E., Madsen, H., Ornbjerg, N., Dunne, D.W. and Vennnervald, B.J. (2003). Efficacy and side effects of praziquantel treatment in a highly endemic *Schistosoma mansoni* focus at Lake Albert, Uganda. *Trans R Soc Trop Med Hyg* 97(5): 599-603.

Kabatereine, N.B., Vennervald, J.B., Ouma, J.H., Kemijumbi, J., Butterworth, A.E., Dunne, D.W. and Fulford, A.J.C. (1999). Adult resistance to schistosomiasis mansoni: age-dependence of reinfection remains constant in communities with diverse exposure patterns. *Parasitology* 118: 101-105.

Kaech, S.M. and Wherry, E.J. (2007). Heterogeneity and cell-fate decisions in effector and memory CD8+ T cell differentiation during viral infection. *Immunity* 27(3): 393-405.

Kariuki, H.C., Madsen, H., Ouma, J.H., Butterworth, A.E., Dunne, D.W., Booth, M., Kimani, G., Mwatha, J.K., Muchiri, E. and Vennervald, B.J. (2013). Long term study on the effect of mollusciciding with niclosamide in stream habitats on the transmission of Schistosomiasis *mansoni* after community-based chemotherapy in Makueni District, Kenya. *Parasit Vectors* 6(1): 107.

Kariuki, T.M., Farah, I.O., Yole, D.S., Mwenda, J.M., Van Dam, G.J., Deelder, A.M., Wilson, R.A. and Coulson, P.S. (2004). Parameters of the attenuated schistosome vaccine evaluated in the olive baboon. *Infect Immun* 72(9): 5526-5529.

Kariuki, T.M., Van Dam, G.J., Deelder, A.M., Farah, I.O., Yole, D.S., Wilson, R.A. and Coulson, P.S. (2006). Previous or ongoing schistosome infections do not compromise the efficacy of the attenuated cercaria vaccine. *Infect Immun* 74(7): 3979-3986.

Kato-Hayashi, N., Kirinoki, M., Iwamura, Y., Kanazawa, T., Kitikoon, V., Matsuda, H. and Chigusa, Y. (2010). Identification and differentiation of human schistosomes by polymerase chain reaction. *Exp Parasitol* 124(3): 325-329.

Keiser, J., Silue, K.D., Adiossan, L.K., N'Guessan, N.A., Monsan, N., Utzinger, J. and N'Goran, E.K. (2014). Praziquantel, mefloquine-praziquantel, and mefloquine-artesunate-praziquantel against *Schistosoma haematobium*: a randomized, exploratory, open-label trial. *PLoS Negl Trop Dis* 8(7): e2975.

Keiser, J., Silue, K.D., Adiossan, L.K., N'Guessan, N.A., Monsan, N.C., Utzinger, J. and N'Goran,

E.K. (2014). Praziquantel, mefloquine-praziquantel, and mefloquine-artesunate-praziquantel against *Schistosoma haematobium*: A randomized, exploratory, open-label trial. *PLoS Negl Trop Dis* 8(7).

Kern, P., Burchard, G.D. and Dietrich, M. (1984). Comparative study of oltipraz versus praziquantel for treatment of schistosomiasis with intestinal manifestation in the Gabon (*Schistosoma intercalatum* and *S. haematobium*). *Tropenmed Parasitol* 35(2): 95-99.

Khalife, J., Capron, M., Capron, A., Grzych, J.M., Butterworth, A.E., Dunne, D.W. and Ouma, J.H. (1986). Immunity in human *Schistosomiasis mansoni*: regulation of protective immune-mechanisms by IgM blocking antibodies. *J Exp Med* 164(5): 1626-1640.

Kiliku, F.M., Kimura, E., Muhoho, N., Migwi, D.K. and Katsumata, T. (1991). The usefulness of urinalysis reagent strips in selecting *Schistosoma haematobium* egg positives before and after treatment with praziquantel. *J Trop Med Hyg* 94(6): 401-406.

King, C.H. and Dangerfield-Cha, M. (2008). The unacknowledged impact of chronic schistosomiasis. *Chronic Illn* 4(1): 65-79.

King, C.H., Dickman, K. and Tisch, D.J. (2005). Reassessment of the cost of chronic helmintic infection: a meta-analysis of disability-related outcomes in endemic schistosomiasis. *Lancet* 365(9470): 1561-1569.

King, C.H., Keating, C.E., Muruka, J.F., Ouma, J.H., Houser, H., Siongok, T.K. and 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(4): 361-368.

King, C.H., Sutherland, L.J. and Bertsch, D. (2015). Systematic review and meta-analysis of the impact of chemical-based mollusciciding for control of *Schistosoma mansoni* and *S. haematobium* transmission. *PLoS Negl Trop Dis* 9(12): e0004290.

Kloos, H., Higashi, G.I., Cattani, J.A., Schlinski, V.D., Mansour, N.S. and Murrell, K.D. (1983). Water contact behavior and schistosomiasis in an upper Egyptian village. *Soc Sci Med* 17(9): 545-562.

Knopp, S., Corstjens, P.L., Koukounari, A., Cercamondi, C.I., Ame, S.M., Ali, S.M., de Dood, C.J., Mohammed, K.A., Utzinger, J., Rollinson, D. and van Dam, G.J. (2015). Sensitivity and specificity of a urine circulating anodic antigen test for the diagnosis of *Schistosoma haematobium* in low endemic settings. *PLoS Negl Trop Dis* 9(5): e0003752.

Knowles, S.C.L., Webster, B.L., Garba, A., Sacko, M., Diaw, O.T., Fenwick, A., Rollinson, D. and Webster, J.P. (2015). Epidemiological interactions between urogenital and intestinal human

schistosomiasis in the context of praziquantel treatment across three West African countries. *PLoS Negl Trop Dis* 9(10): e0004019.

Kohn, R.R. (1971). Effect of antioxidants on life-span of C57BL mice. *J Gerontol* 26(3): 378-380.

Koukounari, A., Gabrielli, A.F., Toure, S., Bosque-Oliva, E., Zhang, Y.B., Sellin, B., Donnelly, C.A., Fenwick, A. and Webster, J.P. (2007). *Schistosoma haematobium* infection and morbidity before and after large-scale administration of praziquantel in Burkina Faso. *J Infect Dis* 196(5): 659-669.

Kutner, M.H. (2005). Applied linear statistical models. Boston, McGraw-Hill Irwin.

Lamberton, P.H., Hogan, S.C., Kabatereine, N.B., Fenwick, A. and Webster, J.P. (2010). In vitro praziquantel test capable of detecting reduced in vivo efficacy in *Schistosoma mansoni* human infections. *Am J Trop Med Hyg* 83(6): 1340-1347.

Lardans, V. and Dissous, C. (1998). Snail control strategies for reduction of schistosomiasis transmission. *Parasitol Today* 14(10): 413-417.

Leenstra, T., Coutinho, H.M., Acosta, L.P., Langdon, G.C., Su, L., Olveda, R.M., McGarvey, S.T., Kurtis, J.D. and Friedman, J.F. (2006). *Schistosoma japonicum* reinfection after praziquantel treatment causes anemia associated with inflammation. *Infect Immun* 74(11): 6398-6407.

Lelo, A.E., Mburu, D.N., Magoma, G.N., Mungai, B.N., Kihara, J.H., Mwangi, I.N., Maina, G.M., Kinuthia, J.M., Mutuku, M.W., Loker, E.S., Mkoji, G.M. and Steinauer, M.L. (2014). No apparent reduction in schistosome burden or genetic diversity following four years of school-based mass drug administration in Mwea, Central Kenya, a heavy transmission area. *PLoS Negl Trop Dis* 8(10).

Lemon, S.C., Roy, J., Clark, M.A., Friedmann, P.D. and Rakowski, W. (2003). Classification and regression tree analysis in public health: Methodological review and comparison with logistic regression. *Ann Behav Med* 26(3): 172-181.

Lewis, R.J. (2000). An introduction to Classification and Regression Tree (CART) analysis. Annual Meeting of the Society for Academic Emergency Medicine. San Francisco, California.

Li, Y.S., He, Y.K., Zeng, Q.R. and McManus, D.P. (2003). Epidemiological and morbidity assessment of *Schistosoma japonicum* infection in a migrant fisherman community, the Dongting Lake region, China. *Trans R Soc Trop Med Hyg* 97(2): 177-181.

Lichtenbergova, L., Kolbekova, P., Kourilova, P., Kasny, M., Mikes, L., Haas, H., Schramm, G., Horak, P., Kolarova, L. and Mountford, A.P. (2008). Antibody responses induced by *Trichobilharzia regenti* antigens in murine and human hosts exhibiting cercarial dermatitis. *Parasite Immunol* 30(11-12): 585-595.

Lin, D.D., Tian, F., Wu, H.W., Gao, Y.N., Wu, J.J., Zhang, D.H., Ji, M.J., McManus, D.P., Driguez, P. and Wu, G.L. (2011). Multiple vaccinations with UV- attenuated cercariae in pig enhance protective immunity against *Schistosoma japonicum* infection as compared to single vaccination. *Parasit Vectors* 4.

Little, R.J.A. and Rubin, D.B. (2002). Statistical analysis with missing data. Hoboken, N.J., Wiley.

Liu, J.L., Fontaine, J., Capron, A. and Grzych, J.M. (1996). Ultrastructural localization of Sm28 GST protective antigen in *Schistosoma mansoni* adult worms. *Parasitology* 113(4): 377-391.

Liu, R., Dong, H.F., Guo, Y., Zhao, Q.P. and Jiang, M.S. (2011). Efficacy of praziquantel and artemisinin derivatives for the treatment and prevention of human schistosomiasis: a systematic review and meta-analysis. *Parasit Vectors* 4: 201.

Loh, W.Y. (2011). Classification and regression trees.

Loker, E.S. (1983). A comparative study of the life-histories of mammalian schistosomes. *Parasitology* 87(2): 343-369.

Loker, E.S. and Hofkin, B.V. (2015). Parasitology: A Conceptual Approach.

Loker, E.S. and Mkoji, G.M. (2005). Schistosomes and their snail hosts. Schistosomiasis. Secor, W. E. and Colley, D. G. New York, Springer: 1-11.

Mallett, R., Hagen-Zanker, J., Slater, R. and Duvendack, M. (2012). The benefits and challenges of using systematic reviews in international development research. *J Dev Effect* 4(3): 445-455.

Mangold, B.L. and Dean, D.A. (1984). The migration and survival of gamma-irradiated *Schistosoma mansoni* larvae and the duration of host-parasite contact in relation to the induction of resistance in mice. *Parasitology* 88(2): 249-266.

McKeand, J.B. (2000). Vaccine development and diagnostics of *Dictyocaulus viviparus*. *Parasitology* 120(Suppl): S17-23.

Mclaren, D.J. and Smithers, S.R. (1985). *Schistosoma mansoni* - challenge attrition during the lung phase of migration in vaccinated and serum-protected rats. *Exp Parasitol* 60(1): 1-9.

McMahon, B.J., Dentinger, C.M., Bruden, D., Zanis, C., Peters, H., Hurlburt, D., Bulkow, L., Fiore, A.E., Bell, B.P. and Hennessy, T.W. (2009). Antibody levels and protection after hepatitis B vaccine: results of a 22-year follow-up study and response to a booster dose. *J Infect Dis* 200(9): 1390-1396.

McManus, D.P. (2005). Prospects for development of a transmission blocking vaccine against *Schistosoma japonicum*. *Parasite Immunol* 27(7-8): 297-308.

McWilliam, H.E., Driguez, P., Piedrafita, D., McManus, D.P. and Meeusen, E.N. (2012). Novel immunomic technologies for schistosome vaccine development. *Parasite Immunol* 34(5):

276-284.

Meeusen, E.N., Walker, J., Peters, A., Pastoret, P.P. and Jungersen, G. (2007). Current status of veterinary vaccines. *Clin Microbiol Rev* 20(3): 489-510.

Mendes, E. and Mendes, N.F. (1982). High-levels of IgE in patients with Schistosomiasis *mansoni*: quantitation of total and specific IgE and IgG. *Trop Geogr Med* 34(2): 133-137.

Meurs, L., Mbow, M., Vereecken, K., Menten, J., Mboup, S. and Polman, K. (2012). Bladder Morbidity and Hepatic Fibrosis in Mixed Schistosoma haematobium and S. mansoni Infections: A Population-Wide Study in Northern Senegal. *PLoS Negl Trop Dis* 6(9).

Midzi, N., Sangweme, D., Zinyowera, S., Mapingure, M.P., Brouwer, K.C., Kumar, N., Mutapi, F., Woelk, G. and Mduluza, T. (2008). Efficacy and side effects of praziquantel treatment against *Schistosoma haematobium* infection among primary school children in Zimbabwe. *Trans R Soc Trop Med Hyg* 102(8): 759-766.

Minard, P., Dean, D.A., Vannier, W.E. and Murrell, K.D. (1978). Effect of immunization on migration of *Schistosoma mansoni* through lungs. *Am J Trop Med Hyg* 27(1 Pt 1): 87-93.

Mitchell, K.M., Mutapi, F., Savill, N.J. and Woolhouse, M.E. (2011). Explaining observed infection and antibody age-profiles in populations with urogenital schistosomiasis. *PLoS Comput Biol* 7(10): e1002237.

Mitchell, K.M., Mutapi, F., Savill, N.J. and Woolhouse, M.E.J. (2012). Protective immunity to *Schistosoma haematobium* infection is primarily an anti-fecundity response stimulated by the death of adult worms. *Proc Natl Acad Sci USA* 109(33): 13347-13352.

Moloney, N.A., Garcia, E.G. and Webbe, G. (1985). The strain specificity of vaccination with Ultra-Violet attenuated cercariae of the chinese strain of *Schistosoma japonicum*. *Trans R Soc Trop Med Hyg* 79(2): 245-247.

Moloney, N.A., Webbe, G. and Hinchcliffe, P. (1987). The induction of species-specific immunity against *Schistosoma japonicum* by exposure of rats to ultra-violet attenuated cercariae. *Parasitology* 94: 49-54.

Mone, H., Minguez, S., Ibikounle, M., Allienne, J.F., Massougbodji, A. and Mouahid, G. (2012). Natural Interactions between *S. haematobium* and *S. guineensis* in the Republic of Benin. *ScientificWorldJournal*: 793420.

Morrison, A., Polisena, J., Husereau, D., Moulton, K., Clark, M., Fiander, M., Mierzwinski-Urban, M., Clifford, T., Hutton, B. and Rabb, D. (2012). The effect of English-language restriction on systematic review-based meta-analyses: A systematic review of empirical studies. *Int J Technol Assess Health Care* 28(2): 138-144.

Morrison, C. (2015). Landmark green light for Mosquirix malaria vaccine. *Nat Biotechnol* 33(10): 1015-1016.

Mountford, A.P., Coulson, P.S., Pemberton, R.M., Smythies, L.E. and Wilson, R.A. (1992). The generation of interferon-gamma-producing T lymphocytes in skin-draining lymph nodes, and their recruitment to the lungs, is associated with protective immunity to *Schistosoma mansoni*. *Immunology* 75(2): 250-256.

Mountford, A.P. and Jenkins, S.J. (2005). Vaccine development lessons from the study of initial immune responses to invading larvae. Schistosomiasis. Colley, D. G. and Secor, W. E. New York, NY, Springer: 65-79.

Muhumuza, S., Olsen, A., Katahoire, A., Kiragga, A.N. and Nuwaha, F. (2014). Effectiveness of a pre-treatment snack on the uptake of mass treatment for schistosomiasis in Uganda: A cluster randomized trial. *PLoS Med* 11(5): e1001640.

Muller-Graf, C.D.M., Collins, D.A., Packer, C. and Woolhouse, M.E.J. (1997). *Schistosoma mansoni* infection in a natural population of olive baboons (*Papio cynocephalus anubis*) in Gombe Stream National Park, Tanzania. *Parasitology* 115(6): 621-627.

Mutapi, F. (2001). Heterogeneities in anti-schistosome humoral responses following chemotherapy. *Trends Parasitol* 17(11): 518-524.

Mutapi, F., Billingsley, P.F. and Secor, W.E. (2013). Infection and treatment immunizations for successful parasite vaccines. *Trends Parasitol* 29(3): 135-141.

Mutapi, F., Burchmore, R., Mduluza, T., Foucher, A., Harcus, Y., Nicoll, G., Midzi, N., Turner, C.M. and Maizels, R.M. (2005). Praziquantel treatment of individuals exposed to *Schistosoma haematobium* enhances serological recognition of defined parasite antigens. *J Infect Dis* 192(6): 1108-1118.

Mutapi, F., Hagan, P., Woolhouse, M.E.J., Mduluza, T. and Ndhlovu, P.D. (2003). Chemotherapy-induced, age-related changes in antischistosome antibody responses. *Parasite Immunol* 25(2): 87-97.

Mutapi, F., Mduluza, T., Gomez-Escobar, N., Gregory, W.F., Fernandez, C., Midzi, N. and Maizels, R.M. (2006). Immuno-epidemiology of human *Schistosoma haematobium* infection: preferential IgG3 antibody responsiveness to a recombinant antigen dependent on age and parasite burden. *BMC Infect Dis* 6: 96.

Mutapi, F., Ndhlovu, P.D., Hagan, P., Spicer, J.T., Mduluza, T., Turner, C.M., Chandiwana, S.K. and Woolhouse, M.E. (1998). Chemotherapy accelerates the development of acquired immune responses to *Schistosoma haematobium* infection. *J Infect Dis* 178(1): 289-293.

Mutapi, F., Ndhlovu, P.D., Hagan, P. and Woolhouse, M.E. (1997). A comparison of humoral responses to *Schistosoma haematobium* in areas with low and high levels of infection. *Parasite Immunol* 19(6): 255-263.

Mutapi, F., Ndhlovu, P.D., Hagan, P. and Woolhouse, M.E. (2000). Anti-schistosome antibody responses in children coinfected with malaria. *Parasite Immunol* 22(4): 207-209.

Mutapi, F., Rujeni, N., Bourke, C., Mitchell, K., Appleby, L., Nausch, N., Midzi, N. and 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(5): e1143.

N'Goran, E.K., Gnaka, H.N., Tanner, M. and Utzinger, J. (2003). Efficacy and side-effects of two praziquantel treatments against *Schistosoma haematobium* infection, among schoolchildren from Cote d'Ivoire. *Ann Trop Med Parasitol* 97(1): 37-51.

Navaratnam, A.M., Sousa-Figueiredo, J.C., Stothard, J.R., Kabatereine, N.B., Fenwick, A. and 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(7): 400-407.

Norton, A.J., Gower, C.M., Lamberton, P.H., Webster, B.L., Lwambo, N.J., Blair, L., Fenwick, A. and 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(4): 951-957.

Nour, N.M. (2010). Schistosomiasis: health effects on women. *Rev Obstet Gynecol* 3(1): 28-32.

Oliveira, R.R., Figueiredo, J.P., Cardoso, L.S., Jabar, R.L., Souza, R.P., Wells, M.T., Carvalho, E.M., Fitzgerald, D.W., Barnes, K.C., Araujo, M.I. and Glesby, M.J. (2012). Factors associated with resistance to *Schistosoma mansoni* infection in an endemic area of Bahia, Brazil. *Am J Trop Med Hyg* 86(2): 296-305.

Olliaro, P.L., Vaillant, M., Diawara, A., Coulibaly, J.T., Garba, A., Keiser, J., King, C.H., Knopp, S., Landoure, A., N'Goran, E.K., Raso, G., Scherrer, A.U., Sousa-Figueiredo, J.C., Stete, K., Zhou, X.N. and Utzinger, J. (2015). Toward measuring schistosoma response to praziquantel treatment with appropriate descriptors of egg excretion. *PLoS Negl Trop Dis* 9(6): e0003821.

Olliaro, P.L., Vaillant, M.T., Belizario, V.J., Lwambo, N.J.S., Ouldabdallahi, M., Pieri, O.S., Amarillo, M.L., Kaatano, G.M., Diaw, M., Domingues, A.C., Favre, T.C., Lapujade, O., Alves, F. and Chitsulo, L. (2011). A multicentre randomized controlled trial of the efficacy and safety of single-dose praziquantel at 40 mg/kg vs. 60 mg/kg for treating intestinal schistosomiasis in

the Philippines, Mauritania, Tanzania and Brazil. *PLoS Negl Trop Dis* 5(6): e1165.

Omedo, M., Ogutu, M., Awiti, A., Musuva, R., Muchiri, G., Montgomery, S.P., Secor, W.E. and Mwinzi, P. (2014). The effect of a health communication campaign on compliance with mass drug administration for schistosomiasis control in Western Kenya - the score project. *Am J Trop Med Hyg* 91(5): 982-988.

Othman, A.A. and Soliman, R.H. (2015). Schistosomiasis in Egypt: A never-ending story? *Acta Trop* 148: 179-190.

Pediatric Praziquantel Consortium. (2016) Pediatric praziquantel consortium. Retrieved Aug., 2016, from http://www.pediatricpraziquantelconsortium.org/.

Phillips, S.M. and Reid, W.A. (1980). *Schistosoma mansoni*: immune response to normal and irradiated cercariae or soluble stage-specific surface immunogens. *Int J Nucl Med Biol* 7(2): 173-186.

Pica-Mattoccia, L., Orsini, T., Basso, A., Festucci, A., Liberti, P., Guidi, A., Marcatto-Maggi, A.L., Nobre-Santana, S., Troiani, A.R., Cioli, D. and Valle, C. (2008). *Schistosoma mansoni*: Lack of correlation between praziquantel-induced intra-worm calcium influx and parasite death. *Exp Parasitol* 119(3): 332-335.

Pinot de Moira, A., Fulford, A.J., Kabatereine, N.B., Ouma, J.H., Booth, M. and 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(9): e820.

Pointier, J.P. and Guyard, A. (1992). Biological control of the snail intermediate hosts of *Schistosoma mansoni* in Martinique, French West Indies. *Trop Med Parasitol* 43(2): 98-101.

Pointier, J.P. and Mccullough, F. (1989). Biological-control of the snail hosts of *Schistosoma mansoni* in the caribbean area using *Thiara spp*. *Acta Trop* 46(3): 147-155.

Polderman, A.M., Gryseels, B. and De Caluwe, P. (1988). Cure rates and egg reduction in treatment of intestinal schistosomiasis with oxamniquine and praziquantel in Maniema, Zaire. *Trans R Soc Trop Med Hyg* 82(1): 115-116.

Ratard, R.C., Kouemeni, L.E., Bessala, M.M., Ndamkou, C.N., Greer, G.J., Spilsbury, J. and Cline, B.L. (1990). Human schistosomiasis in Cameroon. I. Distribution of schistosomiasis. *Am J Trop Med Hyg* 42(6): 561-572.

Reed, S.G., Orr, M.T. and Fox, C.B. (2013). Key roles of adjuvants in modern vaccines. *Nat Med* 19(12): 1597-1608.

Rihet, P., Demeure, C.E., Bourgois, A., Prata, A. and Dessein, A.J. (1991). Evidence for an association between human resistance to *Schistosoma mansoni* and high anti-larval IgE levels. *Eur J*

Immunol 21(11): 2679-2686.

Riley, S., Carabin, H., Belisle, P., Joseph, L., Tallo, V., Balolong, E., Willingham, A.L., Fernandez, T.J., Gonzales, R.O., Olveda, R. and McGarvey, S.T. (2008). Multi-host transmission dynamics of *Schistosoma japonicum* in Samar province, the Philippines. *PLoS Med* 5(1): e18.

Riveau, G., Deplanque, D., Remoue, F., Schacht, A.M., Vodougnon, H., Capron, M., Thiry, M., Martial, J., Libersa, C. and Capron, A. (2012). Safety and immunogenicity of rSh28GST antigen in humans: phase 1 randomized clinical study of a vaccine candidate against urinary schistosomiasis. *PLoS Negl Trop Dis* 6(7): e1704.

Rother, E.T. (2007). Systematic literature review X narrative review. *Acta Paul Enferm* 20(2): 7-8.

Rudge, J.W., Stothard, J.R., Basanez, M.G., Mgeni, A.F., Khamis, I.S., Khamis, A.N. and 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(1): 45-54.

Ruganuza, D.M., Mazigo, H.D., Waihenya, R., Morona, D. and Mkoji, G.M. (2015). *Schistosoma mansoni* among pre-school children in Musozi village, Ukerewe Island, North-Western-Tanzania: prevalence and associated risk factors. *Parasit Vectors* 8: 377.

Rujeni, N., Nausch, N., Midzi, N., Mduluza, T., Taylor, D.W. and Mutapi, F. (2012). *Schistosoma haematobium* infection levels determine the effect of praziquantel treatment on anti-schistosome and anti-mite antibodies. *Parasite Immunol* 34(6): 330-340.

Sabah, A.A., Fletcher, C., Webbe, G. and Doenhoff, M.J. (1985). *Schistosoma mansoni*: reduced efficacy of chemotherapy in infected T-cell-deprived mice. *Exp Parasitol* 60(3): 348-354.

Sacko, M., Magnussen, P., Traore, M., Landoure, A., Doucoure, A., Reimert, C.M. and Vennervald, B.J. (2009). The effect of single dose versus two doses of praziquantel on *Schistosoma haematobium* infection and pathology among school-aged children in Mali. *Parasitology* 136(13): 1851-1857.

Sallusto, F., Lanzavecchia, A., Araki, K. and Ahmed, R. (2010). From vaccines to memory and back. *Immunity* 33(4): 451-463.

Santini-Oliveira, M., Coler, R.N., Parra, J., Veloso, V., Jayashankar, L., Pinto, P.M., Ciol, M.A., Bergquist, R., Reed, S.G. and Tendler, M. (2016). Schistosomiasis vaccine candidate Sm14/GLA-SE: Phase 1 safety and immunogenicity clinical trial in healthy, male adults. *Vaccine* 34(4): 586-594.

Satti, M.Z., Sulaiman, S.M., Homeida, M.M.A., Younis, S.A. and Ghalib, H.W. (1996). Clinical,

parasitological and immunological features of canal cleaners hyper-exposed-to *Schistosoma mansoni* in the Sudan. *Clin Exp Immunol* 104(3): 426-431.

Scherrer, A.U., Sjoberg, M.K., Allangba, A., Traore, M., Lohourignon, L.K., Tschannen, A.B., N'Goran, E.K. and Utzinger, J. (2009). Sequential analysis of helminth egg output in human stool samples following albendazole and praziquantel administration. *Acta Trop* 109(3): 226-231.

Schutte, C.H., Osman, Y., Van Deventer, J.M. and Mosese, G. (1983). Effectiveness of praziquantel against the South African strains of *Schistosoma haematobium* and *S. mansoni*. *S Afr Med J* 64(1): 7-10.

Seder, R.A., Chang, L.J., Enama, M.E., Zephir, K.L., Sarwar, U.N., Gordon, I.J., Holman, L.A., James, E.R., Billingsley, P.F., Gunasekera, A., Richman, A., Chakravarty, S., Manoj, A., Velmurugan, S., Li, M.L., Ruben, A.J., Li, T., Eappen, A.G., Stafford, R.E., Plummer, S.H., Hendel, C.S., Novik, L., Costner, P.J.M., Mendoza, F.H., Saunders, J.G., Nason, M.C., Richardson, J.H., Murphy, J., Davidson, S.A., Richie, T.L., Sedegah, M., Sutamihardja, A., Fahle, G.A., Lyke, K.E., Laurens, M.B., Roederer, M., Tewari, K., Epstein, J.E., Sim, B.K.L., Ledgerwood, J.E., Graham, B.S., Hoffman, S.L. and Team, V.S. (2013). Protection against malaria by intravenous immunization with a nonreplicating sporozoite vaccine. *Science* 341(6152): 1359-1365.

Shamseer, L., Moher, D., Clarke, M., Ghersi, D., Liberati, A., Petticrew, M., Shekelle, P., Stewart, L.A. and Grp, P.-P. (2015). Preferred reporting items for systematic review and meta-analysis protocols (PRISMA-P) 2015: elaboration and explanation. *BMJ* 349.

Shi, F., Zhang, Y., Lin, J., Zuo, X., Shen, W., Cai, Y., Ye, P., Bickle, Q.D. and Taylor, M.G. (2002). Field testing of *Schistosoma japonicum* DNA vaccines in cattle in China. *Vaccine* 20(31-32): 3629-3631.

Shi, Y.E., Jiang, C.F., Han, J.J., Li, Y.L. and Ruppel, A. (1990). *Schistosoma japonicum*: an ultraviolet-attenuated cercarial vaccine applicable in the field for water buffaloes. *Exp Parasitol* 71(1): 100-106.

Shi, Y.E., Jiang, C.F., Han, J.J., Li, Y.L. and Ruppel, A. (1993). Immunization of pigs against infection with *Schistosoma japonicum* using ultraviolet-attenuated cercariae. *Parasitology* 106(5): 459-462.

Siddiqui, A.A., Ahmad, G., Damian, R.T. and Kennedy, R.C. (2008). Experimental vaccines in animal models for schistosomiasis. *Parasitol Res* 102(5): 825-833.

Silveira, A.M., Bethony, J., Gazzinelli, A., Kloos, H., Fraga, L.A., Alvares, M.C., Prata, A., Guerra,

H.L., Loverde, P.T., Correa-Oliveira, R. and Gazzinelli, G. (2002). High levels of IgG4 to *Schistosoma mansoni* egg antigens in individuals with periportal fibrosis. *Am J Trop Med Hyg* 66(5): 542-549.

Simonsen, P.E., Nega, A. and Furu, P. (1990). Intestinal schistosomiasis among children in a labor village of wonji sugar estate, Ethiopia. *East Afr Med J* 67(8): 532-538.

Sinha, M.K. and Montori, V.M. (2006). Reporting bias and other biases affecting systematic reviews and meta-analyses: a methodological commentary. *Expert Rev Pharmacoecon Outcomes Res* 6(5): 603-611.

Siqueira, L.M.V., Gomes, L.I., Oliveira, E., de Oliveira, E.R., de Oliveira, A.A., Enk, M.J., Carneiro, N.F., Rabello, A. and Coelho, P.M.Z. (2015). Evaluation of parasitological and molecular techniques for the diagnosis and assessment of cure of *Schistosomiasis mansoni* in a low transmission area. *Mem Inst Oswaldo Cruz* 110(2): 209-214.

Smith, J.H. and Christie, J.D. (1986). The pathobiology of *Schistosoma haematobium* infection in humans. *Hum Pathol* 17(4): 333-345.

Smithers, S.R. (1962). Immunizing effect of irradiated cercariae of *Schistosoma mansoni* in rhesus monkeys. *Nature* 194(4834): 1146-1147.

Snyman, J.R. and Sommers de, K. (1998). Effect of levamisole on the immune response of patients with schistosomiasis after treatment with praziquantel. *Clin Drug Investig* 15(6): 483-489.

Soler, E. and Houdebine, L.M. (2007). Preparation of recombinant vaccines. *Biotechnol Annu Rev* 13: 65-94.

Song, F., Parekh, S., Hooper, L., Loke, Y.K., Ryder, J., Sutton, A.J., Hing, C., Kwok, C.S., Pang, C. and Harvey, I. (2010). Dissemination and publication of research findings: an updated review of related biases. *Health Technol Assess* 14(8): 1-+.

Sousa-Figueiredo, J.C., Betson, M., Atuhaire, A., Arinaitwe, M., Navaratnam, A.M., Kabatereine, N.B., Bickle, Q. and Stothard, J.R. (2012). Performance and safety of praziquantel for treatment of intestinal schistosomiasis in infants and preschool children. *PLoS Negl Trop Dis* 6(10): e1864.

Sousa-Figueiredo, J.C., Pleasant, J., Day, M., Betson, M., Rollinson, D., Montresor, A., Kazibwe, F., Kabatereine, N.B. and Stothard, J.R. (2010). Treatment of intestinal schistosomiasis in Ugandan preschool children: best diagnosis, treatment efficacy and side-effects, and an extended praziquantel dosing pole. *Int Health* 2(2): 103-113.

Southgate, V.R. (1997). Schistosomiasis in the Senegal river basin: Before and after the construction of the dams at Diama, Senegal and Manantali, Mali and future prospects. *J Helminthol* 71(2):

125-132.

Sow, S., de Vlas, S.J., Stelma, F., Vereecken, K., Gryseels, B. and 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.

Stauffer, J.R., Madsen, H., McKaye, K., Konings, A., Bloch, P., Ferreri, C.P., Likongwe, J. and Makaula, P. (2006). Schistosomiasis in Lake Malawi: Relationship of fish and intermediate host density to prevalence of human infection. *Ecohealth* 3(1): 22-27.

Steinmann, P., Keiser, J., Bos, R., Tanner, M. and Utzinger, J. (2006). Schistosomiasis and water resources development: systematic review, meta-analysis, and estimates of people at risk. *Lancet Infect Dis* 6(7): 411-425.

Stelma, F.F., Talla, I., Sow, S., Kongs, A., Niang, M., Polman, K., Deelder, A.M. and Gryseels, B. (1995). Efficacy and side effects of praziquantel in an epidemic focus of *Schistosoma mansoni*. *Am J Trop Med Hyg* 53(2): 167-170.

Stevens, W.J., Feldmeir, H., Bridts, C.H. and Daffalla, A.A. (1983). IgG and IgE circulating immune complexes, total serum IgE and parasite related IgE in patients with mono- or mixed infection with *Schistosoma mansoni* and/or *S. haematobium*. Influence of therapy. *Clin Exp Immunol* 52(1): 144-152.

Stothard, J.R., Sousa-Figueiredo, J.C. and Navaratnam, A.M. (2013). Advocacy, policies and practicalities of preventive chemotherapy campaigns for African children with schistosomiasis. *Expert Rev Anti Infect Ther* 11(7): 733-752.

Sulaiman, S.M., Traore, M., Engels, D., Hagan, P. and Cioli, D. (2001). Counterfeit praziquantel. *Lancet* 358(9282): 666-667.

Sutton, A.J., Duval, S.J., Tweedie, R.L., Abrams, K.R. and Jones, D.R. (2000). Empirical assessment of effect of publication bias on meta-analyses. *BMJ* 320(7249): 1574-1577.

Takougang, I., Meli, J. and Angwafo, F., 3rd (2006). Field trials of low dose Bayluscide on snail hosts of schistosome and selected non-target organisms in sahelian Cameroon. *Mem Inst Oswaldo Cruz* 101(4): 355-358.

Takougang, I., Meli, J., Pone, J.W. and Angwafo, F. (2007). Community acceptability of the use of low-dose niclosamide (Bayluscide (R)), as a molluscicide in the control of human schistosomiasis in Sahelian Cameroon. *Ann Trop Med Parasitol* 101(6): 479-486.

Talla, I., Kongs, A., Verle, P., Belot, J., Sarr, S. and Coll, A.M. (1990). Outbreak of Intestinal Schistosomiasis in the Senegal River Basin. *Ann Soc Belg Med Trop* 70(3): 173-180.

Taylor, M.G., Huggins, M.C., Shi, F., Lin, J., Tian, E., Ye, P., Shen, W., Qian, C.G., Lin, B.F. and

Bickle, Q.D. (1998). Production and testing of *Schistosoma japonicum* candidate vaccine antigens in the natural ovine host. *Vaccine* 16(13): 1290-1298.

Taylor, M.G., Nelson, G.S. and Andrews, B.J. (1972). A case of natural infection of *S. haematobium* in a Senegalese baboon (*Papio sp.*). *Trans R Soc Trop Med Hyg* 66(1): 16-17.

Taylor, P., Murare, H.M. and Manomano, K. (1988). Efficacy of low doses of praziquantel for *Schistosoma mansoni* and *S. haematobium*. *J Trop Med Hyg* 91(1): 13-17.

Tchuem, L.A.T., Momo, S.C., Stothard, J.R. and Rollinson, D. (2013). Efficacy of praziquantel and reinfection patterns in single and mixed infection foci for intestinal and urogenital schistosomiasis in Cameroon. *Acta Trop* 128(2): 275-283.

Tchuente, L.A., Shaw, D.J., Polla, L., Cioli, D. and Vercruysse, J. (2004). Efficacy of praziquantel against *Schistosoma haematobium* infection in children. *Am J Trop Med Hyg* 71(6): 778-782.

Tchuente, L.A.T., Momo, S.C., Stothard, J.R. and Rollinson, D. (2013). Efficacy of praziquantel and reinfection patterns in single and mixed infection foci for intestinal and urogenital schistosomiasis in Cameroon. *Acta Trop* 128(2): 275-283.

Thompson, S.G. and Higgins, J.P.T. (2002). How should meta-regression analyses be undertaken and interpreted? *Stat Med* 21(11): 1559-1573.

Tinto, H., D'Alessandro, U., Sorgho, H., Valea, I., Tahita, M.C., Kabore, W., Kiemde, F., Lompo, P., Ouedraogo, S., Derra, K., Ouedraogo, F., Ouedraogo, J.B., Ballou, W.R., Cohen, J., Guerra, Y., Heerwegh, D., Jongert, E., Lapierre, D., Leach, A., Lievens, M., Ofori-Anyinam, O., Olivier, A., Vekemans, J., Agnandji, S.T., Lell, B., Fernandes, J.F., Abossolo, B.P., Kabwende, A.L., Adegnika, A.A., Mordmuller, B., Issifou, S., Kremsner, P.G., Loembe, M.M., Bache, E., Alabi, A., Owusu-Agyei, S., Asante, K.P., Boahen, O., Dosoo, D., Asante, I., Yidana, Z., Anim, J., Adeniji, E., Yawson, A.K., Kayan, K., Chandramohan, D., Greenwood, B., Ansong, D., Agbenyega, T., Adjei, S., Boateng, H.O., Rettig, T., Sylverken, J., Sambian, D., Badu-Prepah, A., Kotey, A., Buabeng, P., Paintsil, V., Enimil, A., Hamel, M.J., Kariuki, S., Oneko, M., Odero, C., Otieno, K., Awino, N., Muturi-Kioi, V., Omoto, J., Sang, T., Odhiambo, S., Laserson, K.F., Slutsker, L., Otieno, W., Otieno, L., Otsyula, N., Gondi, S., Ochola, J., Okoth, G., Mabunde, D.C., Wangwe, A., Otieno, A., Oyieko, J., Cowden, J., Ogutu, B., Njuguna, P., Marsh, K., Akoo, P., Kerubo, C., Maingi, C., Bejon, P., Olotu, A., Chilengi, R., Tsofa, B., Lang, T., Gitaka, J., Awuondo, K., Martinson, F., Hoffman, I., Mvalo, T., Kamthunzi, P., Nkomo, R., Tembo, T., Tegha, G., Chawinga, C., Banda, T., Khan, S., Mwambakulu, S., Mzembe, E., Sacarlal, J., Aide, P., Madrid, L., Mandjate, S., Aponte, J.J., Bulo, H., Massora, S., Varela, E., Macete, E., Alonso, P., Lusingu, J., Gesase, S., Malabeja,

A., Abdul, O., Mahende, C., Liheluka, E., Lemnge, M., Theander, T.G., Drakeley, C., Mbwana, J., Olomi, R., Mmbando, B., Abdulla, S., Salim, N., Mtoro, A., Ahmed, S., Hamad, A., Kafuruki, S., Minja, R., Tanner, M., Maganga, M., Mdemu, A., Gwandu, C., Mohammed, A., Kaslow, D., Leboulleux, D., Savarese, B., Schellenberg, D. and Partnership, R.S.C.T. (2015). Efficacy and safety of RTS,S/AS01 malaria vaccine with or without a booster dose in infants and children in Africa: final results of a phase 3, individually randomised, controlled trial. *Lancet* 386(9988): 31-45.

Tuhebwe, D., Bagonza, J., Kiracho, E.E., Yeka, A., Elliott, A.M. and Nuwaha, F. (2015). Uptake of mass drug administration programme for schistosomiasis control in Koome Islands, Central Uganda. *PLoS One* 10(4): e0123673.

Tukahebwa, E.M., Vennervald, B.J., Nuwaha, F., Kabatereine, N.B. and Magnussen, P. (2013). Comparative efficacy of one versus two doses of praziquantel on cure rate of *Schistosoma mansoni* infection and re-infection in Mayuge District, Uganda. *Trans R Soc Trop Med Hyg* 107(6): 397-404.

Uman, L.S. (2011). Systematic reviews and meta-analyses. *J Can Acad Child Adolesc Psychiatry* 20(1): 57-59.

Utzinger, J., Booth, M., N'Goran, E.K., Muller, I., Tanner, M. and Lengeler, C. (2001). Relative contribution of day-to-day and intra-specimen variation in faecal egg counts of *Schistosoma mansoni* before and after treatment with praziquantel. *Parasitology* 122(5): 537-544.

Utzinger, J., N'Goran, E.K., N'Dri, A., Lengeler, C. and Tanner, M. (2000). Efficacy of praziquantel against *Schistosoma mansoni* with particular consideration for intensity of infection. *Trop Med Int Health* 5(11): 771-778.

Utzinger, J., Raso, G., Brooker, S., De Savigny, D., Tanner, M., Ornbjerg, N., Singer, B.H. and N'Goran E, K. (2009). Schistosomiasis and neglected tropical diseases: towards integrated and sustainable control and a word of caution. *Parasitology* 136(13): 1859-1874.

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. and Anderson, T.J. (2013). Genetic and molecular basis of drug resistance and species-specific drug action in schistosome parasites. *Science* 342(6164): 1385-1389.

Van Damme, P., Leroux-Roels, G., Crasta, P., Messier, M., Jacquet, J.M. and Van Herck, K. (2012). Antibody persistence and immune memory in adults, 15 years after a three-dose schedule of a combined hepatitis A and B vaccine. *J Med Virol* 84(1): 11-17.

Van Lieshout, L., Stelma, F.F., Guisse, F., Falcao Ferreira, S.T., Polman, K., van Dam, G.J., Diakhate, M., Sow, S., Deelder, A. and Gryseels, B. (1999). The contribution of host-related factors to low cure rates of praziquantel for the treatment of *Schistosoma mansoni* in Senegal. *Am J Trop Med Hyg* 61(5): 760-765.

Van Schaijk, B.C., Ploemen, I.H., Annoura, T., Vos, M.W., Foquet, L., van Gemert, G.J., Chevalley-Maurel, S., van de Vegte-Bolmer, M., Sajid, M., Franetich, J.F., Lorthiois, A., Leroux-Roels, G., Meuleman, P., Hermsen, C.C., Mazier, D., Hoffman, S.L., Janse, C.J., Khan, S.M. and Sauerwein, R.W. (2014). A genetically attenuated malaria vaccine candidate based on *P. falciparum* b9/slarp gene-deficient sporozoites. *Elife* 3: e03582.

Vendrame, C.M.V., Carvalho, M.D.T., Yamamoto, C.R.F., Nakhle, M.C., Carvalho, S.A. and Chieffi, P.P. (2001). Evaluation of anti-*Schistosoma mansoni* IgG antibodies in patients with chronic *Schistosomiasis mansoni* before and after specific treatment. *Rev Inst Med Trop Sao Paulo* 43: 153-159.

Vennervald, B.J., Booth, M., Butterworth, A.E., Kariuki, H.C., Kadzo, H., Ireri, E., Amaganga, C., Kimani, G., Kenty, L., Mwatha, J., Ouma, J.H. and Dunne, D.W. (2005). Regression of hepatosplenomegaly in Kenyan school-aged children after praziquantel treatment and three years of greatly reduced exposure to *Schistosoma mansoni*. *Trans R Soc Trop Med Hyg* 99(2): 150-160.

Vercruysse, J. and Gabriel, S. (2005). Immunity to schistosomiasis in animals: an update. *Parasite Immunol* 27(7-8): 289-295.

Vereecken, K., Naus, C.W., Polman, K., Scott, J.T., Diop, M., Gryseels, B. and Kestens, L. (2007). Associations between specific antibody responses and resistance to reinfection in a Senegalese population recently exposed to *Schistosoma mansoni*. *Trop Med Int Health* 12(3): 431-444.

Vittinghoff, E. (2012). Regression methods in biostatistics : linear, logistic, survival, and repeated measures models. New York, Springer.

Voller, A., Bartlett, A. and Bidwell, D.E. (1978). Enzyme immunoassays with special reference to ELISA techniques. *J Clin Pathol* 31(6): 507-520.

Waine, G.J. and McManus, D.P. (1997). Schistosomiasis vaccine development-the current picture. *BioEssays* 19(5): 435-443.

Walker, E., Hernandez, A.V. and Kattan, M.W. (2008). Meta-analysis: Its strengths and limitations. *CCJM* 75(6): 431-439.

Walter, K., Fulford, A.J.C., McBeath, R., Joseph, S., Jones, F.M., Kariuki, H.C., Mwatha, J.K.,

Kimani, G., Kabatereine, N.B., Vennervald, B.J., Ouma, J.H. and Dunne, D.W. (2006). Increased human IgE induced by killing *Schistosoma mansoni* in vivo is associated with pretreatment Th2 cytokine responsiveness to worm antigens. *J Immunol* 177(8): 5490-5498.

Wang, W., Dai, J.R., Li, H.J., Shen, X.H. and Liang, Y.S. (2012). The sensitivity of *Schistosoma japonicum* to praziquantel: a field evaluation in areas with low endemicity of China. *Am J Trop Med Hyg* 86(5): 834-836.

Ward, R.D., Lewis, F.A., Yoshino, T.P. and Dunn, T.S. (1988). *Schistosoma mansoni*: relationship between cercarial production levels and snail host susceptibility. *Exp Parasitol* 66(1): 78-85.

Webster, B.L., Diaw, O.T., Seye, M.M., Faye, D.S., Stothard, J.R., Sousa-Figueiredo, J.C. and Rollinson, D. (2013). Praziquantel treatment of school children from single and mixed infection foci of intestinal and urogenital schistosomiasis along the Senegal River Basin: monitoring treatment success and re-infection patterns. *Acta Trop* 128(2): 292-302.

Webster, M., Correa-Oliveira, R., Gazzinelli, G., Viana, I.R., Fraga, L.A., Silveira, A.M. and Dunne, D.W. (1997). Factors affecting high and low human IgE responses to schistosome worm antigens in an area of Brazil endemic for *Schistosoma mansoni* and hookworm. *Am J Trop Med Hyg* 57(4): 487-494.

WHO (2002). Prevention and control of schistosomiasis and soil-transmitted helminthiasis. WHO Technical Report Series. Geneva, Switzerland, World Health Organization.

WHO (2006). Preventive chemotherapy in human helminthiasis. Coordinated use of anthelminthic drugs

in control interventions: a manual for health professionals and programme managers. Geneva, Switzerland, World Health Organization.

WHO (2011). Report of a meeting to review the results of studies on the treatment of schistosomiasis in preschool-age children. Geneva, Switzerland, World Health Organization.

WHO (2013). Assessing the efficacy of anthelminthic drugs against schistosomiasis and soil-transmitted helminthiases. Geneva, Switzerland, World Health Organization.

WHO. (2015) Schistosomiasis control and preventive chemotherapy. Retrieved Dec., 2015, from http://www.who.int/schistosomiasis/strategy/en/.

WHO. (2015) Schistosomiasis Fact sheet N°115. Retrieved Aug., 2015, from http://www.who.int/mediacentre/factsheets/fs115/en/.

WHO. (2016) Tables of malaria vaccine projects globally. Retrieved 8th Februaly, 2016, from http://www.who.int/immunization/research/development/Rainbow_tables/en/.

WHO. (2016) WHO urges increased access to praziquantel as schistosomiasis treatment coverage

improves. Retrieved August, 2016, from http://www.who.int/neglected_diseases/news/WHO_urges_increased_access_to_praziquantel/en/.

Wilkins, H.A., Goll, P.H., Marshall, T.F. and Moore, P.J. (1984). Dynamics of *Schistosoma haematobium* infection in a Gambian community. I. The pattern of human infection in the study area. *Trans R Soc Trop Med Hyg* 78(2): 216-221.

Wilson, R.A. and Coulson, P.S. (1999). Strategies for a schistosome vaccine: can we manipulate the immune response effectively? *Microbes Infect* 1(7): 535-543.

Wilson, R.A. and Coulson, P.S. (2006). The development of a schistosome vaccine. Parasitic flatworms molecular biology, biochemistry, immunology and physiology. Maule, A. G. and Marks, N. J. Cambridge, CABI Pub: 303-326.

Wilson, R.A., Coulson, P.S. and Dixon, B. (1986). Migration of the schistosomula of *Schistosoma mansoni* in mice vaccinated with radiation-attenuated cercariae, and normal mice - an attempt to identify the timing and site of parasite death. *Parasitology* 92(1): 101-116.

Wilson, S., Jones, F.M., Fofana, H.K.M., Doucoure, A., Landoure, A., Kimani, G., Mwatha, J.K., Sacko, M., Vennervald, B.J. and Dunne, D.W. (2013). Rapidly boosted plasma IL-5 induced by treatment of human schistosomiasis haematobium is dependent on antigen dose, IgE and eosinophils. *PLoS Negl Trop Dis* 7(3).

Woolhouse, M.E. (1992). A theoretical framework for the immunoepidemiology of helminth infection. *Parasite Immunol* 14(6): 563-578.

Woolhouse, M.E. (1998). Patterns in parasite epidemiology: the peak shift. *Parasitol Today* 14(10): 428-434.

Woolhouse, M.E. and Hagan, P. (1999). Seeking the ghost of worms past. *Nat Med* 5(11): 1225-1227.

Xia, J., Yuan, Y., Xu, X., Wei, F., Li, G., Liu, M., Li, J., Chen, R., Zhou, Z. and Nie, S. (2014). Evaluating the effect of a novel molluscicide in the endemic schistosomiasis japonica area of China. *Int J Environ Res Public Health* 11(10): 10406-10418.

Xiao, S.H., Mei, J.Y. and Jiao, P.Y. (2009). The in vitro effect of mefloquine and praziquantel against juvenile and adult *Schistosoma japonicum*. *Parasitol Res* 106(1): 237-246.

Yang, G.J., Li, W., Sun, L.P., Wu, F., Yang, K., Huang, Y.X. and Zhou, X.N. (2010). Molluscicidal efficacies of different formulations of niclosamide: result of meta-analysis of Chinese literature. *Parasit Vectors* 3: 84.

Yole, D.S., Pemberton, R., Reid, G.D.F. and Wilson, R.A. (1996). Protective immunity to *Schistosoma mansoni* induced in the olive baboon *Papio anubis* by the irradiated cercaria

vaccine. *Parasitology* 112(1): 37-46.

Yuan, Y., Dong, H.F., Xu, X.J., Li, G.L., Wei, F.H., Zhao, Y.B., Tu, Z.W., Liu, M., Cao, M.M., He, H., Tang, L., Zhu, H. and Fan, H.P. (2011). Evaluation of a new molluscicide for counteracting the intermediate snail host of *Schistosoma japonicum*. *Malacologia* 53(2): 217-227.

Zhang, Z., Wu, H., Chen, S., Hu, L., Xie, Z., Qiu, Y., Su, C., Cao, J.P., Wu, Y., Zhang, S. and Wu, G. (1997). Association between IgE antibody against soluble egg antigen and resistance to reinfection with *Schistosoma japonicum*. *Trans R Soc Trop Med Hyg* 91(5): 606-608.

Zhou, L.Y., Deng, Y., Steinmann, P. and Yang, K. (2013). The effects of health education on schistosomiasis japonica prevalence and relevant knowledge in the People's Republic of China: a systematic review and meta-analysis. *Parasitol Int* 62(2): 150-156.

Zwang, J. and Olliaro, P.L. (2014). Clinical efficacy and tolerability of praziquantel for intestinal and urinary schistosomiasis-a meta-analysis of comparative and non-comparative clinical trials. *PLoS Negl Trop Dis* 8(11): e3286.