Novel methods to expedite schistosome development

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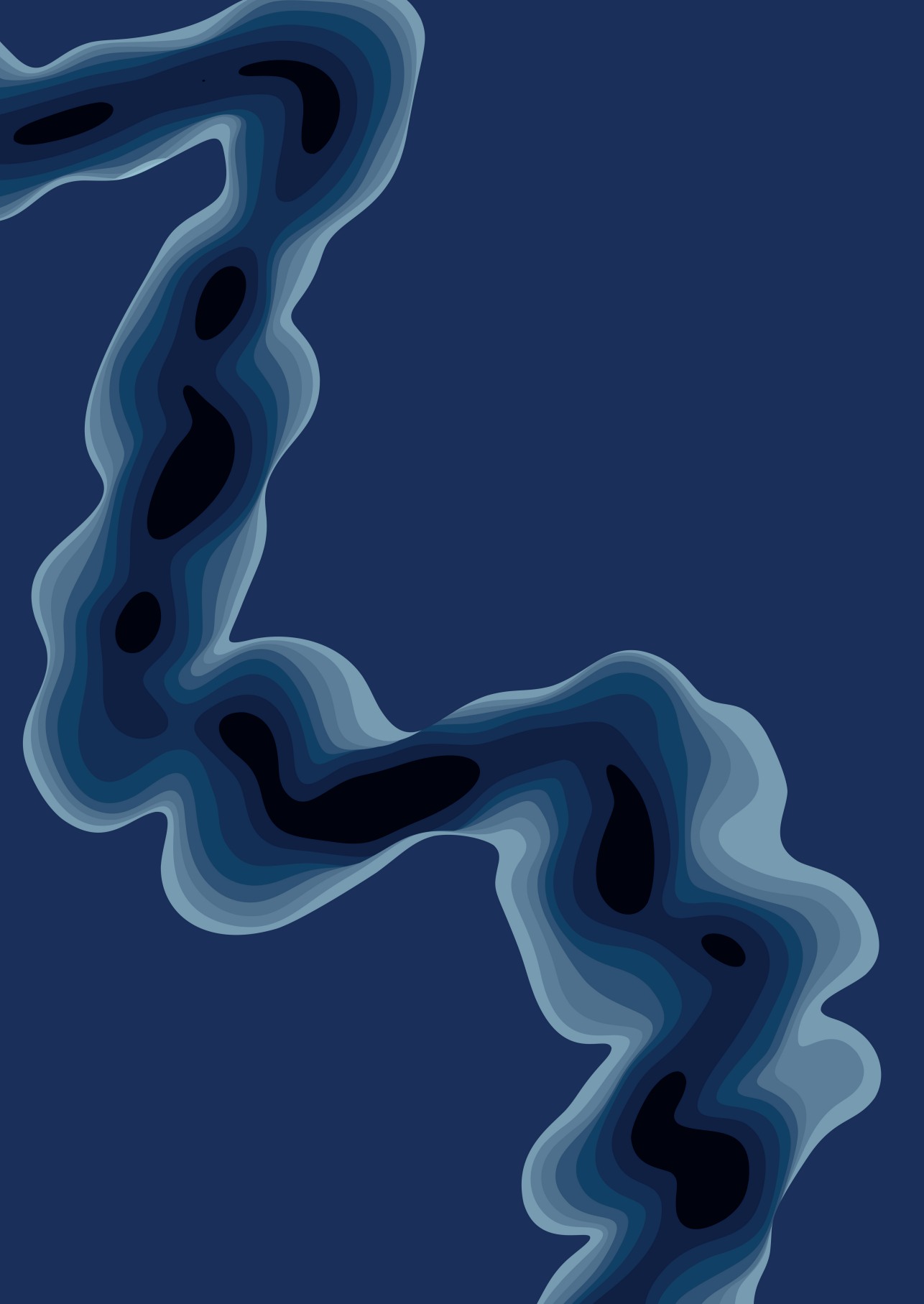

Chapter 1

General Introduction

Schistosomiasis, a parasitic infection with blood flukes of the genus *Schistosoma*, remains highly prevalent, despite extensive control efforts. With more than 150 million people infected with schistosomes worldwide (1), it ranks among the most important neglected tropical diseases (2). Infection occurs through contact with (fresh)water that contains cercariae, the infective larval stage of *Schistosoma* worms, which penetrate the skin, migrate through the lung and end up in and the vessels around the gut (for *Schistosoma mansoni* and *Schistosoma japonicum*) or genito-urinary tract (for *Schistosoma haematobium*). Here they mate and start producing eggs, which are subsequently excreted through faeces or urine into the environment, where they hatch and continue to infect snails, completing the lifecycle. The initial or acute stages of infection are characterised by potential local skin reactions, i.e. cercarial dermatitis, soon after exposure, and acute schistosomiasis syndrome, a transient systemic inflammatory response with flu-like symptoms to the migrating and maturing schistosome (3-8 weeks after infection) (3). Later stages of (chronic) infection are characterised by egg production and egg deposition in tissue triggering a local granulomatous, inflammatory reaction, which are at the core of most severe morbidity observed, such as portal hypertension and haematuria (4).

Schistosomiasis can be diagnosed in various ways (5). The most commonly used method is egg microscopy in stool or urine. The number of eggs is used to determine the infection intensity which ranges from mild, moderate to heavy (6). Other ways to diagnose infection include antigen-based tests, antibody tests, and molecular diagnostics. In particular, the circulating anodic antigen (CAA) test that detects worm-derived CAA in serum or urine is increasingly used in research and clinical settings for diagnosis and treatment evaluation, due to its high sensitivity. For diagnosis of schistosomiasis in returning travellers in non-endemic settings, antibody testing is also suitable, however because antibodies remain detectable for longer periods of time, it is challenging to discriminate active vs previous infection or to monitor treatment effects. Control of schistosomiasis in endemic settings relies on intermittent praziquantel (PZQ) treatment in mass drug administration (MDA) programs (7). However, this approach falls short because reinfection rates are high (8) and praziquantel is ineffective against juvenile worms (9).

ELIMINATING SCHISTOSOMIASIS AS A PUBLIC HEALTH PROBLEM

In its 2020 roadmap, the World Health Organization has set an ambitious target of eliminating schistosomiasis as a public health problem by 2030 through scaling up mass drug administration, WASH interventions, environmental interventions, and behavioural change interventions to reduce transmission and to improve testing (10). Although many national control programs have incorporated various abovementioned measures, decreasing disease burden will be a tremendous challenge and will require sustained effort. Disruptions in schistosomiasis control programs, as seen during the COVID-19 pandemic, can potentially delay achieving elimination of schistosomiasis as a public health problem by up to two years depending on the epidemiological setting (11). This should make us mindful that the commendable progress in reaching disease-control targets that has been achieved in recent years (12) is precarious and new tools for disease control should be pursued.

VACCINES AGAINST SCHISTOSOMIASIS

Unlike for many other infectious diseases, there is currently no licensed vaccine available for schistosomiasis. Modelling studies project that the addition of an efficacious vaccine will increase the likelihood of achieving elimination of schistosomiasis as a public health problem (13). However, the development of vaccines against parasitic infections is complicated for different reasons. Biologically, helminths are complex, multicellular organisms that undergo several developmental stages in humans, yet that have at each stage developed intricate mechanisms to evade immune attack by the human host (14, 15). Moreover, funding into schistosomiasis vaccine research, as for many other neglected tropical diseases, is limited by the lack of commercial interest. Yet despite these hurdles, four vaccine candidates have gone into clinical testing, namely Sh28GST, Sm-14, Sm-TSP2-2 and Sm-p80. All predominantly target *Schistosoma mansoni,* except Sh28GST which targets *Schistosoma haematobium*. An overview of these vaccine candidates and study results is given in **Table 1**. Of these, only Sh28GST has progressed to a phase III study in Senegalese schoolchildren, but unfortunately failed to demonstrate efficacy, despite good immunogenicity (16).

Table 1. Overview of schistosomiasis vaccine candidates in clinical testing.

Vaccine (target)	Adjuvant	Phase I	Phase II	Phase III
rSh28GST	Alhydrogel®	France, 1999 **Safe, immunogenic** (NCT01512277) (17)	Senegal, year unclear, data not published	Senegal 2009-2012 **Safe, immunogenic, but not efficacious** (NCT00870649) (16)
rSm-14	GLA-SE	Brazil, 2011-2014 **Safe, immunogenic** (NCT01154049) (18, 19)	Senegal (2022-now) (NCT05658614)	-
rSm-TSP-2	Alhydrogel® and/or GLA-AF	US 2015-2017, Brazil 2018-2019 **Safe, immunogenic** (NCT02337855; NCT03110757) (20, 21)	Uganda (2019-now) (NCT03910972)	-
rSm-p80	GLA-SE	US (2022-now) (NCT05292391)	Madagascar & Burkina Faso (2023-now) (NCT05762393)	-

NCT numbers refer to clinicaltrials.gov numbers. Colour coding: blue = completed and published, yellow = ongoing

CONTROLLED HUMAN INFECTIONS WITH SCHISTOSOMES

Traditionally, vaccine candidates sequentially move through different testing phases (I, II, to III) that require increasingly large sample sizes and resources. Unfortunately, only few candidates show sufficient efficacy in phase III studies to ultimately become licensed, incurring a substantial waste of resources along the way. To remedy this, controlled human infections can be used where a small number of (healthy) volunteers are intentionally exposed to a pathogen at a fixed time. By doing so, they provide an early estimate of vaccine efficacy in a relatively short period of time that can guide selection of vaccine candidates for larger field studies.

Controlled human infection models (CHIMs) have been established for a wide variety of pathogens, including other helminths (22), but (prior to this work) had not been established for schistosomes. CHIMs are carefully designed with various ethical considerations in mind. It should, for instance, not lead to irreversible harm in participants (23). Although a controlled human infection with schistosomes (CHI-S) would be valuable for schistosomiasis vaccine development, accumulation of eggs and resulting symptomology presents a significant health risk for participants. As such, a mixed-sex CHI-S,

i.e. exposing participants to male and female cercariae with resulting egg production, would not be considered acceptable. A way to avoid these risks, is to expose participants to a single-sex infection with only male or only female cercariae. Single-sex cercariae are produced by exposing a snail to a single miracidium (male or female) (24). After about five weeks, an infected snail will then start shedding cercariae that are either male or female. The sex cannot be distinguished microscopically, and therefore needs to be determined by PCR on the W1 repeat and ITS region. After passing rigorous quality checks including microbial burden of snails, the cercariae can then be administered to participants by pipetting a pre-defined dose onto the skin in mineral water resembling the natural route of infection **(Figure 1)**. After exposure, participants frequently visit the study centre for safety checks and sample collection. In the absence of eggs, infection status is determined by measuring serum circulating anodic antigen (CAA) excreted by juvenile and adult worms. Afterwards, participants are treated with PZQ to cure infection.

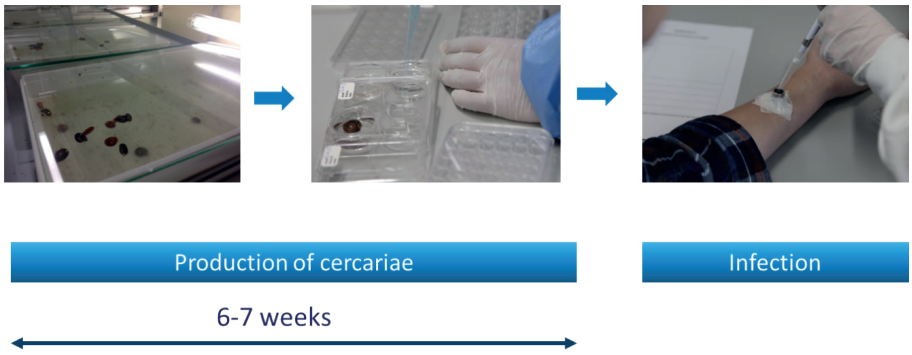

Figure 1. Production of single-sex cercariae for controlled human infection with schistosomes (CHI-S). To produce single-sex cercariae, a single snail is exposed to a single miracidium (mono-miricidial) and housed separately. After five weeks, successfully infected snails start shedding cercariae that are either male or female. The sex is determined by PCR. After quality checks, that include microbial burden assessment, the cercariae are released by our pharmacist. The controlled infection takes place 6-7 weeks after mono-miricidial snail infection by pipetting a pre-defined number of single-sex cercariae in water onto the skin of study participants.

CHALLENGES AND OPPORTUNITIES FOR CHI-S IN SCHISTOSOMIASIS VACCINE DEVELOPMENT

The merits of CHI-S are not limited to vaccine evaluation, but also lie in exploring host-pathogen interactions to help identify potential new vaccine targets and (diagnostic) biomarkers (25). In particular, early stages of infection

have been difficult to study in endemic settings or in returning travellers where timing of exposure is often unclear or diagnosis is only made in later stages of (egg-producing) infection. By design, CHI-S differ substantially from natural infection, because of the single-sex nature, infectious dose, and challenge strain, similar to any other experimental (animal) model. Nevertheless, CHI-S enables us to explore human schistosomiasis in novel ways, for instance in repeat infection studies. A better understanding of immune responses to reinfections may provide leads for new vaccine targets or strategies. Similarly, comparing single-sex male infections and single-sex female infections can help shed light on sex-specific immune responses.

Previously, controlled human infections for tropical diseases were usually performed in non-endemic settings in participants without any prior exposure to the pathogen. However, because of variations in genetics, environmental factors, and (previous) pathogen exposure, responses to vaccines may differ between endemic and non-endemic populations, as seen with malaria, BCG, yellow fever, and rotavirus vaccines (26). Fortunately, there has been a push towards strengthening research capacity to perform controlled human infections in endemic settings (27). To explore establishment of CHI-S in Uganda, where schistosomiasis endemicity is high, a stakeholder's meeting was held in 2017 with regulators, community members, researchers, and policy-makers (28). CHI-S in Uganda was thought to be both feasible and desirable. Several key next steps were formulated before implementation, including a risk assessment for importing laboratory vector snails and schistosome strains from The Netherlands.

To further increase chances of successful vaccine licensure, immunological and methodological aspects of schistosomiasis vaccine development, particularly when transitioning from phase II to phase III, need to be carefully considered. For instance, prior exposure to schistosomes or other infections may affect vaccine efficacy. Moreover, there is ongoing debate on the role of praziquantel (pre-) treatment on protective vaccine responses to the point that praziquantel codelivery was identified as a potential reason for Sh28GST vaccine failure (16). Early identification of these potential interactions can steer study design choices that improve study quality and impact.

SCOPE AND OUTLINE OF THIS THESIS

This thesis explores novel methods to expedite schistosomiasis vaccine development, particularly using controlled human infections with schistosomes. In **chapter 2** and **chapter 3**, we describe the development of single-sex CHIM with *Schistosoma mansoni* using male or female cercariae, respectively. Both were dose-finding studies in which *Schistosoma*-naïve participants were exposed to pre-defined low doses (10, 20 or 30) of cercariae and followed-up over time to assess safety and infectivity.

Given the potential influence of prior exposure to schistosomes, among others, on (vaccine) immune responses, we think it is valuable to transfer the infection model to endemic settings. **Chapter 4** discusses the potential risks related to implementing single-sex controlled human infections in Uganda and aims to provide mitigation strategies to minimise these risks.

Chapter 5 and **chapter 6** focus on the use of CHI-S for vaccine development. In **chapter 5,** we have used the controlled human infection model to investigate whether repeated exposure and treatment leads to protection from reinfection and to identify potential correlates of protection or novel vaccine targets. In **chapter 6**, we present the study protocol for a CHI-S vaccine study. This study will assess the protective efficacy of three immunisations with Sm-p80 + GLA-SE against controlled infection male *Schistosoma mansoni* cercariae in healthy Schistosome-naïve volunteers.

In **chapter 7** and **chapter 8**, we reflect on immunological and methodological aspects of schistosomiasis vaccine development, particularly transitioning from phase II to phase III.

Finally, in the discussion (**chapter 9**) we summarise the main findings of this thesis and discuss these in a broader context.

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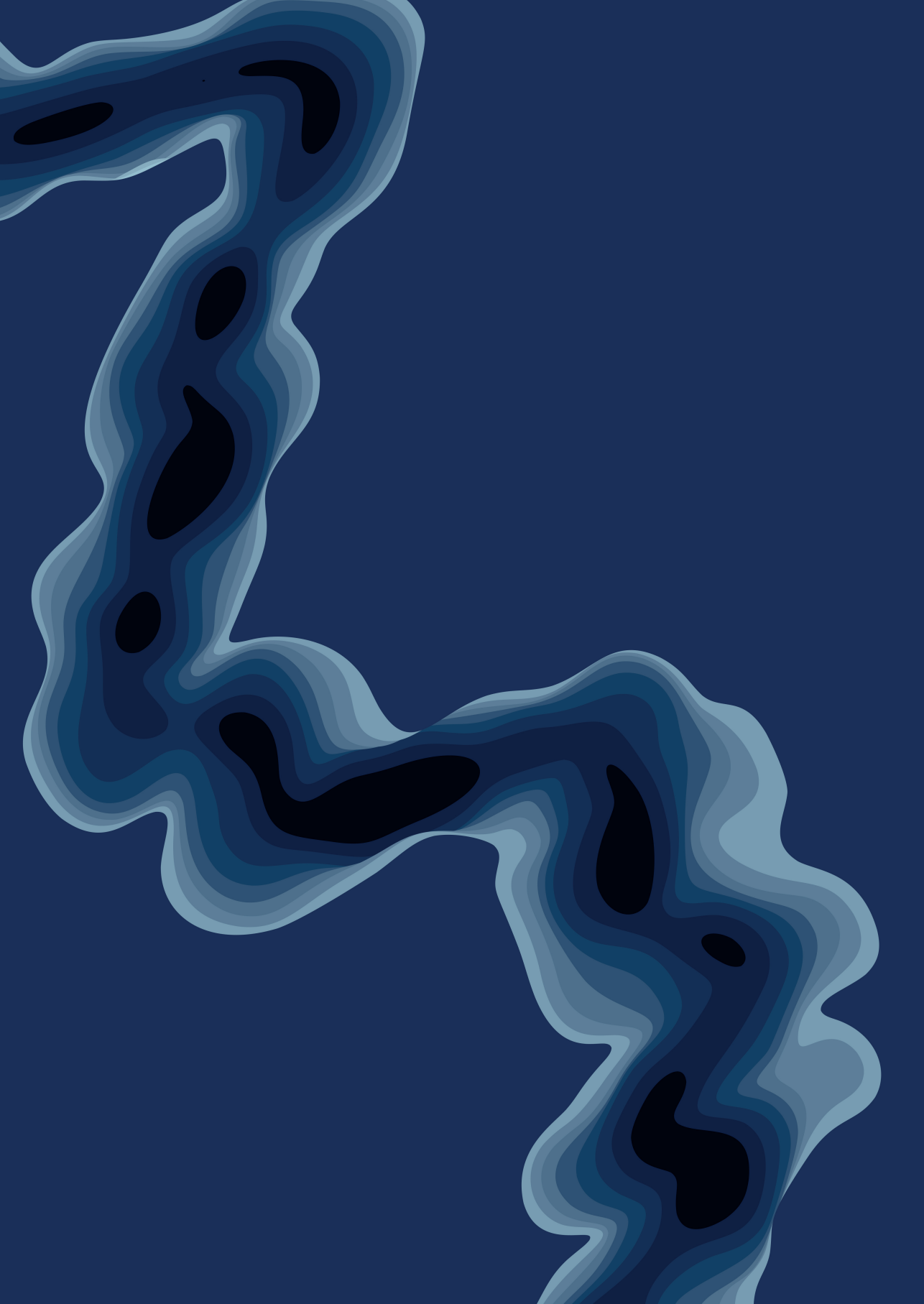

Chapter 2

A controlled human *Schistosoma mansoni* infection model to advance novel drugs, vaccines and diagnostics

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ABSTRACT

Schistosomiasis treatment relies on the use of a single drug, praziquantel, which is insufficient to control transmission in highly endemic areas (1). Novel medicines and vaccines are urgently needed (2, 3). An experimental human model for schistosomiasis could accelerate the development of these products. We performed a dose-escalating clinical safety trial in 17 volunteers with male *Schistosoma mansoni* cercariae, which do not produce eggs (clinicaltrials.gov NCT02755324), at the Leiden University Medical Center, the Netherlands. The primary endpoints were adverse events and infectivity. We found a dose-related increase in adverse events related to acute schistosomiasis syndrome, which occurred in 9 of 17 volunteers. Overall, 5 volunteers (all 3 of the high dose group and 2 of 11 of the medium dose group) reported severe adverse events. Worm-derived circulating anodic antigen, the biomarker of the primary infection endpoint, peaked in 82% of volunteers at 3–10 weeks following exposure. All volunteers showed IgM and IgG1 seroconversion and worm-specific cytokine production by $CD4^+$ T cells. All volunteers were cured with praziquantel provided at 12 weeks after exposure. Infection with 20 *Schistosoma mansoni* cercariae led to severe adverse events in 18% of volunteers and high infection rates. This infection model paves the way for fast-track product development for treatment and prevention of schistosomiasis.

INTRODUCTION

Worldwide, 290 million people are infected with schistosomes, mainly *Schistosoma haematobium* and *Schistosoma mansoni* (4). The endemicity is determined by the presence of the fresh water snail intermediate host. Snail-derived cercariae penetrate the human skin and migrate into the vascular system, where mature male and female worms mate and produce ~300 eggs per day. *S. mansoni* eggs provoke inflammatory responses, which can lead to liver cirrhosis and portal hypertension (5).

Current treatment and control of schistosomiasis relies on the use of a single drug, praziquantel. Mass drug administration with praziquantel does not protect from reinfection (1) and thus provides insufficient control in highly endemic areas, creating the need for a vaccine (2).

Several schistosome antigens have been put forward as possible *S. mansoni* vaccine candidates, of which three are in clinical development: Sm-TSP-2, rSm14/GLA-SE and Sm-p80 (6-8) . These candidates aim for >40% reduction in worm load for World Health Organization endorsement (9), but higher levels are preferred (10, 11). To obtain efficacy data, large phase II and III field trials in *Schistosoma*-endemic areas are needed (12). In addition, increasing concerns of praziquantel resistance create a need for anti-schistosomal drug development (3). Controlled human infection (CHI) trials can select drug and vaccine candidates early in clinical development and help prevent late clinical failure (12). We thus aimed to develop a schistosome CHI model to aid vaccine and drug development and better characterize human anti-schistosome immune responses.

Between September 2016 and January 2018, 35 healthy adult volunteers were screened, of which 17 were included in the trial and completed follow up (**Figure 1**). Baseline demographics between dose groups were comparable (**Supplementary Tables 1 and 2**).

Volunteers were exposed to 10, 20 or 30 cercariae in water on the forearm skin for 30 min, after which they were followed bi-weekly for adverse events and infectivity. After exposure, water was pipetted off the skin and inspected for remaining cercariae. We found tails of roughly half the number of the cercariae that they were exposed to, with clear differences between exposure groups (r = 0.70, P = 0.002; **Supplementary Table 1**).

RESULTS

Safety

The majority of volunteers (15 of 17, 88%) experienced pruritus during or after exposure, but no topical treatment was required (**Supplementary Table 3**). All but one volunteer (16 of 17, 94%) developed a mild local cercarial dermatitis within 2 d after exposure (**Figure 2a–c**), which lasted longer in higher dose groups (10 cercariae: median 1 week (range 1–2); 20 cercariae: 3 weeks (1–9); 30 cercariae: 6 weeks (2–7), $r = 0.45$, $P = 0.07$).

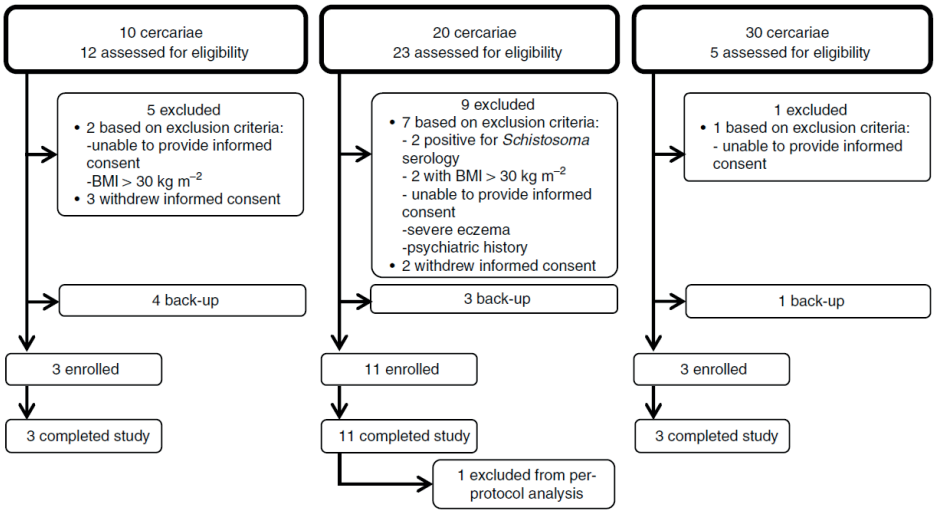

Figure 1. Study flow diagram. The inclusion and exclusion of volunteers. The first group (n = 3 volunteers) was exposed to 10 cercariae and the second group (n = 3 volunteers) to 30 cercariae. Thereafter the dose was de-escalated to 20 cercariae for the third group (n = 3 volunteers) and validated in a fourth group (n = 8 volunteers). In total eight volunteers served as backup for included volunteers in case an included volunteer might drop out before exposure to cercariae.

There were no serious adverse events (AEs) (an event that is life-threatening or requires hospitalization), but nine severe related AEs (resulting in the inability to perform daily activity) were reported in volunteers from the 20 ($n = 2$) or 30 cercariae ($n = 3$) group. Seven of these severe AEs were symptoms of an acute schistosomiasis syndrome ($n = 5$). In the 30 cercariae group all volunteers ($n = 3$) experienced severe AEs, starting 2.5 to 5.0 weeks after exposure (**Figure 2d–f**) as follows: headache ($n = 2$), fever ($n = 2$), syncope ($n = 1$), nausea ($n = 1$) and elevated liver enzymes ($n = 1$) (**Supplementary Table 3**). In one volunteer this episode was followed by mild to moderate headaches, malaise, fatigue and nocturnal sweats for up to 6 weeks as previously described [13], but steroid

treatment was declined. Given the burden of these prolonged symptoms of an acute schistosomiasis syndrome (Katayama symptoms), defined as moderate to severe symptoms of malaise, fatigue, fever, night sweats, flu-like symptoms or headache, between 2 to 7 weeks after exposure, the dose was de-escalated to 20 cercariae. Subsequently, 2 of 11 (18%) volunteers reported one severe symptom of an acute schistosomiasis syndrome (headache, nocturnal fever and sweats).

In addition to these five volunteers with severe AEs there were four volunteers, all exposed to 20 cercariae, with moderate symptoms of an acute schistosomiasis syndrome (flu-like symptoms, *n* = 3; and malaise, *n* = 2). Eight volunteers did not experience any symptoms of an acute schistosomiasis syndrome.

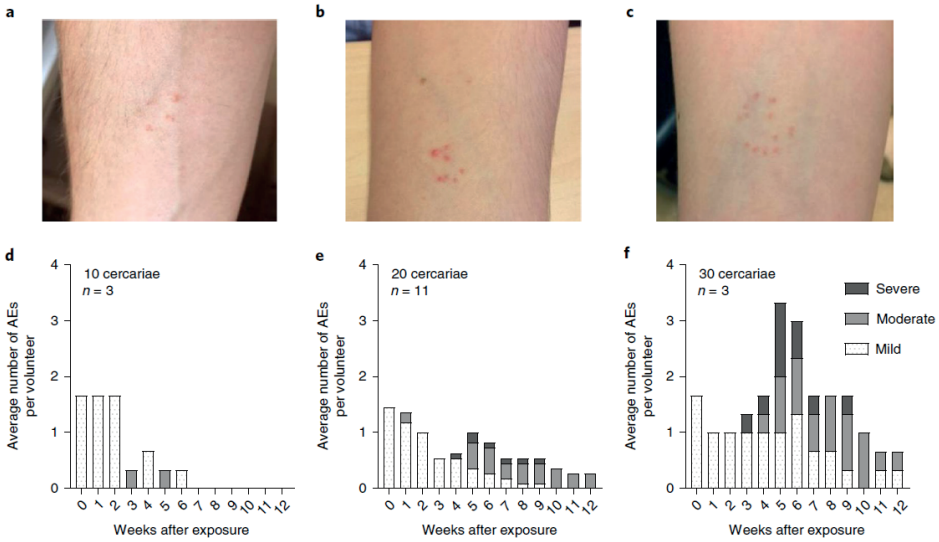

Figure 2. Adverse events. a–c, Pictures of volunteers with the most pronounced cercarial dermatitis taken 5 d after infection from the group infected with 10 cercariae (**a**), 20 cercariae (**b**) or 30 cercariae (**c**). **d–f**, The average number of related AEs per volunteer at each week for volunteers exposed to 10 cercariae (**d**), 20 cercariae (**e**) or 30 cercariae (**f**). For each AE the highest grading score is plotted. White bar, mild AE; light gray bar, moderate AE; dark gray bar, severe AE.

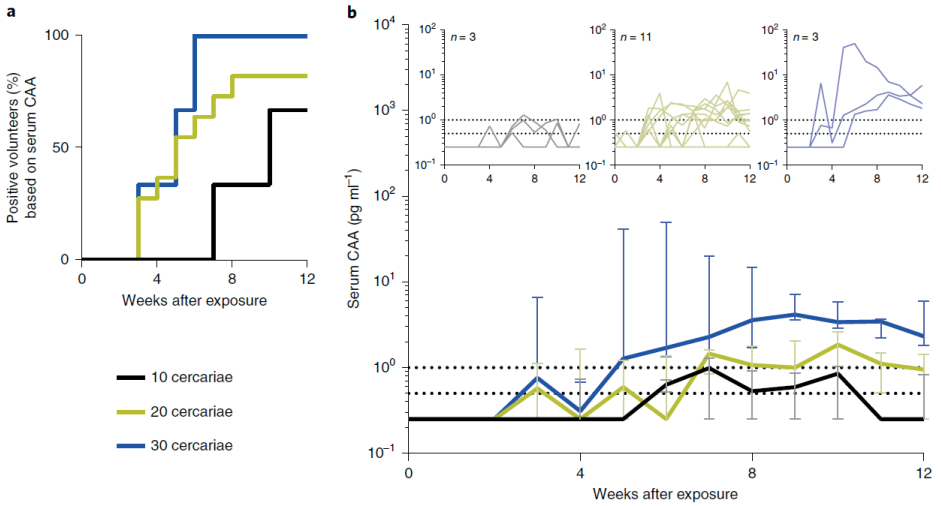

Figure 3. Pre-patent period and serum CAA levels. a, The time to patency (serum CAA level > 1 pg ml^{-1}) in weeks per dose group (log-rank test, P = 0.21). **b**, The serum CAA levels for individual volunteers in pg ml^{-1} (top) and the median serum CAA levels and interquartile range for groups infected with 10, 20 or 30 cercariae (bottom). The gray, yellow and blue lines represent data of individual volunteers or groups exposed to 10 (n = 3), 20 (n = 11) or 30 (n = 3) cercariae respectively.

Eosinophils

Eosinophils increased in 11 of 17 (65%) volunteers peaking between week 2 and 8 after infection (0.1–8.4 × 10^9 l^{-1}; **Extended Data Figure 1**). Eosinophils were not related to the dose or symptoms (**Extended Data Figure 2a**).

Infection rates by antigen detection assays

Both worm-excreted circulating anodic antigen (CAA) and circulating cathodic antigen (CCA) were measured to determine the presence and degree of infection. In two of three volunteers exposed to 10 cercariae the serum CAA was higher than 1 pg ml^{-1}. In the higher dose groups, 9 out of 11 (82%) volunteers exposed to 20 cercariae and all volunteers exposed to 30 cercariae crossed this threshold. The time to patency was comparable between these groups (range 3–8 weeks, **Figure 3a**).

Cercarial dose and serum CAA levels were related (10 cercariae median at week 7–12: 0.4 pg ml^{-1} (range 0.3–0.8); 20 cercariae: 1.2 pg ml^{-1} (0.3–1.9); and 30 cercariae: 3.6 pg ml^{-1} (2.0–6.5), r = 0.70, P = 0.002) (**Figure 3b**). The volunteer experiencing the most severe AEs had at least sevenfold higher serum CAA levels (maximum 49.9 pg ml^{-1}) than other volunteers. There was no

significant correlation between serum CAA levels and symptoms of an acute schistosomiasis syndrome (**Extended Data Figure 2b**) or eosinophils (data not shown).

Urine CAA levels were variable (**Extended Data Figure 3a,b**), but correlated with serum CAA ($r = 0.58$, $P < 0.0001$). The point-of-care rapid test for CCA (POC-CCA) was positive in 12% (2 of 17) of volunteers within 12 weeks after exposure and did not correlate with serum or urine CAA levels.

Praziquantel treatment

After a single 40 mg kg^{-1} dose of praziquantel treatment 12 weeks after exposure, serum CAA levels dropped below the detection limit in 8 out of 14 (57%) positive volunteers. The remaining 43% of volunteers were treated again with praziquantel, after which all remained undetectable until 1 year after exposure (**Extended Data Figure 4a–c**).

Serology

All volunteers showed seroconversion of IgM against adult worms by immunofluorescence assay (IFA) (**Figure 4a,b**). Seroconversion became apparent at week 4 in two volunteers and by week 6 in all. In addition, seven volunteers showed anti-soluble egg antigen (SEA) IgG seroconversion respectively at week 4 (1 of 16), week 12 (1 of 16) or week 20 (5 of 16). The absence of eggs was confirmed by a negative feces *Schistosoma* PCR at week 12 in all volunteers.

All volunteers showed an increase in adult worm antigen (AWA)-specific total IgG over time, with seroconversion above 2× s.d. of all baseline values in 12 of 16 volunteers at week 20. These responses were primarily IgG1, with seroconversion in all volunteers by week 16 (**Figure 4c,d**). There was a clear dose response in AWA-specific total IgG and IgG1 levels (IgG week 20: $r = 0.70$, $P = 0.003$; IgG1 week 16: $r = 0.56$, $P = 0.02$), and a trend toward higher IgG1 levels and symptoms of an acute schistosomiasis syndrome ($P = 0.08$) (**Extended Data Figure 2c**). No changes over time were found in total IgE or AWA-specific IgE and IgG4 compared to baseline (data not shown).

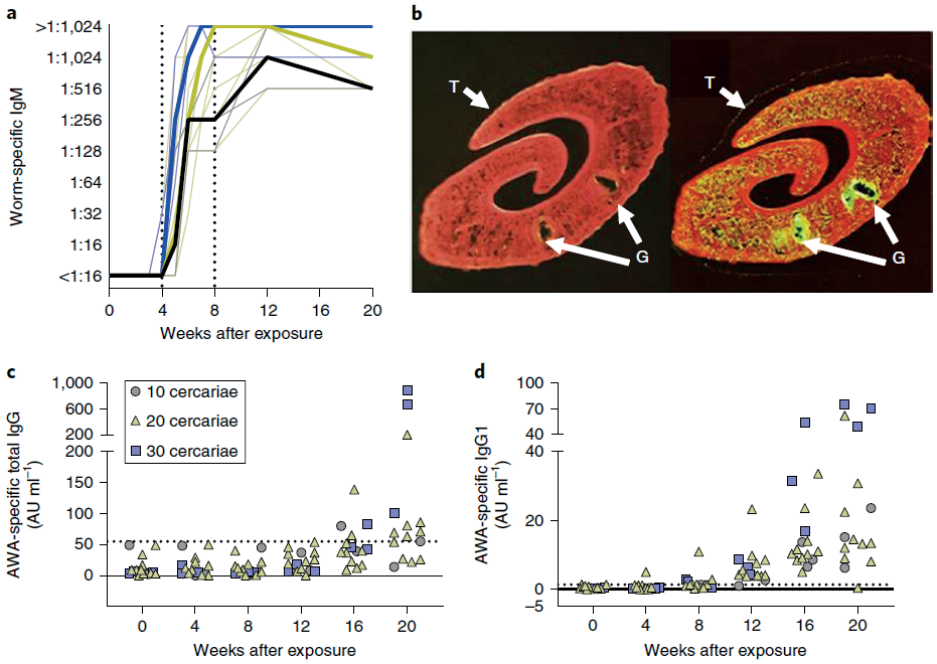

Figure 4. Humoral immune response. a, IgM against adult worm antigens over time. The gray, yellow and blue lines represent data of individual volunteers exposed to 10 (n = 3), 20 (n = 10) or 30 (n = 3) cercariae respectively and the darker lines represent the median of each group. **b**, IFA showing reactivity of volunteer IgM antibodies to Rossman-fixed S. mansoni adult worm sections (right) and pre-exposure negative serum (left). T, tegument; G, gastrodermis. **c**, AWA-specific total IgG for individual volunteers over time. **d**, AWA-specific IgG1 for individual volunteers over time. The gray circles, yellow triangles and blue squares represent data of individual volunteers exposed to 10 (n = 3), 20 (n = 10) or 30 (n = 3) cercariae respectively.

Cytokines

Cytokine and chemokine measurements in serum revealed increases in innate chemokines interferon (IFN)-γ- inducible protein (IP)-10 (**Figure 5a,b**) and macrophage inflammatory protein (MIP)-1β (**Figure 5c**), the latter of which was significantly higher in volunteers with symptoms of an acute schistosomiasis syndrome (P = 0.01 at week 8, **Figure 5d**). There were no detectable changes in the other circulating chemokines or cytokines measured in serum (data not shown).

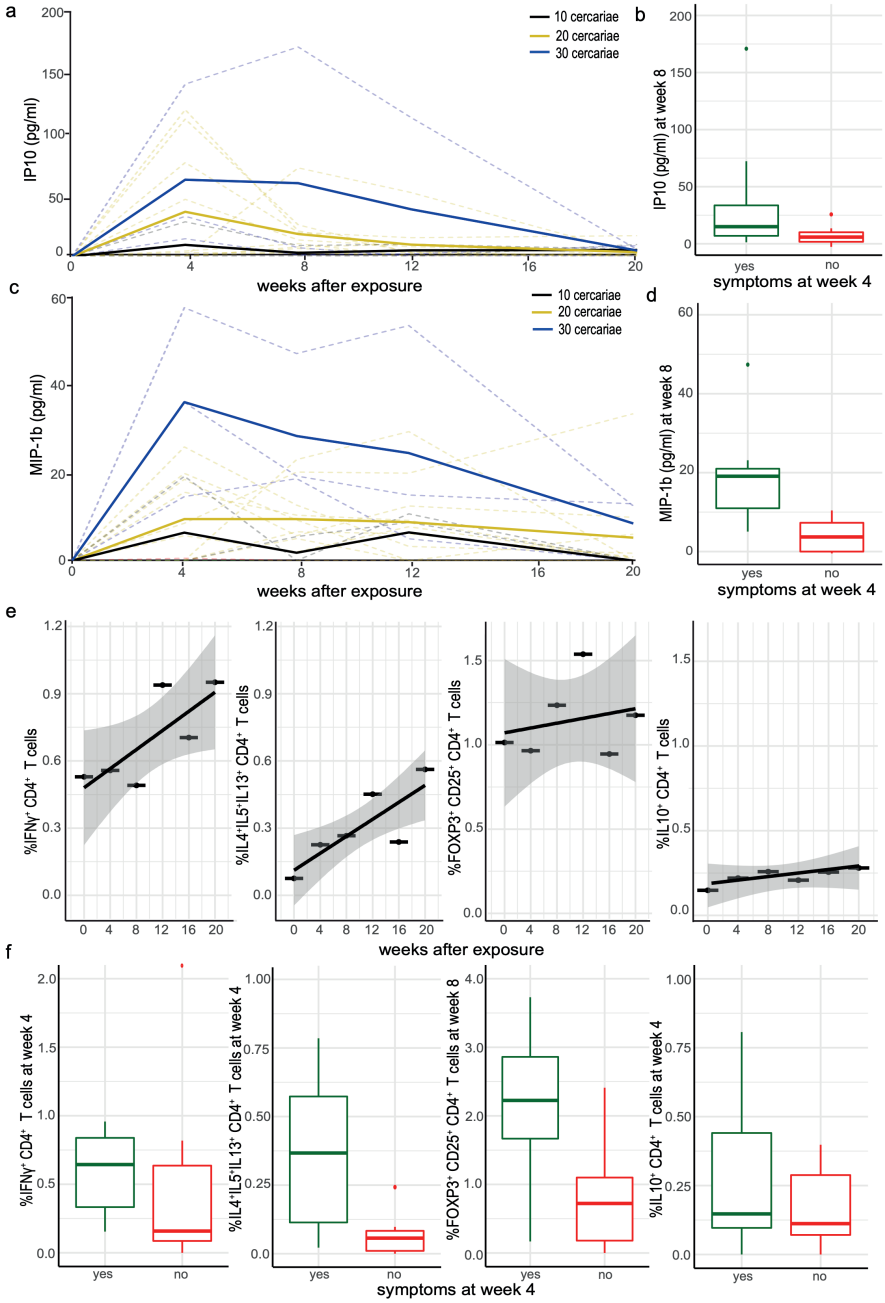

Figure 5. Cytokine responses. a, Ex vivo IP-10 over time for individual volunteers (dotted line) and the mean of each group (line). **b**, IP-10 levels at week 8 in volunteers with (yes, n = 8) or without (no, n = 8) symptoms of an acute schistosomiasis syndrome (two-sided Mann–Whitney U-test, P = 0.16). **c**, Ex vivo MIP-1β over time for individual volunteers (dotted line) and the mean

of each group (line). **d**, MIP-1β levels at week 8 in volunteers with (yes, n = 8) or without (no, n = 8) symptoms of an acute schistosomiasis syndrome at week 4 (two-sided Mann–Whitney U-test, P = 0.01). **e**, The percentage of IFN-γ-producing $CD4^+$ T cells (P = 0.01), Th2 cytokine (IL-4, IL-5 and IL-13)-producing $CD4^+$ T cells (P = 0.004), $FOXP3^+CD25^+CD4^+$ T cells (P = 0.49) and IL-10-producing $CD4^+$ T cells (P = 0.91) over time for all volunteers (n = 16), showing average of all volunteers at each week and fitted linear regression line with 95% confidence interval (linear mixed model with two-sided Student's t-test, using the Satterthwaite's degrees of freedom method). **f**, The percentage of IFN-γ-producing $CD4^+$ T cells (P = 0.28), Th2 cytokine (IL-4, IL-5 and IL-13)-producing CD4+ T cells (P = 0.01), $FOXP3^+CD25^+CD4^+$ T cells (P = 0.02) and IL-10-producing $CD4^+$ T cells (0.56) in volunteers with (yes, n = 8) or without (no, n = 8) symptoms of an acute schistosomiasis syndrome. In all boxplots, the lower and higher hinge are respectively the first and third quartiles and the middle line is the median (second quartile). The whiskers extend from the hinge to the largest value no further than 1.5× the interquartile range or distance between the first and third quartile. The largest and lowest visible points beyond the whiskers extend this range and can then be considered, respectively, the maxima and minima.

Cellular immunology

Overall, the frequency of antigen-specific IFN-γ (P = 0.01) and Th2 cytokine (interleukin (IL)-4, IL-5 and IL-13, P = 0.004)-producing $CD4^+$ T cells increased over time (**Figure 5e** and **Extended Data Figure 5a,b),** but $FOXP3^+CD25^+CD4^+$ regulatory T cells (P = 0.49) or the production of the regulatory cytokine IL-10 (P = 0.91, **Figure 5e**) did not. However, in volunteers with symptoms of an acute schistosomiasis syndrome, both IFN-γ and Th2 cytokine-producing $CD4^+$ T cells and $CD25^+FOXP3^+$ regulatory T cells were higher at week 4 and week 8 respectively, although not significantly for all (P = 0.28, P = 0.01 and P = 0.02, respectively; **Figure 5f**). There were no differences in IL-10-cytokine-producing $CD4^+$ T cells at week 4 (**Figure 5f**). At all timepoints 17% of IFN- γ^+CD4^+ T cells also produced IL-2.

Data integration

To understand which immunological and microbiological datasets were associated with the occurrence of symptoms of an acute schistosomiasis syndrome, we performed data integration using parallel generalized canonical correlation and partial least squares discriminant analysis. Eosinophil data decreased model accuracy and were thus removed (**Extended Data Figure 6a,b**). At baseline, 9 of 16 (56.3%) participants were accurately predicted by the model with leave-one-out cross-validation, which increased to 13 of 16 (81.3%) correct classifications at week 12 and further to 15 of 16 (93.8%) when data from all timepoints were included (**Figure 6a,b**). Thus, the model was able to accurately classify participants by the presence of an acute schistosomiasis syndrome. Permutation analysis confirmed that symptoms were strongly associated with the measured immunological and microbiological parameters

over the infection course (*n* = 1,000; 99.6th percentile, **Extended Data Figure 6c**). We identified Th2 cytokines at week 4 and FOXP3 regulatory T cells at weeks 12 and 16, MIP-1β at weeks 8 and 12 and levels of serum CAA at week 9 as important features elevated in symptomatic participants by leave-one-out cross-validation (**Figure 6c**). Levels of Th2 cytokines upon stimulation at week 4 correlated with serum CAA at week 9, whereas concentrations of MIP-1β in plasma correlated with FOXP3 regulatory T cell numbers (**Extended Data Figure 6d**).

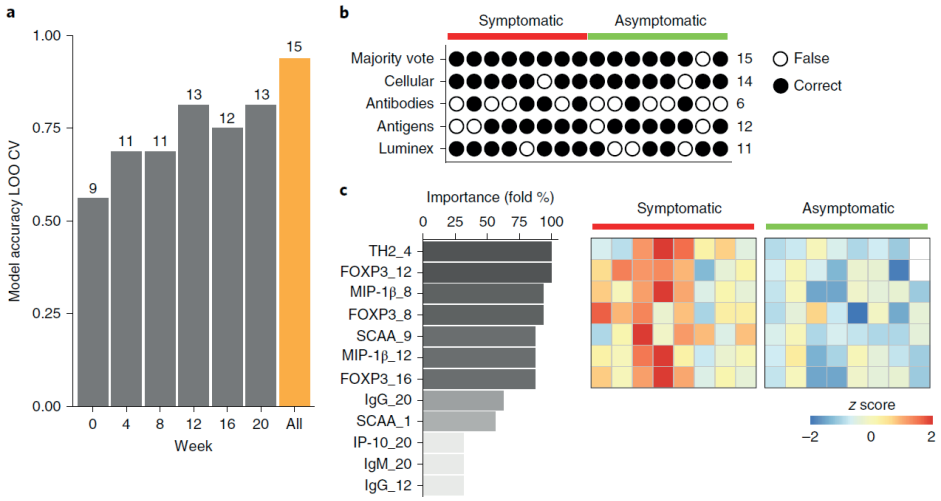

Figure 6. Immunological and microbiological data integration. Cellular, antibody, antigen and Luminex data were combined with generalized canonical correlation analysis and simultaneously associated with presence of symptoms through discriminant analysis. **a**, Proportion of correct predictions of each model assessed using leave-one-out cross-validation. Gray bars indicate data from one specific timepoint and orange bars indicate the model, including all data across timepoints. The number of correct predictions is indicated above each bar. **b**, Individual predictions across folds for the full model per dataset, including majority vote, with weighing to break ties. Each symbol represents one prediction, with volunteers in columns and datasets in rows. Filled circles indicate a correct prediction and open circles a false prediction. Volunteers with (red, n = 8) or without (green, n = 8) symptoms of acute schistosomiasis syndrome are indicated, as are the number of correct predictions per dataset. **c**, Consensus features selected in at least 25% of the folds are indicated and ranked by frequency of presence among folds. The number after each feature indicates the week of measurement. The z score-normalized levels of the seven features present in at least 75% of the folds are indicated in the heat map, where each column corresponds to one volunteer. Missing data points from one asymptomatic participant are depicted in white.

DISCUSSION

This pilot study shows that experimental exposure to 20 male cercariae results in a detectable and well-tolerated *S. mansoni* infection in 82% of volunteers. This infection rate resembles that of other human infection models (14). All volunteers were cured after 1–2 doses of praziquantel.

The dose-escalating design of the study revealed a concomitant increase in AEs. The occurrence of a severe acute schistosomiasis (Katayama) syndrome in one volunteer prompted us to lower the dose. At a dose of 20 cercariae, 2 of 11 volunteers reported severe AEs, which is comparable with other human infection models. For example, in experimental malaria infections, volunteers generally experience three to four AEs, of which one would be severe for several days (15). In typhoid infections ~50% of volunteers report severe symptoms and 55% experiences fever (16) and in cholera infection studies 40% of volunteers experience 1.6–8.0 liters of diarrhea (17). We thus conclude that 20 cercariae may be the optimal dose that was both well tolerated and detectable. A relationship between dose and symptoms, as suggested by epidemiological data (18), cannot be statistically confirmed with the current number of volunteers. Regardless of dose, all symptoms had resolved without sequelae at 12 weeks.

The follow up of volunteers in our study unequivocally showed that anti-adult worm IgM (100% seroconversion by week 6) or serum CAA (10 of 17 positive by week 6) are the earliest diagnostic markers currently available. This is in accordance with previous studies in travelers (19-21). We found levels of serum CAA comparable to those in low endemic settings (22). In nonhuman primate models these levels reflect 5–24 cercariae reaching adulthood (8, 23). Although serum CAA levels were more stable compared to urine CAA, the higher volume input of urine allows for more sensitive detection (24). As suggested in previous studies, the urine POC-CCA rapid test was not suitable to detect very low intensity infection (25). The main advantage of measuring serum CAA levels, as opposed to antibody detection, is the potential to follow up after treatment and confirm cure. In doing so, we found low cure rates with a single dose of 40 mg kg^{-1} praziquantel. Although a 60 mg kg^{-1} dose seemed more effective, the subsequent changes in pharmacokinetic and pharmacodynamic parameters need to be evaluated to conclude this with certainty. Because of the synchronous development of adult worms and the absence of reinfection in our model, the CHI design allows for screening of existing and new (stage-specific) anthelmintics.

The controlled schistosome infection model presented here clearly differs from infections in endemic settings, where doses are not controlled and infection occurs repeatedly. In addition, the single-sex infection lacks production of eggs that drive chronic regulatory and modified Th2 responses (26). The presence of IgG to soluble egg antigen, indicates cross-reactive epitopes between eggs and worms. The induction of chemokines IP-10 and MIP-1β aligned with the increase in both antigen-specific $CD4^+$ T cell IFN-γ and Th2 cytokine production, respectively. The IFN-γ production contrasts with predominantly Th2 profiles in epidemiological studies, which may be driven by egg-related responses[26]. Although we detected increases in $FOXP3^+CD25^+CD4^+$ T cells, these mainly occurred in symptomatic volunteers and were found at a later timepoint than IFN-γ- or Th2 cytokine-producing T cells. This would be in line with the induction of regulatory T cells to prevent deleterious immune responses (27), different from the chronic regulation found in endemic infections. These important differences in antigen exposure and subsequent immunological responses between single-sex infections and endemic chronic egg production may limit the use of the model to study anti-fecundity effects. However, the immunological observations from our model are comparable to acute infection models in travelers and baboons, where a mixed Th1 and Th2 response also dominates (28, 29). Integrated data analysis revealed that acute schistosomiasis symptoms were particularly associated with antigen-specific Th2 cytokine production and circulating MIP-1β and IgM production, but could not be predicted at baseline. In contrast to volunteers with symptoms, those without symptoms predominantly showed IgG1 antibody responses. Similarly to what has been performed for other infectious diseases (30), a trial with repeated controlled infections could address whether these IgM or IgG1 antibodies have a protective effect. Currently, the clinical and immunological data from baseline alone cannot predict who will become symptomatic. However, the analysis of longitudinal responses reveals a clear profile predicting presence or absence of symptoms in 15 out of 16 participants. Unraveling the interplay between symptoms, immune responses and resistance to schistosome reinfection provides an opportunity for identification of new antigens for vaccine development (31).

Immunological models for protection against *S. mansoni* were developed in rodents and nonhuman primates by repeated exposure to radiation attenuated cercariae (23, 32). In humans, three candidate *S. mansoni* vaccines are currently in clinical development (6). A phase I study with rSm14/GLA-SE showed a good safety profile and immunogenicity (7). Phase I safety results for Sm-TSP-2 are expected soon (ClinicalTrials.gov identifier: NCT02337855), while Sm-p80 is

currently entering phase I testing on the basis of promising efficacy data in baboons (8). The limited funding opportunities and large sample size required for phase III testing preclude testing of all three candidates in phase III trials. Despite the differences between chronic *S. mansoni* infection in the field and the controlled human *S. mansoni* infection, the model provides an opportunity to obtain preliminary efficacy data on these vaccines and reduce the costs by allowing selection of the most promising candidates, which may be co-formulated to maximize benefit (12).

Future vaccine studies aim at a 75% infection reduction in worm burden and egg output (10). As there is a clear relationship between worm burden and serum CAA levels (24, 33), we propose that the reduction in worm burden can be determined by measuring the median serum CAA level between week 7–12. Assuming an 80% power and $\alpha = 0.05$, this would require a group size of 11 individuals per arm. Currently the main limitation of our model is the use of male schistosomes only. Consequently vaccine targets that are more commonly expressed on females, such as the Sm-p80 vaccine candidate (8), cannot be fully evaluated. A female worm infection model would be of value to dissect mechanisms of action and sex-specificity of vaccines.

We conclude that this controlled human *S. mansoni* infection model results in an 82% infection rate with few severe side effects at a dose of 20 cercariae. In addition, this model provides insight into the onset of symptoms of a schistosome infection, the ensuing immune response and the performance of diagnostic tests over time. Notably, this model paves the way for cost-effective and rapid proof-of-concept testing of new vaccines and drugs.

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METHODS

Study design

This phase I trial (ClinicalTrials.gov identifier: NCT02755324) was an open-label dose-escalation study at the Leiden University Medical Center (LUMC).

Male cercariae were produced as previously reported (1). The initial dose was 10 cercariae (*n* = 3), which was escalated to 30 (*n* = 3) and subsequently de-escalated to 20 cercariae (*n* = 3) on the basis of AEs. The 20 cercariae infection was then validated in another group (*n* = 8). The viability of cercariae was confirmed by imaging of cercariae penetrating skin explants (2). The cercariae were applied to the volunteer's forearm in 0.5–1 ml of water for 30 min, the number of remaining cercariae were counted by microscopy. Volunteers were observed for 30 min after exposure.

Volunteers were followed bi-weekly between week 0–24 and on week 52. During each visit, AEs were recorded. Symptoms of an acute schistosomiasis syndrome were defined as moderate to severe symptoms of malaise, fatigue, fever, night sweats, flu-like symptoms or headache, between 2–7 weeks after exposure. Safety reports were reviewed regularly by an external safety monitor, who advised on dose escalation. Blood and urine samples were collected at all visits.

The pre-patent period was defined as the time until serum CAA levels were above 1.0 pg ml^{-1} [24]. At week 12 all volunteers were treated with 40 mg kg^{-1} praziquantel in two doses. A second regimen of 60 mg kg^{-1} praziquantel in two doses was provided if serum CAA levels persisted 3–6 weeks after treatment. Cure was defined as serum CAA levels ≤0.5 pg ml^{-1}.

The study was approved by the LUMC Institutional Medical Ethical Research Committee (Institutional Review Board P16.111). It was performed according to the European Clinical Trial Directive 2001/20/EC, in accordance with ICH-GCP guidelines and the Declaration of Helsinki (3, 4).

Informed consent procedure

Healthy 18–45-year-old *Schistosoma*-naïve volunteers were screened by medical history, general physical examination and safety laboratory tests. Informed consent was obtained from all volunteers.

Through advertisements, volunteers provided their email address and received written information. When they so wished, volunteers could schedule a

screening visit at least 3 d after having received the information. They were then requested to complete an application form, which included a questionnaire regarding their health.

During the 1.5–2-h screening visit, the study purpose and procedures were explained and questions answered. The possible AEs and right of withdrawal were explained to the volunteers. The informed consent form was signed and a full physical exam was performed. All volunteers were required to consent to an HIV, hepatitis B (HBV) and hepatitis C (HCV) serological screening, urine toxicology and (for females), a pregnancy test at screening.

At the infection day (14 d to 23 weeks after screening), informed consent was reconfirmed, and a final check of inclusion and exclusion criteria was performed, including a focused physical exam. Volunteers were exposed to male *Schistosoma mansoni* cercariae after baseline assessment and safety laboratory tests.

The first volunteer was included on 27 October 2015 and the last volunteer was included on 1 February 2018.

All three volunteers gave permission to use the photographs (**Figure 2a–c**) taken of their skin after cercarial exposure for publication.

Inclusion and exclusion criteria

Inclusion criteria

- Volunteer is aged ≥ 18 and ≤ 45 years and is in good health.
- Volunteer has adequate understanding of the procedures of the study and agrees to abide strictly thereby.
- Volunteer is able to communicate well with the investigator and is available to attend all study visits.
- Volunteer will remain within Europe (excluding Corsica) during the study period and is reachable by mobile telephone from week 3 to week 12 of the study period.
- Volunteer agrees to refrain from blood donation to Sanquin or for other purposes throughout the study period.
- For females: volunteer agrees to use adequate contraception and not to breastfeed for the duration of study.
- Volunteer has signed informed consent form.

Exclusion criteria

- Any history, or evidence at screening, of clinically significant symptoms, physical signs or abnormal laboratory values suggestive of systemic conditions, such as cardiovascular, pulmonary, renal, hepatic, neurological, dermatological, endocrine, malignant, hematological, infectious, immune-deficient, psychiatric and other disorders, which could compromise the health of the volunteer during the study or interfere with the interpretation of the study results. These include, but are not limited to, any of the following:
- body weight <50 kg or body mass index <18 or >30 kg m^{-2} at screening;
- positive HIV, HBV or HCV screening tests;
- the use of immune-modifying drugs within 3 months before study onset (inhaled and topical corticosteroids and oral anti-histamines exempted) or expected use of such during the study period;
- history of malignancy of any organ system (other than localized basal cell carcinoma of the skin), treated or untreated, within the past 5 years;
- any history of treatment for severe psychiatric disease by a psychiatrist in the past year;
- history of drug or alcohol abuse interfering with normal social function in the period of 1 year before study onset;
- any clinically significant abnormalities (including extended QT interval) on electrocardiogram.
- The chronic use of any drug known to interact with praziquantel, artesunate or lumefantrine (artesunate combined with lumefantrine served as alternative treatment of schistosomiasis in an earlier phase of infection) metabolism (for example, phenytoin, carbamazepine, phenobarbital, primidone, dexamethasone, rifampicin, cimetidine, flecainide, metoprolol, imipramine, amitriptyline, clomipramine, class IA and III anti-arrythmics, antipsychotics, antidepressants, macrolides, fluorquinolones, imidazole- and triazole antimycotics and anti-histamines). Because lumefantrine may cause extension of QT-time, chronic use of drugs with effect on QT interval are excluded from the study.
- For female volunteers: positive urine pregnancy test at screening
- Any history of schistosomiasis or treatment for schistosomiasis
- Positive serology for schistosomiasis or elevated serum or urine CAA at baseline
- Known hypersensitivity to or contraindications (including co-medication) for use of praziquantel, artesunate or lumefantrine

- Being an employee or student of the Department of Parasitology or Infectious Diseases of the LUMC

Adverse events

Grading

- Mild (grade 1): awareness of symptoms that are easily tolerated and do not interfere with usual daily activity
- Moderate (grade 2): discomfort that interferes with or limits usual daily activity
- Severe (grade 3): disabling, with subsequent inability to perform usual daily activity, resulting in absence or required bed rest
- Serious AE: any untoward medical occurrence in a patient or trial participant, which does not have a causal relationship with the treatment, and:
- is fatal, and/or
- is life-threatening for the volunteer, and/or
- makes hospital admission or an extension of the admission necessary, and/or
- causes persistent or significant invalidity or work disability, and/or
- manifests itself in a congenital abnormality or malformation, and/or
- could, according to the person that carries out the research, have developed to a serious undesired medical event, but was, however, prevented due to premature interference.

Causality

Unrelated

- Not related: a relationship to the administration of *the S. mansoni* male cercariae cannot be reasonably established; another etiology is known to have caused the AE or is highly likely to have caused it.
- Unlikely related: a relationship to the administration *of S. mansoni* male cercariae is unlikely; however, it cannot be ruled out.

Related

- Possibly related: there is a potential association between the event and administration of the *S. mansoni* male cercariae; however, there is an alternative etiology that is more likely.
- Probably related: administration of the *S. mansoni* male cercariae is the most likely cause; however, there are alternative reasonable explanations, even though less likely.

- Definitely related: administration of the *S. mansoni* male cercariae is the cause; another etiology causing the adverse event is not known.

Parasitological assays

CAA was measured in serum and urine by the previously described upconverting phosphor lateral flow (UCP-LF CAA) assay (5, 6). The presence of urine CCA was determined by the POC-CCA (Rapid Medical Diagnostics). The *Schistosoma* PCR on feces was performed according to previous descriptions (ISO 15189:2012-certified) (7).

Antigen detection assays

Upconverting phosphor lateral flow assay for circulating anodic antigen

Both serum and urine were analyzed for schistosome CAA using a UCP-LF CAA assay. The assay was performed as described previously (5, 6). In brief, 500 µl of serum (or 4 ml of urine) samples or standards were diluted 1:1 in 4% trichloroacetic acid (TCA) (or diluted 5:1 in 12% TCA for urine samples) and incubated for 5 min at room temperature. Serum samples were centrifuged for 10 min at 13,000 r.p.m. (and urine samples were centrifuged for 45 min at 4,000 r.p.m.). Then 500 µl of supernatants of serum (or 4 ml of urine) was applied to 0.5-ml (or 4.0-ml) Amicon filtration devices (Amicon Ultra-0.5 (or Amicon Ultra4), Millipore) and concentrated to approximately 20 µl by centrifugation for 25 min at 13,000 r.p.m. for serum (or 60 min at 4,000 r.p.m. for urine). The concentrates were diluted 1:5 in LF assay buffer and incubated in microtiter plate wells at 37 °C for 1 h while shaking. LF strips were inserted into the wells and incubated for 3 h or overnight, before being read on a modified Packard FluoroCount microtiter plate reader[24]. A TCA-soluble fraction of *S. mansoni* adult worm antigen with known CAA concentration was used as a reference standard for the quantification of the antigen. Predefined cutoff values were used, where a serum CAA concentration above 1 pg ml^{-1} was defined as positive, below 0.5 pg ml^{-1} as negative and between 0.5 and 1.0 as undecisive (5).

Point-of-care circulating cathodic antigen

Urine samples were tested for the presence of schistosome CCA using a commercially available rapid diagnostic test (POC-CCA, batch no. 170622073, Rapid Medical Diagnostics) according to the manufacturer's procedure. Readings above a trace line were considered as positive.

Immune responses

Clinical diagnostics

Schistosome-specific antibodies, IgM against adult worms and anti-SEA IgG were determined according to ISO 15189:2012-certified routine diagnostic in-house IFA and ELISA respectively, which have been in use for the clinical diagnosis of schistosomiasis at the LUMC for decades (8, 9).

IgM adult worm antibodies by IFA

IgM antibodies against adult worms were detected by an in-house IFA assay as previously described (10). This IFA, as well as the ELISA described below, are currently in use at LUMC as the routine antibody detecting assays for the diagnosis of imported schistosomiasis and feature in all laboratory quality assessment requirements (ISO 15189:2012-certified), including successful participation in an external quality assessment scheme (UK-NEQAS). In brief, sections of Rossman's fixed male adult worms were incubated with a twofold dilution series of serum samples starting at 1:8 dilution. Following incubation with goat anti-human IgM (u-chain specific)-FITC antibody (Sigma-Aldrich; F5384), slides were examined using a fluorescence microscope. A negative control and a positive reference serum were run in parallel at each slide. The titer was determined as the dilution of the sample at which the fluorescence of adult worm gut epithelium was still visible. Samples were considered positive if titers were above 1:8. Previous studies showed that the IgM detected in this IFA is mainly directed against CCA (10).

IgG against soluble egg antigen

IgG antibodies directed against *S. mansoni* SEA were detected by a previously described in-house ELISA with some minor modifications (8). Crude SEA was prepared from *S. mansoni* eggs collected from the livers of infected hamsters (11, 12). A concentration of 5 µg protein ml^{-1} was diluted in 100 µl of 0.1 M sodium carbonate buffer (pH 9.6) and coated overnight at 4 °C in 96-well plates (Polysorb NUNC). Plates were stored at −80 °C until use. After thawing, plates were washed and blocked in 1% BSA in PBS for 1 h at 37 °C. A dilution series of serum samples from 1:16 to 1:2,048 in a solution with 5% FCS and 0.05% Tween in PBS were incubated for 1 h at 37 °C. Plates were washed and incubated for 1 h at 37 °C with mouse anti-human IgG alkaline phosphatase 1:10,000 (Sigma-Aldrich; A2064) in 4% BSA and 0.05% Tween/PBS. Para-nitrophenyl phosphate (pNPP) substrate (Sigma-Aldrich; P5994) in 0.1 M diethanolamine buffer (pH 9.6) (Merck) was added for 1 h at room temperature after washing. Plates were read with the Multiskan EX reader at

405 nm. A negative control and a positive reference serum were run in parallel at each plate. The titer was determined as the dilution of the sample at which the extinction is higher or equal to the reference standard. Samples were considered positive if titers were above 1:16.

Experimental immunological assays

Serum was analyzed for total IgE, *S. mansoni* AWA-specific IgE and IgG by ELISA (13-15). Data were expressed as arbitrary units (AU ml^{-1}). Peripheral blood mononuclear cells (PBMCs) were evaluated for their phenotype and function after 24-h stimulation with crude *S. mansoni* AWA by flow cytometry.

Adult worm antigen-specific IgE, IgG, IgG1 and IgG4

S. mansoni AWA-specific IgE and IgG was measured by ELISA modified from previous protocols (14, 15). In brief, adult *S. mansoni* worms were collected from hamsters and crude AWA was prepared as described previously (16). MaxiSorp plates (Nunc) were coated overnight with 5 µg ml^{-1} AWA diluted in carbonate buffer pH 9.6. After blocking with 5% BSA/PBS, the sera were diluted with a solution with 5% FCS and 0.05% Tween in PBS and the presence of IgG1 or IgG4 was shown by using horseradish peroxidase (HRP)-labeled anti-human IgG1 or HRP-labeled anti-human IgG4 (1:3,000 dilution; Sanquin). For measuring total anti-AWA IgG antibody, alkaline phosphatase-conjugated anti-human IgG (1:5,000 dilution; Sigma) was added, whereas for the anti-AWA IgE assay, the plate was incubated with biotinylated goat anti-human IgE (1:1,000 dilution; Vector Laboratories) followed by streptavidin HRP conjugate (1:10,000 dilution; Sanquin). IgG1 and IgG4 assays were developed using tetramethylbenzine, stopped with 10% H_2SO_4 and the absorbencies were measured at 450 nm. For total IgG and IgE, the color was developed by addition of pNPP (Sigma) diluted in diethanolamine buffer and optical density was measured at 405 nm. For all four assays, the levels of antibody present in a given sample were expressed in AU ml^{-1} or International Units (IU ml^{-1}) according to the standard curve of pooled sera from inhabitants of an *S. mansoni* endemic area in Ghana. The levels of antibody present in a given samples were expressed in AU ml^{-1} for total IgG, IgG1 and IgG4 or in IU ml^{-1} for IgE. Seroconversion was defined as antibody levels above 2× s.d. of the baseline.

Total IgE

Total IgE levels were measured as previously described (15). Briefly, MaxiSorp plates were coated overnight with rabbit anti-human IgE (Dako). Plates were blocked with PBS 5% BSA followed by incubation of diluted samples in PBS

0.05% Tween-20. As a reference, the World Health Organization standard of human serum IgE (NIBSC) was used, starting at a concentration of 90 IU ml^{-1}. After a washing step, the plates were incubated with IgE biotinylated goat anti-human IgE antibody (Vector Laboratories) followed by an incubation with streptavidin alkaline phosphatase conjugate (Boehringer Mannheim). The color was developed by addition of pNPP (Boehringer Mannheim) diluted in diethanolamine buffer and optical density was measured at 405 nm. The results were expressed in IU ml^{-1}. Seroconversion was defined as antibody levels above 2× s.d. of the baseline.

Ex vivo cytokines

Serum samples were tested for the presence of different cytokines using a commercially ProcartaPlex Multiplex Immunoassay (17-plex, lot 178863000, Invitrogen) according to the manufacturer instructions. The following cytokines were measured: IFN-α, IFN-β, IL-1β, IL-10, IL-12p70, IL-13, IL-15, IL-2, IL-22, IL-23, IL-4, IL-6, IP-10, MCP-1, MIP-1α, MIP-1β and TNF-α using the Bioplex 200 Luminex (Bio-Rad).

Cellular immunology

Between week 0 and 24 after exposure every 4 weeks, human PBMCs were isolated from whole blood collected in heparin. Cells of two heparin tubes were diluted at least 1:2 with HBSS (ThermoFisher) at room temperature. Ten milliliters of ficoll at room temperature was added, followed by 25 min centrifugation at 400g with low brake. Cells were collected and washed with HBSS, counted and frozen in 10% DMSO, in RPMI Hepes (Invitrogen), with 100 U ml^{-1} penicillin, 100 µg ml^{-1} streptomycin, 1 mM pyruvate/2 mM glutamate and 10% FCS (Bodinco). Subsequently, PBMCs were thawed in RPMI Hepes, with 100 U ml^{-1} penicillin, 100 µg ml^{-1} streptomycin, 1 mM pyruvate/2 mM glutamate and 10% FCS and rested overnight at 37 °C with 5% CO_2. Cells were counted and transferred to a 96-well round bottom plate (Corning) with 500,000 cells per well. Cells were stimulated with AWA (50 µg ml^{-1}) for 24 h. Staphylococcal enterotoxin B (SEB) (Sigma-Aldrich) 200 ng ml^{-1} was used as a positive control and RPMI Hepes (Invitrogen), with 100 U ml^{-1} penicillin, 100 µg ml^{-1} streptomycin, 1 mM pyruvate/2 mM glutamate and 10% FCS as a negative control. After 4 h of incubation 5 mg ml^{-1} brefeldin A (Sigma) was added to SEB-stimulated wells and after 20 h to AWA- and medium-stimulated wells. After a total stimulation of 24 h, the cells were stained with Aqua (Invitrogen) and fixed with 3.9% formaldehyde (Sigma). After fixating, the cells were stained with the following antibodies: CD3, CD4, IFN-γ, IL-2, Th2-cytokines (IL-4, IL-5, IL-13),

TNF and IL-10 (**Supplementary Table 4**). Human FC block was used to avoid nonspecific interactions. The cells were measured with the FACSCanto II (BD Biosciences; **Supplementary Figure 1**). The data were analyzed with FlowJo 10.5 software for MAC OS. The gating was placed with the help of fluorescence minus one controls, the medium as a negative control and SEB as a positive control. The leftover, aqua-stained and fixed cells were frozen in 10% DMSO, in RPMI Hepes (Invitrogen), with 100 U ml^{-1} penicillin, 100 µg ml^{-1} streptomycin, 1 mM pyruvate/2 mM glutamate and 10% FCS (Bodinco) and stored at −80 °C. The cells were thawed at 37 °C and stained with the following antibodies: CD3, CD4, CD25, CD127 and FOXP3 (**Supplementary Table 4**). As before, human FC block was used to avoid nonspecific interactions. The cells were measured with the FACSCanto II (BD Biosciences; **Supplementary Figure 1**). The data were analyzed with FlowJo 10.5 software for MAC OS. The gating was placed with help of fluorescence minus one controls, the medium as a negative control and SEB as a positive control.

Statistical analysis

All 17 volunteers were included in the intention-to-treat analysis (safety analysis and parasitological assays). One volunteer was excluded from the per-protocol analysis (all immunological readouts) on the basis of high baseline AWA-specific IgG and IgG1 levels. Samples from individual volunteers were measured once and plotted as single values.

Demographics and the presence of symptoms between groups were analyzed with a Mann–Whitney *U*-test, time to patency with a log-rank test and correlations with a Spearman's rank test. Changes in the frequency of cytokine-producing cells over time were analyzed using a linear mixed model. Time was considered as the fixed effect and the volunteer ID as a random effect for the intercept. *P* values based on Student's *t*-tests were obtained using the Satterthwaite's degrees of freedom method. In the cytokine boxplots, the negative values (after subtracting the medium condition) were set to zero to prevent a negative cytokine response. However, the statistical analysis was performed on the unaltered data. All statistical tests were two-tailed with α set at the 0.05 level.

Analyses were performed using IBM SPSS statistics for Windows, v.23.0 (IBM Corp.) and R (v.3.5). The R packages used were ggplot2, lme4 and lmerTest (17-19).

P values were considered significant when $P < 0.05$.

MixOmics and data integration

Data integration was performed using the mixOmics package in R (v.6.8.0)(20, 21). This method allowed us to correlate across datasets, while associating features with outcome. Feature selection was performed using Lasso-like penalization for each of the datasets. The number of components was set at two and tuning was performed to find the minimum number of features needed per dataset (in a range from 1–3 per component) and the correlation between datasets was entered into the design matrix. The number of features included in the final model was 3, 3, 2 and 5 for cytokines, antigens, antibodies and cellular responses, respectively. A correlation of 0.75 between datasets was used in the design matrix.

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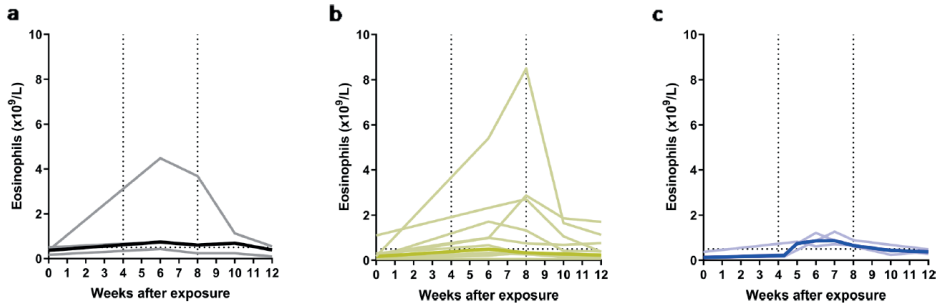
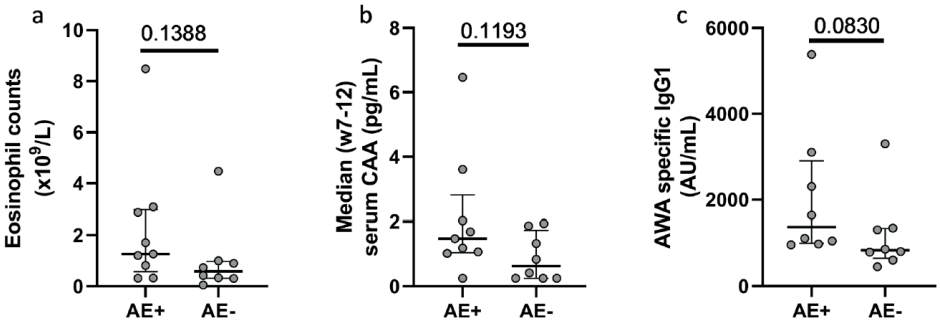
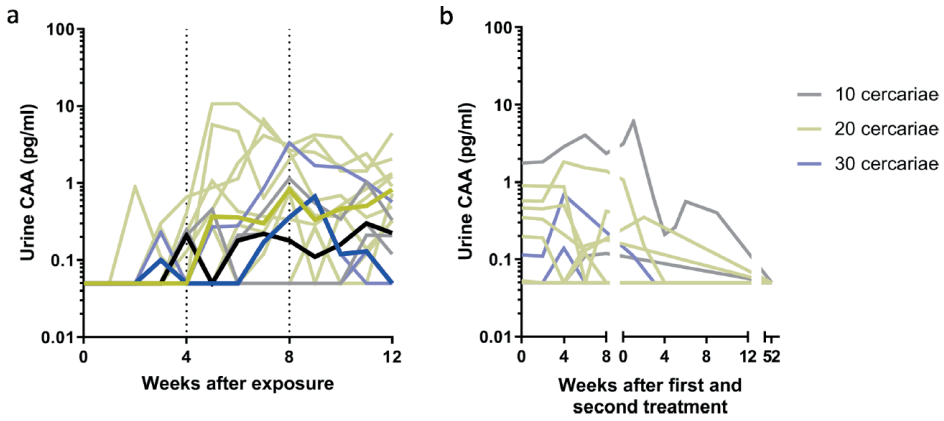
EXTENDED DATA



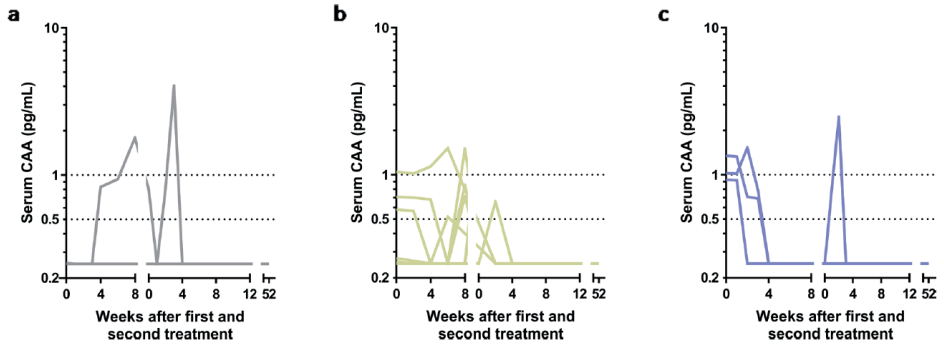
Extended Data Figure 1. Eosinophil counts. a-c. Eosinophil counts (x10^9/L) per volunteer and the median per group. The thin green, red, and blue lines represent data of individual volunteers infected with 10 (n = 3), 20 (n = 11) or 30 (n = 3) cercariae respectively, while thick lines represent the median of each group.



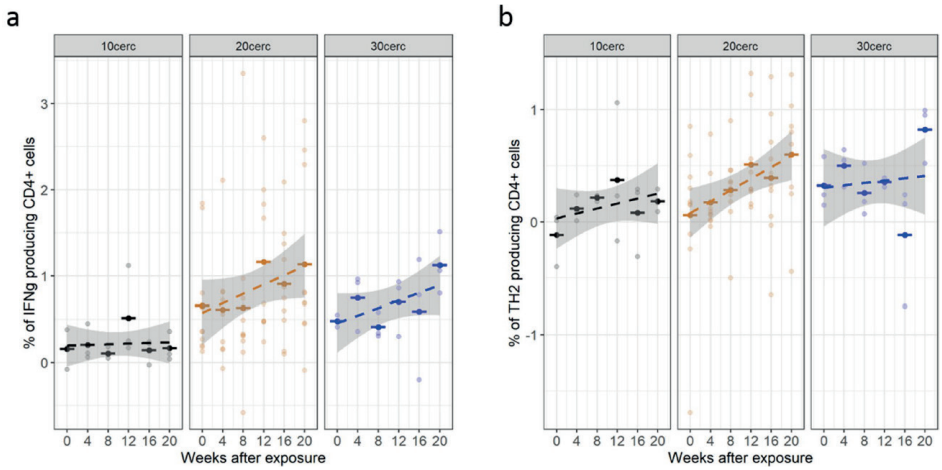
Extended Data Figure 2. Relation between symptoms of an acute schistosomiasis syndrome and immunological readouts. The relation between the presence of symptoms of an acute schistosomiasis infection and **a**. the highest eosinophil count (n = 17), **b**. the median serum CAA level from week 7 to 12 (n = 17), or **c**. the AWA specific IgG1 response at week 16 (n = 16). All using the two-sided Mann-Whitney U test. Individual data is presented as dots, the line represents the median, while the error bars represent the interquartile range of the groups.



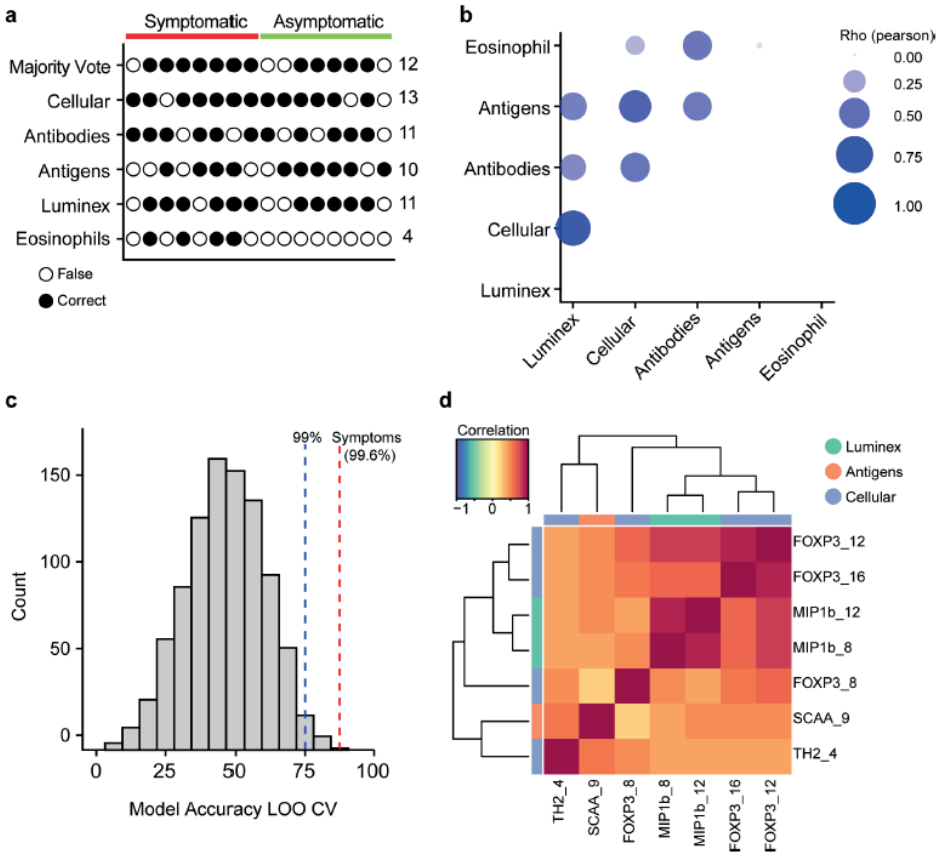
Extended Data Figure 3. Urine CAA levels. a. Urine CAA levels after exposure and **b**. after first and second praziquantel treatment (week 0) and at week 52 after exposure. The thin green, red, and blue lines represent of individual volunteers infected with 10 (n = 3), 20 (n = 11) or 30 (n = 3) cercariae respectively, while thick lines represent the median of each group before treatment.



Extended Data Figure 4. Serum CAA levels after treatment. a-c. Serum CAA levels in pg/ml after the first treatment, second treatment, and at week 52. All values below the detection threshold of 0.5 pg/mL, are plotted at 0.25 pg/mL. The gray, yellow and blue lines represent data of individual volunteers infected with 10 (n = 3), 20 (n = 11) or 30 (n = 3) cercariae respectively.

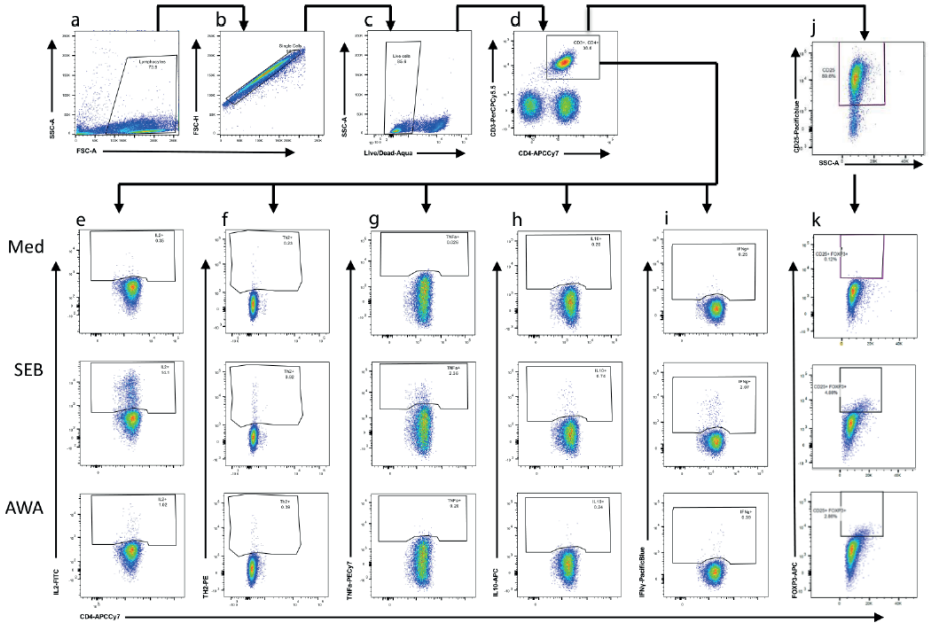


Extended Data Figure 5. IFN-γ and TH2 cytokine producing CD4⁺ T-cells over time. The percentage of **a**. IFN-γ and **b**. Th2-cytokine producing CD4⁺ T-cells over time in weeks after exposure in the 10 cercariae (gray, n = 3), 20 cercariae (yellow, n = 10) and 30 cercariae (blue, n = 3) groups. Dotted lines are linear regression lines, gray areas are confidence intervals, light dots are individual data, and horizontal lines with dots are the average values.



Extended Data Figure 6. Effect of eosinophil data and model performance. a. Individual predictions across folds for the full model per dataset including majority vote. Each symbol represents one prediction, with volunteers in columns and datasets in rows. Filled circles indicate a correct prediction and open circles a false prediction. Symptomatic and asymptomatic volunteers are indicated in red and the number of correct predictions per dataset is indicated. **b**. Mean Pearson correlation score between datasets using the first component of the projection onto the latent space across all folds from the model including all datasets. Size and color of circles reflect the mean rho value. **c**. Permutations analysis (n = 1000) with leave-one-out cross-validation on the full model using all subjects and including the four datasets without eosinophils. Blue and red dashed lines indicate the 99th percentile and the accuracy when comparing symptomatic and asymptomatic individuals (99.6%), respectively. **d**. Spearman correlation matrix of the seven consensus features selected in > 75% of folds in the leave-one-out cross-validation. Features were clustered using hierarchical clustering with complete linkage on Euclidean distance. All graphs are based on n = 16.

SUPPLEMENTARY



Supplementary Figure 1. Flow cytometry gating strategy. Gating of stimulated PBMCs. **a**. gating on lymphocytes. **b**. gating on single cells. **c**. gating of live cells **d**. gating on CD4⁺ cells. **e-i**: gating on selection of IL2, Th2 (IL-4+IL-5+IL-13), TNF-α, IL10, or IFN-γ positive cells. **j**. gating on CD25⁺ cells **k**. gating on FOXP3 positive cells. For cells stimulated with either medium, Staphylococcal Enterotoxin B (SEB) or adult worm antigen (AWA).

Supplementary Table 1. Demographics and cercariae retrieved after exposure

Demographics of included volunteers, (median and range). The number of heads, tails and whole body cercariae retrieved from volunteers' skin after infection (median and range). The 10, 20 and 30 cercariae groups were compared using the two-sided Mann-Whitney U test (top) or the Spearman's rank correlation (bottom).

	10 cercariae (*n*=3)	20 cercariae (*n*=11)	30 cercariae (*n*=3)	All volunteers (*n*=17)	*P*-value
Demographics					
Gender					0.34
·Male	1	5	0	6	
·Female	2	6	3	11	
Age in years (median (range))	20 (19-26)	30 (18-45)	23 (21-25)	25 (18-45)	0.21
BMI in kg/m^2 (median (range))	21.7 (20.5-24.1)	25.1 (20.7-28.5)	20.9 (18.6-24.4)	24.1 (18.6-28)	0.12
Cercariae retrieved after exposure					
Heads (median (range))	0 (0-1)	0 (0-1)	1 (0-1)	0 (0-1)	0.37
Tails (median (range))	5 (4-6)	8 (4-12)	14 (10-19)	8 (4-19)	**0.002**
Whole body cercariae (median (range))	0 (0-1)	0 (0-3)	1 (0-3)	0 (0-3)	0.33

Supplementary Table 2. Volunteer's age and sex

Listing of volunteer code, group allocation, cercarial exposure, sex and age.

Volunteer	Group	Cercarial exposure	Sex	Age
Cs1-501	group 1	10	F	20
Cs1-516	group 1	10	F	26
Cs1-753	group 1	10	M	19
Cs1-106	group 2	30	F	23
Cs1-495	group 2	30	F	21
Cs1-674	group 2	30	F	25
Cs1-069	group 3	20	F	22
Cs1-229	group 3	20	F	35
Cs1-425	group 3	20	M	20
Cs1-004	group 4	20	M	18
Cs1-102	group 4	20	F	30
Cs1-464	group 4	20	M	36
Cs1-759	group 4	20	M	25
Cs1-780	group 4	20	F	39
Cs1-832	group 4	20	F	23
Cs1-923	group 4	20	M	35
Cs1-967	group 4	20	F	45

Supplementary Table 3. Adverse events

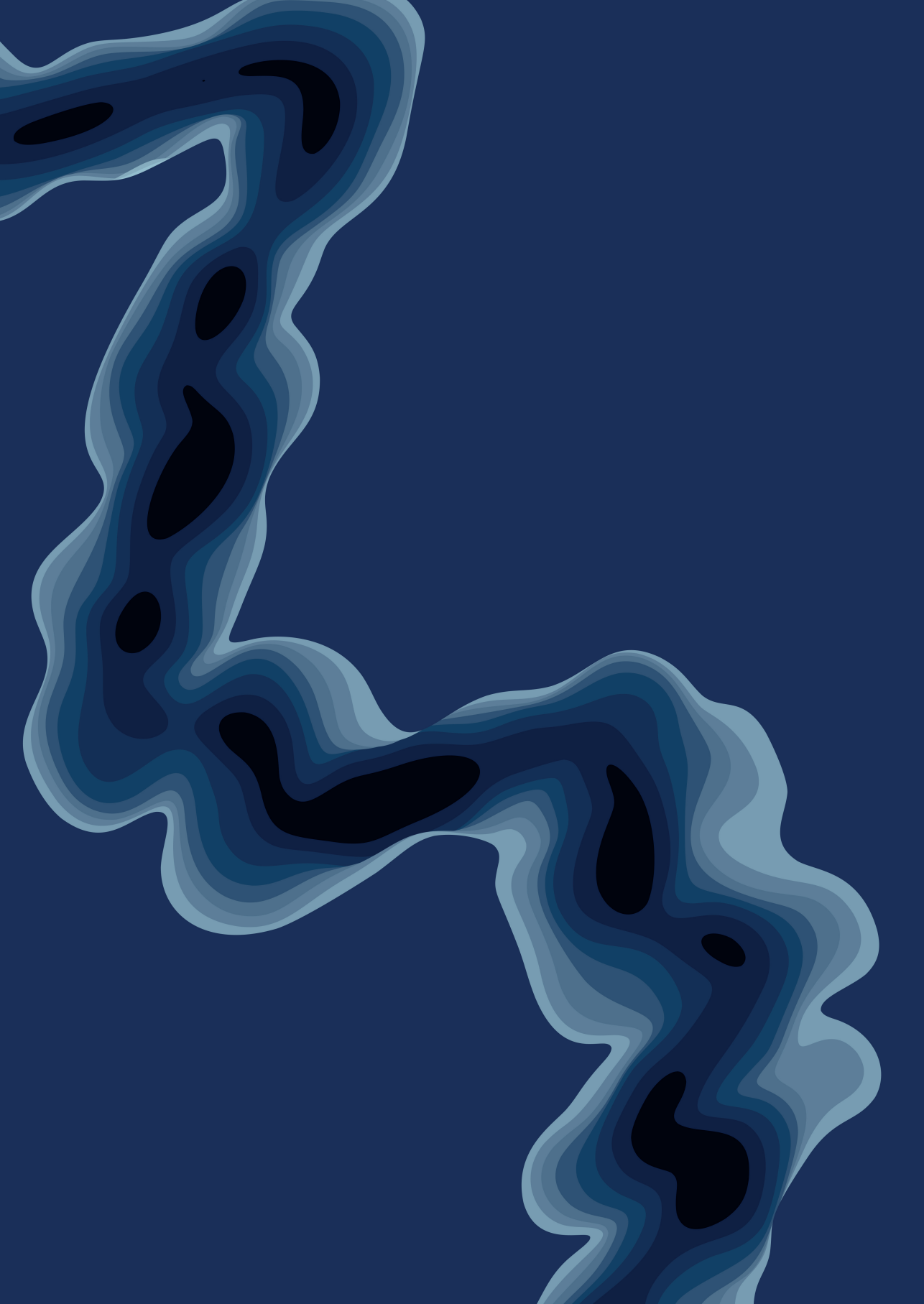
The number of volunteers developing a related (either possibly, probably or definitely related) solicited or unsolicited adverse event (AE) throughout the study period. Data is presented per cercarial dose group and in total. The maximum severity of an AE is presented in the table.

			10 cercariae (*n*=3)			20 cercariae (*n*=11)			30 cercariae (*n*=3)			Total (*n*=17)		
			Mild	Moderate	Severe	Mild	Moderate	Severe	Mild	Moderate	Severe	Mild	Moderate	Severe
Local	solicited	Pruritus	3			10			2			15 (88%)		
		Cercarial dermatitis	3			10			3			16 (94%)		
	unsolicited	Burning feeling				3						3 (18%)		
		Dysesthesia				1						1 (6%)		
Systemic	solicited	Fever	1				1	1			2	1 (6%)	1 (6%)	3 (18%)
		Headache				1	4	1			3	1 (6%)	4 (24%)	4 (24%)
		Fatigue					3						3 (18%)	
		Malaise					2			1			3 (18%)	
		Cough					1						1 (6%)	
		Myalgia				1			1			2 (12%)		
		Night sweats					1						1 (6%)	
		Nausea		1							1		1 (6%)	1 (6%)
		Abdominal pain	1						1			2 (12%)		
		Diarrhea		1						1			2 (12%)	
	unsolicited	Less ability to focus	1									1 (6%)		
		Elevated liver enzymes									1			1 (6%)
		Syncope									1			1 (6%)
		Periorbital edema				1				1		1 (6%)	1 (6%)	
		Pharyngitis/ sore throat				2						2 (12%)		
			None of the volunteers experienced vomiting, urticaria, backpain or anorexia											

Supplementary Table 4.

The antibodies used for staining of the cells.

Antibody	Label	Dilution	Company	Cat.
IL-2	FITC	1:25	BD Biosciences	340448
IL-4	PE	1:20	BD Biosciences	340451
IL-5	PE	1:250	Biolegend	504303
IL-13	PE	1:100	Biolegend	501903
CD4	PerCP-ef710	1:400	eBioscience	46-0047
TNF	PE-Cy7	1:1000	eBioscience	25-7349
IL-10	APC	1:250	BD Biosciences	554707
CD3	APC ef780	1:800	eBioscience	47-0038
IFN-γ	BV421	1:1000	Biolegend	502531
CD3	FITC	1:100	BD Biosciences	555339
CD4	Pe-Cy7	1:150	BD Biosciences	557852
CD25	BV421	1:200	BD Biosciences	562442
CD127	APCe780	1:200	eBioscience	47-1278-42
FOXP3	APC	1:100	eBioscience	17-4776-42
Human FC block	-	1:100	eBioscience	14-9161
Live/Dead Aqua	AmCyan	1:400	ThermoFisher	L34957



Chapter 3

Safety and infectivity of female cercariae in *Schistosoma*-naïve, healthy participants: a controlled human *Schistosoma mansoni* infection study

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SUMMARY

Background: A controlled human infection model for schistosomiasis (CHI-S) can speed up vaccine development and provides insight into early immune responses following schistosome exposure. Recently, we established CHI-S model using single-sex male-only *Schistosoma mansoni (Sm)* cercariae in *Schistosoma*-naïve individuals. Given important differences in antigenic profile and human immune responses to schistosomes of different sex, we pioneered a single-sex female-only CHI-S model for future use in vaccine development.

Methods: We exposed 13 healthy, S*chistosoma*-naïve adult participants to 10 (n=3) or 20 (n=10) female cercariae and followed for 20 weeks, receiving treatment with praziquantel (PZQ) 60 mg/kg at week 8 and 12 after exposure.

Findings: The majority (11/13) participants reported rash and/or itch at the site of exposure, 5/13 had transient symptoms of acute schistosomiasis. Exposure to 20 cercariae led to detectable infection, defined as serum circulating anodic antigen levels >1·0 pg/mL, in 6/10 participants. Despite two rounds of PZQ treatment, 4/13 participants showed signs of persistent infection. Additional one- or three- day PZQ treatment (1x60 mg/kg and 3x60 mg/kg) or artemether did not result in cure, but over time three participants self-cured. Antibody, cellular, and cytokine responses peaked at week 4 post infection, with a mixed Th1, Th2, and regulatory profile. Cellular responses were (most) discriminative for symptoms.

Interpretation: Female-only infections exhibit similar clinical and immunological profiles as male-only infections but are more resistant to PZQ treatment. This limits future use of this model and may have important implications for disease control programs.

Funding: European Union's Horizon 2020 (grant no. 81564)

Keywords: Controlled Human Infection model, Neglected Tropical Diseases, Schistosomiasis, *Schistosoma mansoni*

RESEARCH IN CONTEXT

Evidence before this study

A controlled human infection model for schistosomiasis has the potential to speed up vaccine development and provide insight into early immune responses following schistosome exposure. Previously, a male-only *Schistosoma mansoni (Sm)* infection model has been successfully developed in *Schistosoma*-naïve participants. However, animal studies suggest there are significant differences between immune responses to male and female worms, and differential expression of some vaccine antigens in male and female adult worms suggests important differences in the use of single-sex models for vaccine testing.

Added value of this study

This study aimed to establish a controlled human *Schistosoma mansoni* infection with female cercariae. We show that exposure to 20 female cercariae leads to detectable infection and is well-tolerated, however resulting infections are refractory to (repeated) praziquantel treatment. We observe strong similarities between immune responses in male- and female-only infections, which show a mixed Th1/Th2 immunophenotype contrasting the existing dogma that Th2 responses result from egg deposition

Implications of all the available evidence

Female-only infections exhibit similar clinical and immunological phenotypes profiles, but are more resistant to PZQ treatment than male-only infections. This limits future use of this model for vaccine testing, but may have important implications for disease control programs.

INTRODUCTION

Schistosomiasis, an infection with *Schistosoma* parasites, remains an important neglected tropical disease (NTD) that adversely affects global health with an estimated prevalence of 240 million (1). Infection occurs through contact with fresh water that contains cercariae, the larval stage of *Schistosoma,* which are secreted by freshwater snails. Subsequent egg deposition by the matured male and female worm pair is responsible for schistosome morbidity as it causes local inflammation and granulomas (2). Control of disease relies heavily on mass drug administration (MDA) with praziquantel (PZQ), however reinfections occur rapidly. Modelling studies suggest that an efficacious vaccine against schistosomiasis is needed to improve disease control (3). Currently, four vaccine candidates are in clinical testing (4). A controlled human infection with *Schistosoma* (CHI-S), whereby healthy adult participants are deliberately exposed to schistosomes, may be instrumental to get an early vaccine efficacy estimate and guide vaccine selection and design for larger phase 3 studies (5). To avoid egg-induced pathology, we previously developed a single-sex, CHI-S model with male-only, *Schistosoma mansoni (Sm)* cercariae. In this study, exposure of *Schistosoma*-naïve participants to 20 male cercariae led to detectable infection by worm-derived circulating anodic antigen (CAA) in 9 out of 11 participants (82%) before clearing infection with one to two doses of PZQ (6). However, animal studies suggest that there are significant differences between immune responses to male and female worms (7). In addition, differential expression of vaccine antigens in male and female adult worms may require the development of CHI-S for both schistosome sex for testing of vaccines targeting adult worm antigens (8). We thus aimed to develop a single-sex, female CHI-S model complementary to the previous male model to enable exploration of sex-specific immune responses and future vaccine testing.

MATERIAL AND METHODS

Study design

This open label, dose-escalation study (clinicaltrials.gov identifier: NCT04269915) took place at the Leiden University Medical Centre, The Netherlands between September 2020 and April 2022. Healthy, 18-45 year old schistosome-naïve participants without prior (suspected) exposure to schistosomes and without travel plans to *Schistosoma*-endemic regions during the study period were recruited through advertisements. Participants were excluded if they had a history or evidence of any illness that could compromise

the health of the participant during the study or affect the interpretation of study results. Participants with a known hypersensitivity to or contra-indications for use of PZQ, artesunate or lumefantrine were also excluded. The complete in- and exclusion criteria are given in **Supplementary Table 1**. Participant's sex was self-reported.

Ethics

The study was approved by the local medical ethics review board (METC-LDD: P20.015) and was conducted in accordance with ICH-GCP guidelines and the Declaration of Helsinki. All participants provided written informed consent for participation.

Study procedures

After inclusion, small groups of three people were then exposed to a pre-defined number of female *Sm* cercariae. The doses were based on our previous study with male *Sm* cercariae. Depending on safety and infectivity, dose was either escalated or an additional seven participants were exposed to the same dose after discussions with the safety monitoring committee, taking into account safety data from the study with male cercariae. Sample size was based on the number of other proof-of-concept vaccine efficacy studies, where small groups of 10 subjects are preferred.

Female *Sm* cercariae were produced as described previously (9), with the addition of a confirmatory PCR on 10 individual, hand-picked cercariae from each snail to ascertain female-only cercariae. Cercariae were applied in 0.5 mL mineral water to the skin of the participant's forearm for 30 minutes. Next, microscopy was performed on rinse water to count the remaining cercarial tails.

Participants were followed up weekly until week 16 for adverse event and sample collection. PZQ treatment (60 mg/kg) was provided at week 8 and 12. Long-term visits were at week 18, week 20 and week 52 after infection.

Outcomes

Safety was assessed through adverse events (AEs) reporting and blood tests. Severity of adverse events was assessed as follows: symptoms that do not interfere with daily activities (mild); symptoms that interfere with or limits daily activities (moderate); and symptoms resulting in absence or required bed rest (severe). AEs were assessed as related or unrelated to study procedures

based on clinical judgement. Symptoms of acute schistosomiasis were defined as: fever, urticaria, angioedema, night sweats, myalgia, arthralgia, dry cough, diarrhoea, abdominal pain, and headache occurring between 2-8 weeks after exposure without other clear cause. Classification of acute schistosomiasis (yes/no) was performed separately by two clinicians and in case of disagreement, consensus was reached after discussion.

Patent infection was determined by upconverting reporter particle lateral flow (UCP-LF CAA) assay in serum (10) and defined by at least one value ≥1·0 pg/mL before week 8. Values below the limit of detection (<0·5 pg/mL) were set to 0·25 pg/mL. In addition, schistosome-specific antibodies were measured using in-house adult worm IgM antibody (IFA) and soluble egg IgG antibody (ELISA) assays (11). To rule out egg production, *Schistosoma* PCR was performed on faeces (12). Adult worm antigen (AWA)-specific IgG, IgG1, and IgE were measured by ELISA as previously described (6) with the following modifications: 1) plates were coated with 25µg antigen and 2) pooled positive participants from the previous CHI-S was used as standard (6).

Serum samples were assessed for CCL2, CXCL10, IL-4, CCL4, IL-1β and IL-10 using a custom Luminex kit (LXSAHM-06, R&D systems) according to manufacturer instructions. Cytokines with over 40% of samples under the limit of detection were not included in the analysis (IL-4, IL-1β and IL-10).

PBMCs were isolated using a ficoll gradient, cryopreserved, thawed and stimulated with AWA, media or SEB for 24 hours as previously described [6]. Cells were centrifuged at 400g for 4 minutes, supernatants were cryopreserved at -80°C for cytokine analysis using a Luminex kit (LKTM008, R&D systems) per manufacturer's instructions. The following cytokines were measured: GM-CSF, IFNγ, IL-1β, IL-2, IL-4, IL-5, IL-6, IL-10, IL-12-p40, IL-13 and TNFα. IL-12p40 was excluded from further analysis as all samples were below the LOD. Antibody staining and flow cytometry of cells was performed as previously described [6]. Antibodies used in flow cytometry staining were FcBlock (BD biosciences, 1:100, 564219), CD3 (APC-ef780, eBioscience, 1:800, 47-0038-42), CD4 (PE-Cy5, eBioscience, 1:400, 555348), IFNγ (BV421, BioLegend, 1:1000, 502531), IL-2 (FITC, FITCXBD biosciences, 1:25, 340448), Th2-cytokines IL-4 (PE, BD biosciences, 1:20, 340451), IL-5 (PE, BioLegend, 1:250, 504303), IL-13 (PE, BioLegend, 1:100, 501903), TNFα (PE-Cy7, eBioscience, 1:1000, 25-7349-41) , IL-10 (BV711, BD biosciences, 1:250, 564050), Foxp3 (PE-CF594, BD biosciences, 1:100, 562421) and CD25 (BD biosciences, 1:800, 340907). All antibodies are available commercially and validated by the referenced supplier. Data was analyzed with

FlowJo 10.8, gating scheme in **Supplementary Figure 1**. FMO controls were used for gating, with SEB as a positive control.

Statistical analyses

All 13 participants were included in the intention-to-treat analysis. Given the low number of participants, adverse event, CAA, and antibody data were mostly descriptive and no formal statistical testing was used to compare dose groups Data analyses and visualisation was performed using R (v4.2) and R studio (v2022.02.3). A Kaplan Meier plot was used to describe when participants first become CAA positive. For cytokine analysis, linear mixed models were fitted to compare mean responses within participants over time, with participant as a random effect and time in weeks as a fixed effect (as a factor; weeks 0,4,8,12) using the packages lme4 (version 1.1-29) and lmerTest (version 3.1-3). No other variables, such as dose group, were included in the model. Model assumptions of normality and homogeneity of residuals were checked through residual plots.

Data integration was performed using the 'mixOmics' package in R (version 6.8.0) (13). For comparability, data from both male (6) and female models were separately centered and scaled using the base R scale function. Multiblock sparse partial least squares discriminant analysis (multiblock sPLS-DA) was used to identify correlated variables in multiple dataset 'blocks' predictive of acute schistosomiasis symptoms (13). Feature selection was performed using Lasso-like penalization for all analyses. For both the male-only and combined model the number of components was set to two and tuned to determine the features per block (1-4 per component). Correlations between blocks of 0-1 were trialed, with 0.75 and 1 chosen because of the lowest error rate for the combined and male-only models. For the combined model 3,4,6 and 5 features were chosen from the Luminex, Antigens, Antibodies and Cellular blocks respectively. For the male-only model 4,4,5 and 2 features were chosen from the Luminex, Antigens, Antibodies and Cellular blocks respectively. Weighted votes consider correlation between the latent components of the block and the predicted outcome.

Role of funders

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 815643. The funder had no role in study design, data collection, data analysis, data interpretation, writing the manuscript, and the decision to submit.

RESULTS

Out of 26 individuals screened for eligibility, 13 were included. In line with the adaptive dose design, three were exposed to 10 female *Sm* cercariae, while the remaining ten were exposed to 20 female *Sm* cercariae (**Figure 1**) after discussions with the SMC. There was no loss to follow-up. Eight (62%) participants were female(**Supplementary Table 2**). Median age at infection was 26 years (range: 18-38). Immediately after exposure, rinse water was examined and showed very few remaining cercarial heads (median 0, range 0-2) or whole cercariae (median 0, range 0-1), but many cercarial tails (median 7, range 4-13), suggesting successful skin penetration (**Supplementary Table 3**).

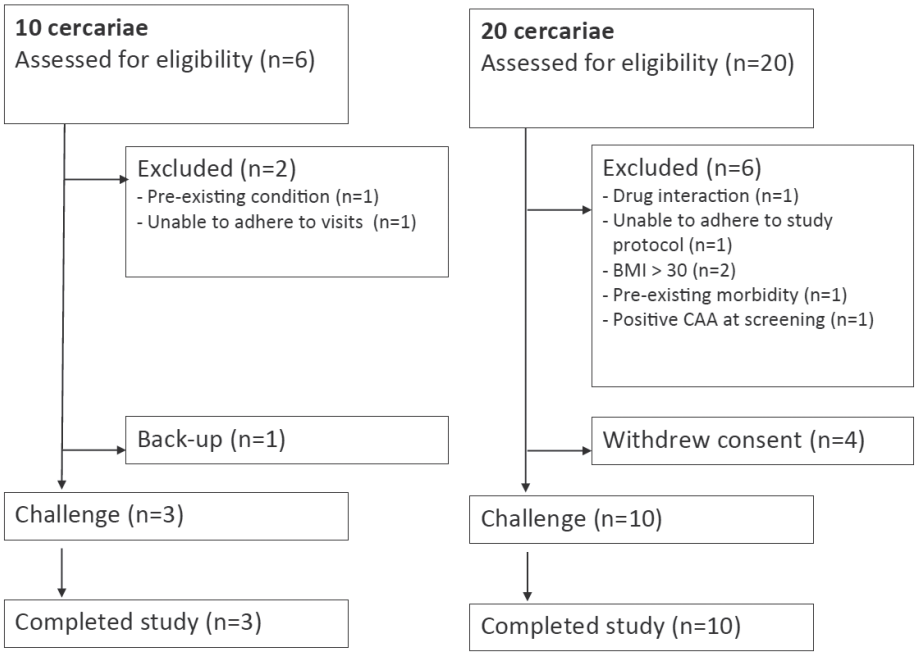

Figure 1. Consort flow diagram of study participants. Participants were exposed to either 10 or 20 female cercariae.

	All (n=13)	10 cercariae group (n=3)	20 cercariae group (n=10)
Age in years, median (range)	26 (18-38)	33 (22-38)	26 (18-35)
Sex, n (%)			
Male	5 (38%)	1 (33%)	4 (40%)
Female	8 (62%)	2 (67%)	6 (60%)

Table 1. Baseline characteristics of study participants

No serious adverse events were reported. Adverse events related to *Schistosoma* infection occurred in all participants (**Figure 2**) of which more than half (55%) were mild. Most participants developed a local skin reaction at the site of infection and reported rash (11/13, 85%) and itching (10/13, 77%) shortly after exposure. While itching resolved within 1-2 days, rash remained visible for median 23 days (range: 1-42). From week three onwards, systemic adverse events occurred (Supplementary **Figure 2**) in 5/10 participants exposed to 20 cercariae, indicative of acute schistosomiasis. One participant experienced moderate symptoms and four participants experienced severe symptoms (median duration: 2 days) that could effectively be managed with common analgesics (paracetamol and NSAIDs). One participant had prolonged symptoms that required treatment with prednisolone, after which symptoms swiftly resolved. During this period, this participant also showed transient elevations of liver enzyme tests (ALT >5 x ULN) without focal abnormalities on abdominal ultrasound, which over the course of three weeks also resolved. Apart from common PZQ side effects, no related adverse events were reported after week 8. Eosinophil counts for both dose groups peaked between 5-8 weeks after exposure (**Supplemental Figure S3**).

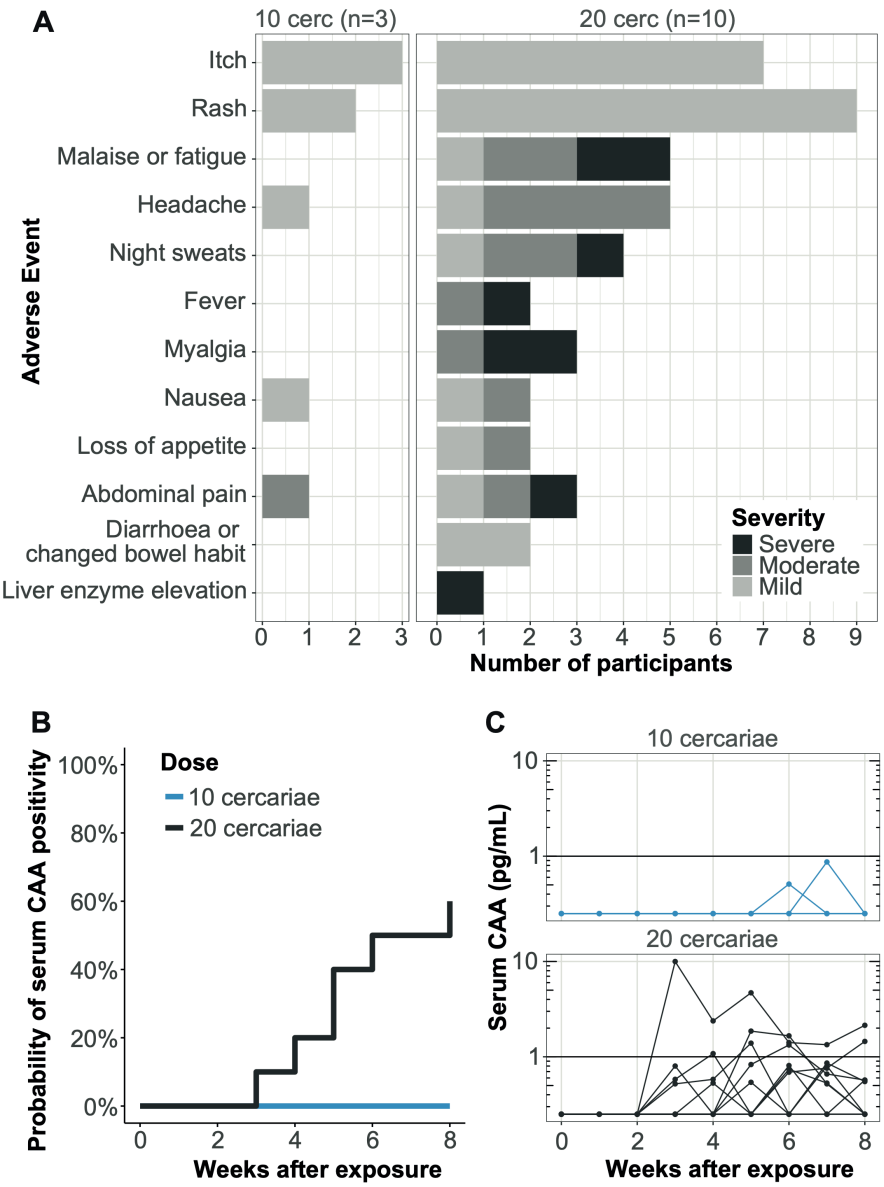

Figure 2 Safety and infectivity of exposure to female cercariae. The number of participants developing Schistosoma-related symptoms (possibly, probably or definitely related) in the eight weeks following exposure by dose and severity (a). Reverse Kaplan–Meier plot for the probability of CAA positivity (CAA ≥1.0 pg/mL, at any time point after exposure) over time per dose group (b). Individual trajectories for CAA levels (c). CAA values < 0.5 pg/mL (LoD) were set to 0.25 pg/mL. Horizontal line at 1.0 pg/mL shows the CAA cut-off for CAA positivity. Cercs = cercariae.

None of the participants exposed to 10 female cercariae showed patent infection (CAA values ≥1·0 pg/mL) at any time point after exposure, whereas in those exposed to 20 cercariae six (out of 10, 60%) did starting at three weeks after exposure (**Figure 2**). *Schistosoma* PCR on faeces at week 8 was negative for all participants indicating no eggs were produced.

After PZQ 60 mg/kg treatment at week 8 and 12, four (out of 10) participants in the 20 cercariae group showed signs of persistent infection without any symptoms (**Figure 3**) at the one-year follow-up timepoint. Three were CAA positive (≥ 1·0 pg/mL) infection, while one had an indeterminate result (CAA between 0·5-1·0 pg/mL). Remarkably, serum CAA levels first fell to below detection limit to then recur. Because repeated treatment with PZQ as a single day schedule was unsuccessful in these participants, a three-day schedule with 60 mg/kg PZQ split into three doses (morning, afternoon, and evening, 20 mg/kg each) was tried in two participants. One of these participants had self-cured already at the time of PZQ distribution and the other was not cured after the three-day course. Another participant was treated with artemether (artemether/lumefantrine 20/120mg, 24 tablets split in 6 equal doses at time = 0, 8, 24, 36, 48, and 60 hours), as artemisinin derivatives have reported to be active also against immature worms (2). Artemether/lumefantrine is the only oral artemisinin derivative registered in The Netherlands. Unfortunately, this also did not result in cure. After 6-12 additional months of follow-up three participants had self-cured, while the remaining participant remains under follow-up.

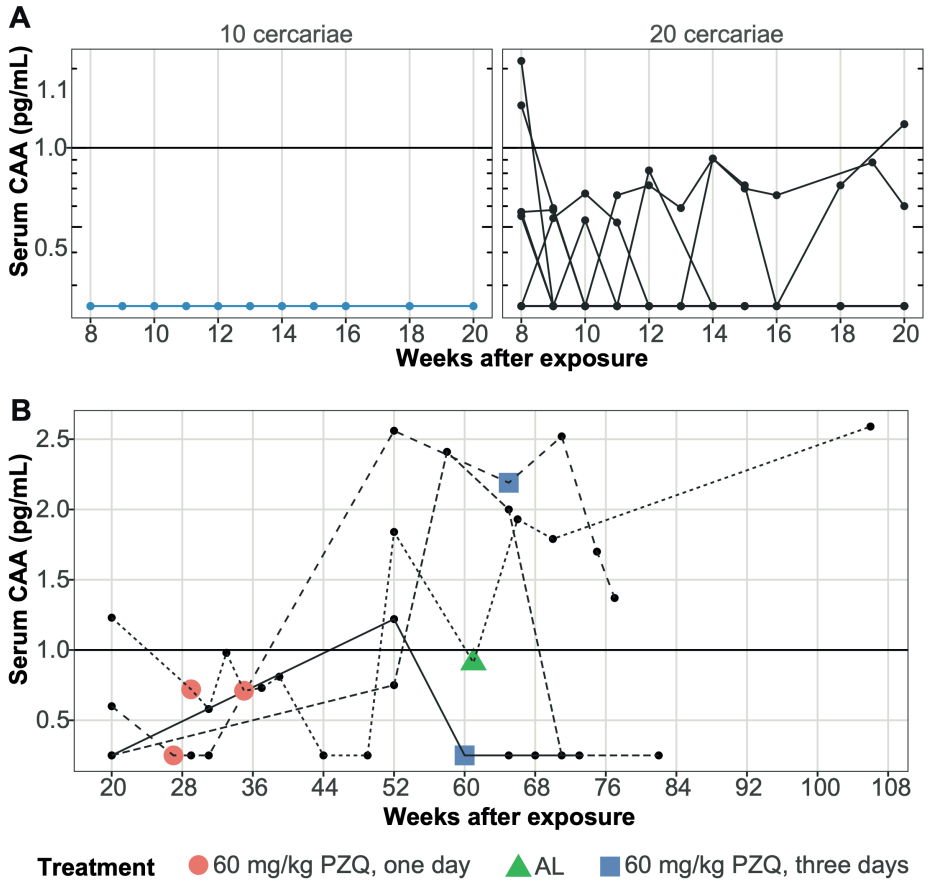

Figure 3 Serum CAA levels after treatment with PZQ. Individual CAA trajectories after treatment with PZQ 60 mg/kg at week 8 and 12 for each dose group (a). Overview of additional treatment regimens in 4 participants with persisting CAA levels (b). CAA values < 0.5 were set to 0.25. Horizontal line at 1.0 pg/mL shows the cut-off for CAA positivity. PZQ: praziquantel. AL: artemether-lumefantrine.

All participants seroconverted for adult-worm IgM between 4-7 weeks after exposure (**Figure 4A**), using IFA. Also total IgG and IgG1 against AWA measured by ELISA increased from week 8 onwards (**Figure 4B+C**), while no changes in IgG4 and IgE against AWA were observed (**Supplementary Figure 4**). Two out of 13 and 10 out of 13 participants showed detectable IgG against soluble egg antigen (SEA) at week 20 and 52, respectively.

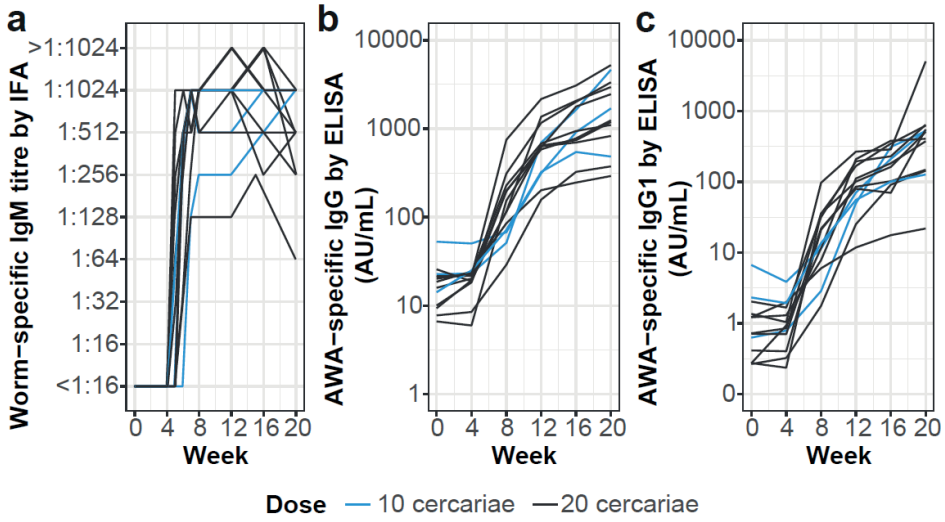

Figure 4 Antibody responses after exposure to female cercariae. Plots show individual changes in antibody levels over time in worm-specific IgM (a), AWA-specific IgG (b), and AWA-specific IgG1 (c).

Cytokines and chemokines in serum revealed an increase in the pro-inflammatory chemokines CXCL10 and CCL4 at week 4 post infection (**Supplementary Figure 5A**). Other serum cytokines measured were either unchanged during infection (CCL2) or below the limit of detection (IL-4, IL-1β and IL-10).

To understand the development of cellular *S. mansoni* specific immune responses peripheral blood mononuclear cells (PBMCs) were stimulated with AWA and both intracellular (**Supplementary Figure 5B**) and secreted (**Supplementary Figure 5C**) cytokines assessed. At week 4 post infection, $CD4^+$ T-cells showed a mixed response to *S. mansoni* AWA by secreting cytokines which encompassed Th1 (IFNγ, TNFα, IL-2 & IL-1β), Th2 (IL-4, IL-5, IL-13) and regulatory responses (IL-10) as well as the regulatory T cell transcription factor Foxp3. AWA-induced Th2 cytokine and Foxp3 expression remained significantly elevated to week 8 post infection.

Next, we wanted to understand how host and parasite parameters were associated with the occurrence of acute schistosomiasis syndrome. In line with our previous study, we used multiblock sparse partial least squares discriminant analysis (multiblock sPLS-DA), to identify correlated variables in

multiple dataset 'blocks' predictive of acute schistosomiasis symptoms (13). Using immunological and parasitological parameters from male-only infection as a training set, we were able to correctly discriminate acute schistosomiasis in 9/13 (69%) of female-only infected participants (**Figure 5A**). To improve performance of this model and potential generalizability to future studies we combined both male and female only infection studies into one multiblock sPLS-DA (**Figure 5B**). This combined model assigned 25 of 29 participants (86%) correctly (**Figure 5B**). Serum CAA and AWA-specific antibodies were poorly discriminative for symptoms. In contrast, serum cytokines performed better (65%) and the cellular block performed as well as the combined model (86% correct) (**Figure 5B**). Important variables, chosen in over 75% of the LOO models, are shown in Figure 5C. Our findings were confirmed by separate sPLS-DA analysis per block, the cellular block remaining the most discriminative (83% correct) (**Supplementary Figure 6**). AWA-specific Th2 cytokines and Foxp3 expression at week 8, the two features chosen in the highly discriminative cellular block of the male and female model (**Figure 5B&C**), together could separate the majority of symptomatic from non-symptomatic individuals (**Figure 5D**). In line with this, there were significantly higher Tregs at week 8 in individuals with acute schistosomiasis (**Figure 5E**).

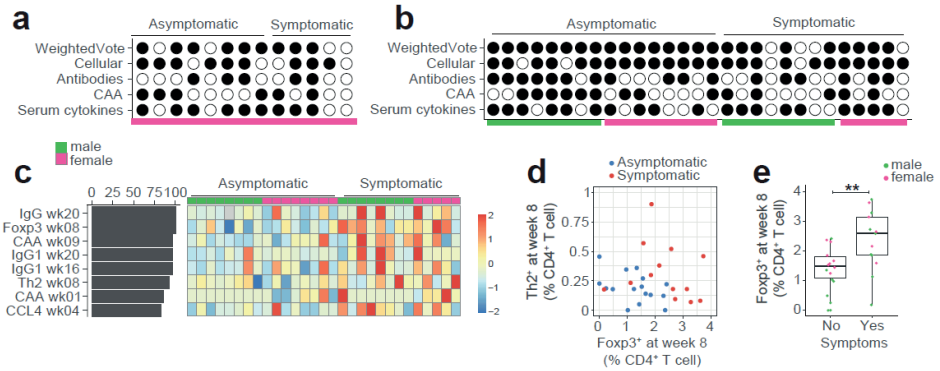

Figure 5 Data integration and symptom prediction. (a–b) Filled circles indicate a correct prediction and open circles a false prediction of acute schistosomiasis using multiblock sPLS-DA. Individual participants shown in columns, blocks in rows. (a) Capacity of multiblock sPLS-DA trained on male-only infection data to predict symptoms during female-only infection (n = 13). (b) Capacity of multiblock sPLS-DA trained on combined male and female infection (n = 29) to predict symptoms, result of leave one out cross validation (LOO-CV). (c) Consensus features present in over 75% of LOO-CV models displayed. The z score-normalized levels of chosen features are indicated in the heat map, one column corresponds to one participant. (d) Scatterplot of AWA-specific Foxp3 and Th2 cytokine expression at week 8. Points represent individual participants, coloured by symptoms. (e) Boxplot displays Foxp3 levels at week 8 in the combined studies, split by symptoms. For plotting, negative values have been placed at zero. Points represent individual participants, coloured by study, showing median, interquartile range, with whiskers extending to the largest or smallest value no further than 1.5X the interquartile range.

DISCUSSION

In this study, we established a single-sex female, *Schistosoma mansoni* controlled human infection model with 60% of participants showing serum CAA detectable infection after exposure to 20 female cercariae. Transient local (rash and itch) and systemic (acute schistosomiasis) symptoms were observed. The resulting female-only infections were refractory to treatment with PZQ. Despite double treatment with PZQ, four (out of 13) participants showed signs of persistent infection, all after exposure to 20 cercariae. Additional one- or three- day PZQ treatment (1x60 mg/kg and 3x60 mg/kg) or artemether did not result in cure, but over time three participants self-cured.

Based on animal studies, unpaired female worms are thought to remain immature and therefore excrete lower amounts of gut-derived antigens (14). However, we did not find evidence of reduced CAA secretion or symptoms in this small group of individuals. Similar attack rates were observed for exposure

to 20 female cercariae (60%, 6 out of 10) and 20 male cercariae (82%, 9 out of 11). (6) Moreover, exposure to female cercariae resulted in similar symptoms as in the single-sex male infection study, with nearly all participants having local skin reactions and roughly 50% developing symptoms of acute schistosomiasis syndrome in the 20 cercariae group, similar to 55% after exposure to 20 male cercariae (6). These symptoms could effectively be managed with standard analgesics and prednisolone. Although this risk of challenge-related symptoms was substantial, symptoms are well-tolerated and of short duration without irreversible harm in line with other controlled human infection model studies (15).

Immune responses to female-only infection were markedly similar to our previous findings during male-only schistosome infection. As observed in male-only infection, antibody responses to female-only infection were characterised by increases in worm-specific IgM, AWA-specific total IgG and IgG1 after four weeks, but not IgE. Cellular and cytokine responses peaked at week 4 post infection, with a mixed response encompassing Th1, Th2 and regulatory profiles (6). These findings contrast the dogma that schistosome infection induces initial Th1 responses, with Th2 responses induced upon egg deposition (16), instead supporting more recent evidence of mixed Th1/Th2 responses to maturing schistosomes (17). The similarity between male- and female-induced immune responses is in line with recent work using long-term male and female schistosome infections in a murine model (18). However, it contrasts prior literature which suggested male-only infections induce a more pro-inflammatory response, whilst female-only infection are characterized by more regulatory profiles (7, 19). However, given the very small set of individuals in both our male- and female-only CHI-S studies, more in- depth immunological assessments will be needed to confirm similarity of immune interaction between male and female worms.

We did not find evidence for (unfertilised) egg excretion in stool by PCR, but did observe egg-specific IgG at later time points (week 20 and 52) which may be suggestive of antibody cross-reactivity, similar to the male-only study, to for instance glycan epitopes that are shared between cercariae and eggs (20). However, we cannot entirely rule out the presence of unfertilised eggs below the PCR detection threshold or that do not penetrate the gut. In contrast to male-only infections where egg production has not been reported, we and others have in rare occasions observed egg production in single-sex female infection of mice (14, 21). The number of eggs recovered in mice were negligible

compared to mixed-sex infections and were never found to contain a viable miracidium.

Integrating immune and parasitological parameters with acute schistosomiasis symptoms revealed that symptoms were related to interindividual variability in host responses rather than the levels of active infection as measured by CAA. Long-lasting (week 8) AWA-specific Treg expansion was particularly discriminatory for symptomatic individuals. We suggest that both week 8 Treg expansion, and week 4 symptoms may result from enhanced inflammation at week 4 in symptomatic individuals, supported by a trend for increased inflammatory chemokine (CCL4) in symptomatic individuals. (Further immunological characterisation is required to establish a definitive causal relationship however. Tregs have been shown to be elevated during endemic *S. mansoni* infection (22), with murine models revealing a crucial role for Tregs in reducing immune pathology following egg-production (23, 24). Whether the enhanced AWA-specific Treg response found in symptomatic individuals in CHI-S could dampen immune responses to subsequent schistosome infection remains to be investigated.

Even though individuals were treated twice with PZQ, infection could not be fully cleared in four out of six individuals. Moreover, repeated treatment with a one- and or three- day PZQ or artemether-lumefantrine did not lead to cure, however three participants self-cleared within 2 years after exposure. The remaining participant, although showing low CAA levels, just above the limit of detection, will remain under follow-up and is also expected to self-clear. Unfortunately, oxamniquine which is also used to treat *Sm* infecton is not registered nor available in The Netherlands and could therefore not be used. Decreased drug susceptibility of single-sex females to PZQ had previously only been reported in animal models and is not well understood (25, 26). One of the explanations proposed is the aforementioned incomplete maturation of the female in the absence of a male worm which would result in decreased sensitivity.. Artemisinin derivatives have reported efficacy against immature stages of the worm (27), but in our participant did not lead to cure. Another explanation for the reduced susceptibility may be the location of the worms: single-sex female worms may be unable to migrate to the mesenteric vessels and stay in the liver, where the effective drug concentration is lower due to high first-pass effect of PZQ. Other individual pharmacogenetic factors, particularly in drug-metabolising cytochrome P450 enzymes, have also been found to influence effective drug concentrations (28), however given the high frequency of PZQ failure in our study, this seems unlikely as a single cause of

drug failure. Despite the excellent safety profile, the lack of cure after PZQ treatment unfortunately precludes the use of the female-only CHI-S model at a larger scale.

The decreased susceptibility of unpaired female worms to PZQ may also have important implications for schistosomiasis control programs in endemic areas. Even though the frequency of single-sex infection in nature is unclear, it seems likely unpaired female worms can survive treatment and can persist for over two years. Recently, Winkelmann et al. hypothesised that female worms may have developed more effective evasion strategies than males as demonstrated through repulsion of the opsonized surface, faster regeneration after PZQ, and upregulation of gene expression associated with tegument maintenance after incubation with serum (29). This was observed in paired (and separated) female worms from a mixed-sex infection as well as in unpaired female worms from a single-sex infection. If natural resistance of female worms to PZQ indeed also holds true for paired worms, surviving females may explain the findings of low cure rates, such as a recent study in schoolchildren investigating repeated PZQ treatment, which found a considerable decrease in CAA in urine but a lack of clearance (30).

An important limitation of this study involves the small sample size and the intrinsic difference to natural, mixed-sex egg-producing infections. Nevertheless, we were able to gain valuable insights into host responses following exposure to female cercariae, which were clinically and immunologically very similar to those in the male-only model. Moreover, this study shows decreased susceptibility of female worms to PZQ in the human host which limits the use of this model for future vaccine testing but may have important implications for disease control programs.

Contributors:

MR acquired funding. JK, MR prepared the research protocol. JK, MR, CH, MY. were involved in study design. JS, MC, IvA, PM, AvD, HH were involved in production and release of cercariae. JK, EH, JJ, MC, OL, JS, CD, SH, AO, VK, GR, LdB, YK generated the data. LvL, LW, GvD, PC were involved in the infection endpoint measurements and interpretation. JK, JJ were involved in data curation, project administration, and accessed and verified the data. JK, EH performed the data analyses and prepared the first draft. SJ provided support for mixomics analyses. All authors have read and approved the final version of the manuscript. All authors have confirmed full access to all data in the study and were responsible for the decision to submit the manuscript for publication.

Data sharing statement: After publication, all data will undergo FAIRification and will be made available anonymised through a LUMC-based fair data point which will be made accessible through data visiting. The study protocol is available as supplementary material with this publication.

Declaration of interests: Authors declare that they have no competing interests

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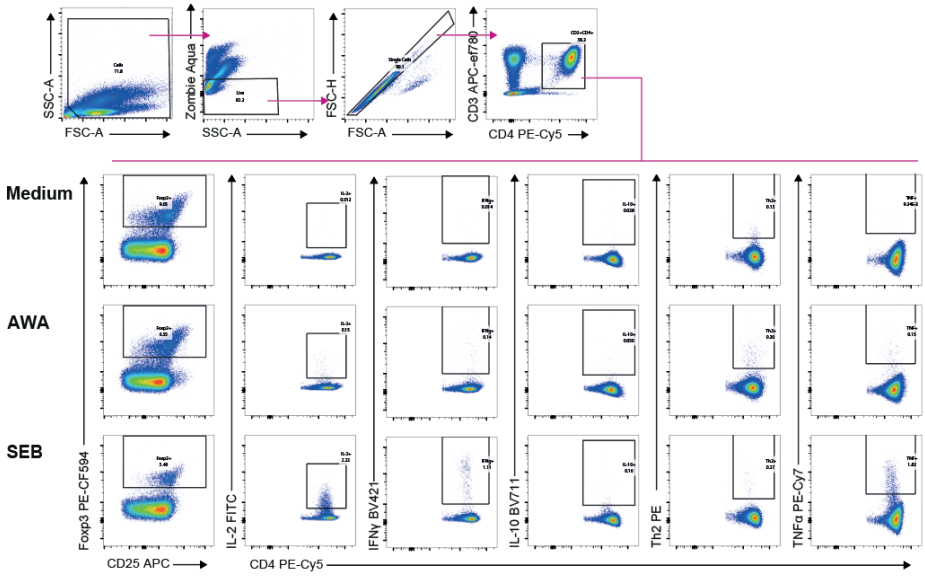
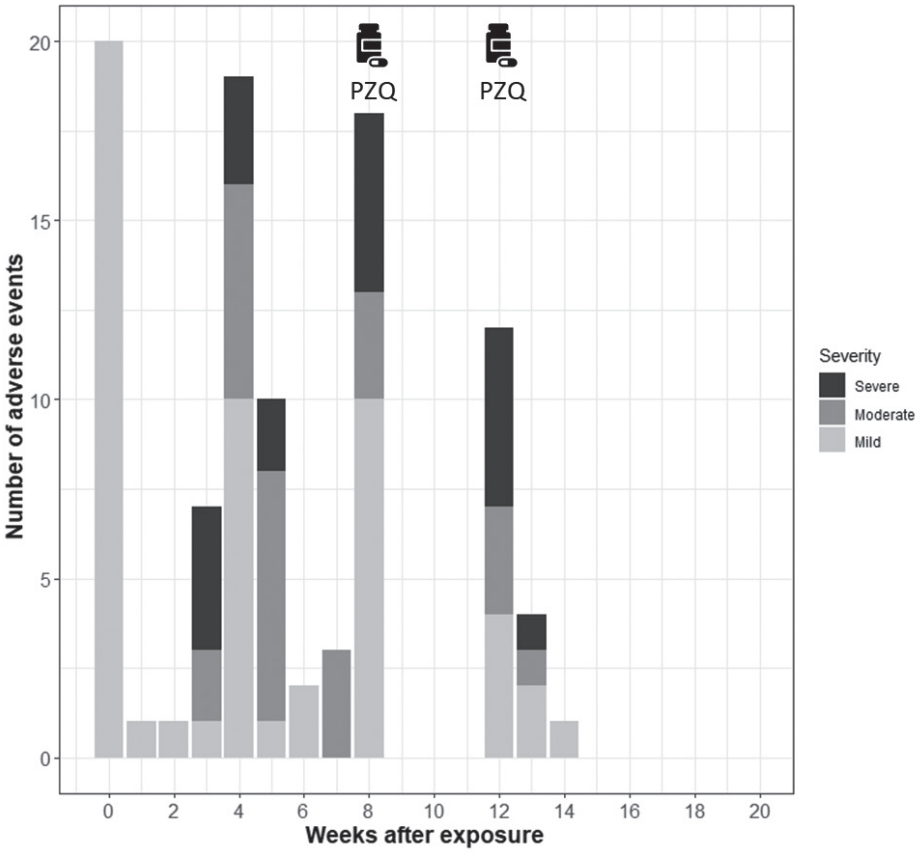
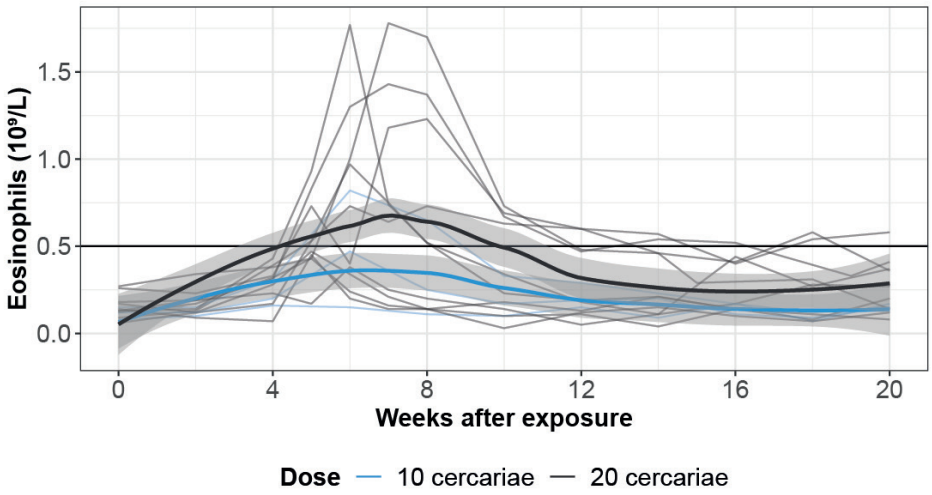
SUPPLEMENTARY MATERIALS



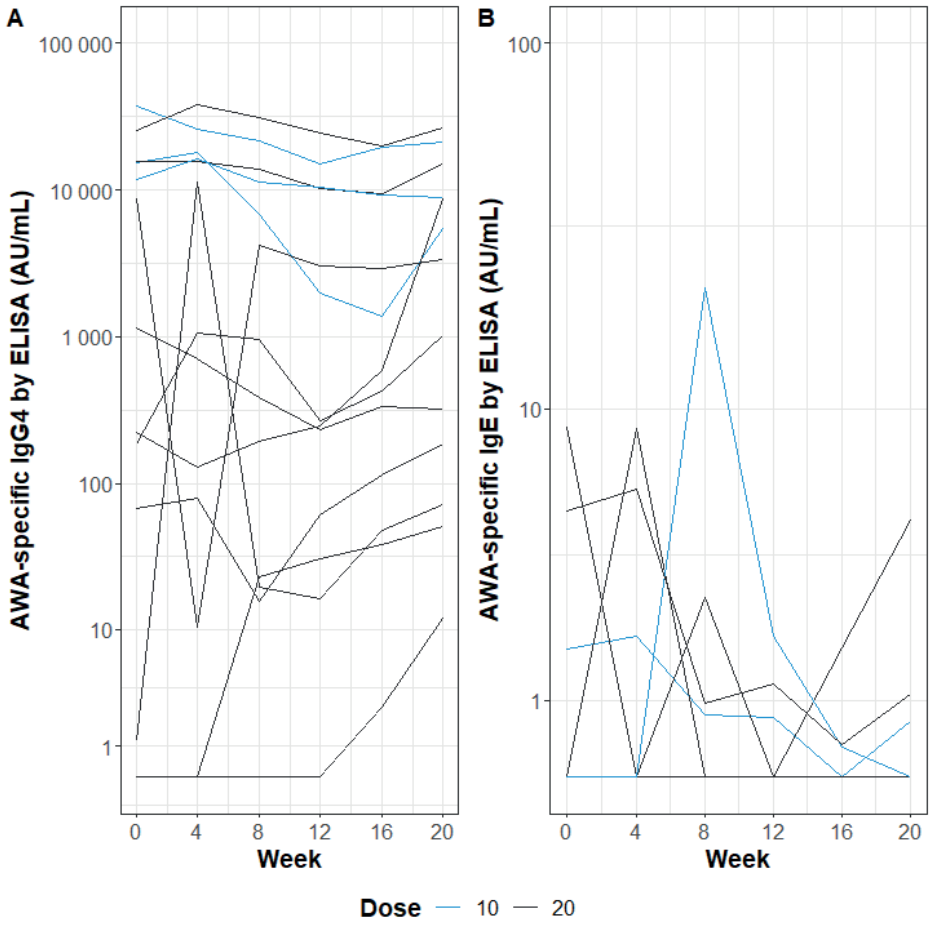
Supplementary Figure 1. Flow cytometry gating strategy. Initial gates chose cells, live cells, single cells and then $CD4^+$ $CD3^+$ T cells. $CD4^+$ $CD3^+$ T cells were then gated on Foxp3, IL2, IFNγ, IL10, Th2 (IL-4, IL-5, IL-13) or TNFα, positive cells. Initial gates are shown from medium stimulated cells. Foxp3 and cytokine gates show cells stimulated with either medium, Staphylococcal Enterotoxin B (SEB) or adult worm antigen (AWA). Magenta arrows relate to order of gating, numbers in plots are frequencies of parent population.



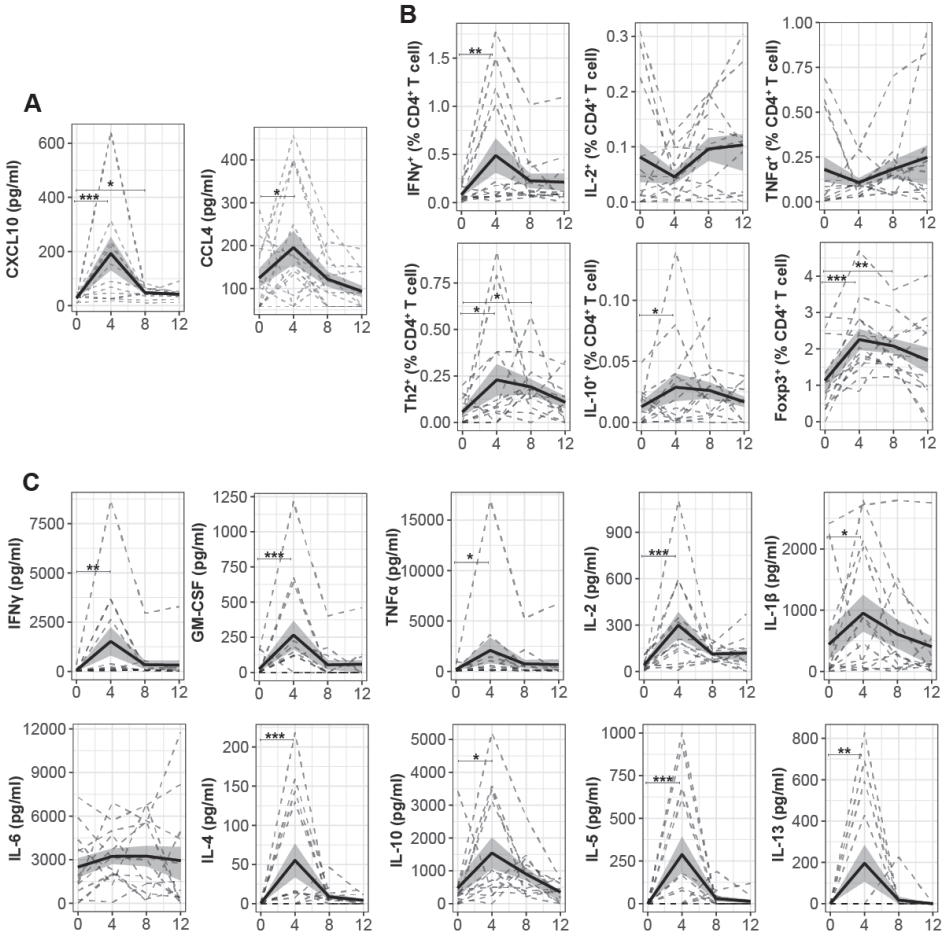
Supplementary Figure 2. Timing of related adverse events after challenge for all participants. This figure shows that a large number of AEs are reported at week 0 (itch and rash). Another increase in reported AEs occurs between weeks 3-5 which correspond to the onset of acute schistosomiasis. Note these only occurred in those exposed to 20 cercariae. High number of adverse events reported at week 8 and 12-14 are common side effects of praziquantel treatment. PZQ: praziquantel



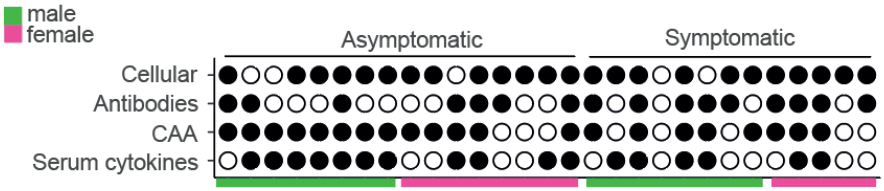
Supplementary Figure 3. Eosinophil counts after exposure to female cercariae. Graph shows the kinetics in eosinophil counts for the two dose groups. The light blue and light grey lines show the individual trajectories, whereas the bold lines show the locally weighted smoothing (LOESS) line with 95% confidence interval. Horizontal line at 0·5 * 10^9 eosinophils/L shows the clinical cut-off for abnormality.



Supplementary Figure 4. Antibody responses after exposure to female cercariae. Plots show individual changes in antibody levels over time in worm-specific IgG4 (A), and AWA-specific IgE (B).



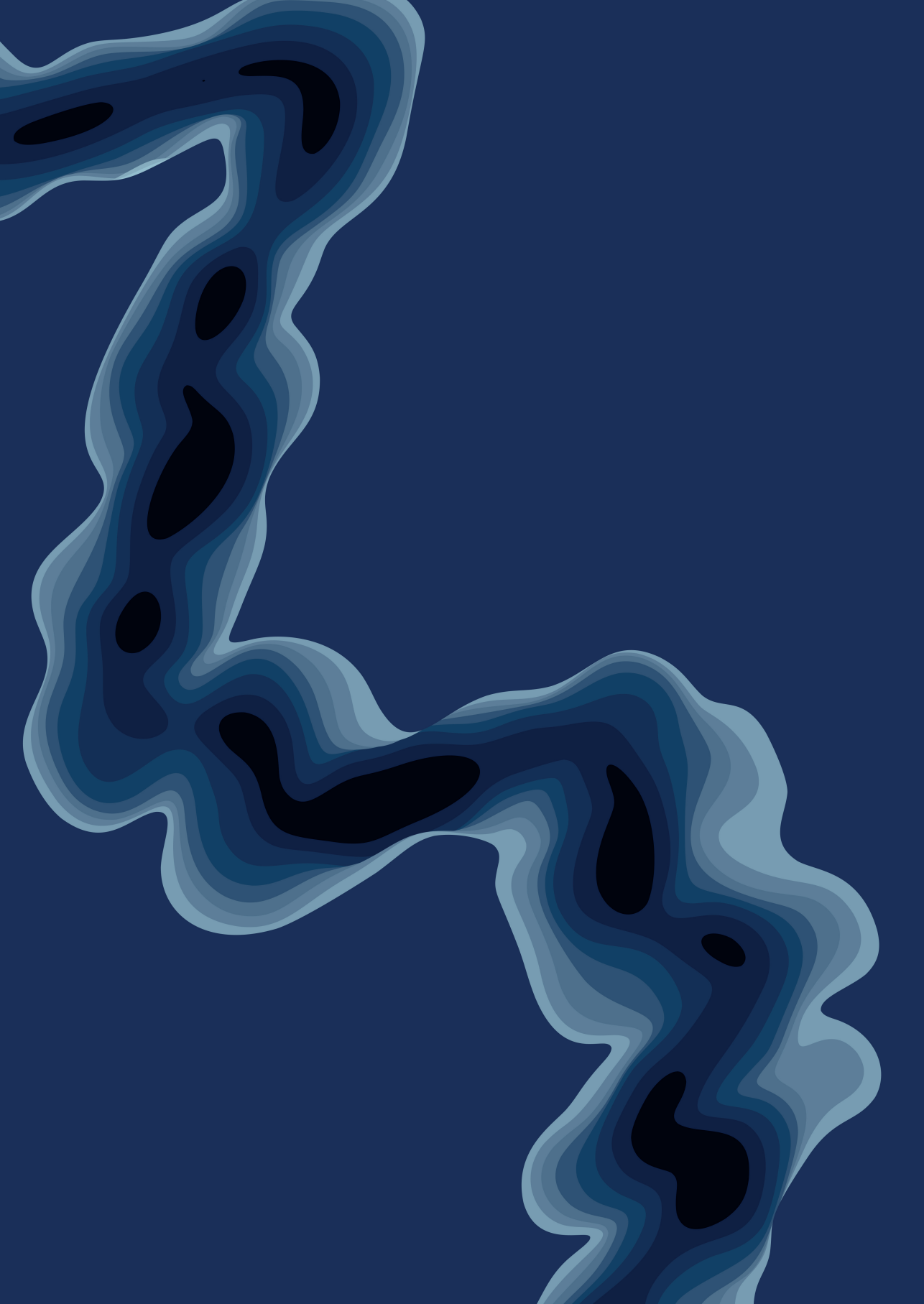
Supplementary Figure 5. Serum cytokines, AWA induced intracellular and secreted cytokines. Serum cytokines CXCL10 and CCL4, measured by Luminex multiplex immunoassay **(A)**. PBMCs were stimulated for 24hrs with 50µg/ml AWA or medium before analysis of intracellular cytokines via flow cytometry **(B)** and secreted cytokines via Luminex multiplex immunoassay **(C).** Responses are displayed post subtraction of corresponding medium stimulated values. Dashed lines represent individual participants, solid line the overall mean and the grey ribbon encompassing the standard error of the mean. For plotting, negative values have been shown as 0. Linear mixed models were fitted, with p-values shown on plots estimated using Satterthwaite's method for degrees of freedom. *p<0·05, **p<0·01, ***p<0·001.



Supplementary Fig 6. Individual sPLS-DA prediction analyses. Filled circles indicate a correct prediction and open circles a false prediction of acute schistosomiasis using individual sPLS-DA analyses per row. Individual participants shown in columns, blocks in rows. Model trained on male and female infection data, predictions result of LOO-CV. Tuning was performed to determine the features chosen within each sPLS-DA, with a range of 1-4 features per component.

Supplementary Table 1. Microscopy counts of cercariae in rinse water after exposure

	10 cercariae (n=3)	20 cercariae (n=10)
Heads, median (range)	1 (0-1)	1 (0-1)
Tails, median (range)	8 (7-9)	7 (4-13)
Whole cercariae, median (range)	0 (0-2)	0 (0-1)



Chapter 4

Risk assessment for the implementation of controlled human *Schistosoma mansoni* infection trials in Uganda

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ABSTRACT

Schistosomiasis is a parasitic infection highly prevalent in sub-Saharan Africa, and a significant cause of morbidity; it is a priority for vaccine development. A controlled human infection model for *Schistosoma mansoni* (CHI-S) with potential to accelerate vaccine development has been developed among naïve volunteers in the Netherlands. Because responses both to infections and candidate vaccines are likely to differ between endemic and non-endemic settings, we propose to establish a CHI-S in Uganda where *Schistosoma mansoni* is endemic. As part of a "road-map" to this goal, we have undertaken a risk assessment. We identified risks related to importing of laboratory vector snails and schistosome strains from the Netherlands to Uganda; exposure to natural infection in endemic settings concurrently with CHI-S studies, and unfamiliarity of the community with the nature, risks and rationale for CHI. Mitigating strategies are proposed. With careful implementation of the latter, we believe that CHI-S can be implemented safely in Uganda. Our reflections are presented here to promote feedback and discussion.

Keywords: *Schistosoma mansoni*, Controlled Human Infection Studies, Uganda, risk assessment

BACKGROUND

Schistosomiasis is a parasitic infection affecting approximately 230 million people worldwide (1). Infection is caused by trematodes (flukes) of the genus *Schistosoma.* Because the infection is responsible for considerable morbidity worldwide, particularly in Africa, schistosomiasis was recently listed among the top 10 infections for which a vaccine should urgently be developed (2).

Controlled human infection (CHI) studies are an important tool for vaccine development. They provide a platform to safely and swiftly test vaccine candidates for the pathogen in question. Furthermore, they can contribute to understanding host-pathogen interactions and help to unravel the nature of protective immunity. They have been used successfully for a substantial number of infectious diseases, including malaria, dengue, and influenza (3). A CHI model has now been developed for schistosomiasis at Leiden University Medical Center, where Dutch volunteers with no previous exposure to schistosomiasis participated (3) . However, the response to schistosome infection, and to candidate vaccines, is likely to be different in endemic countries. In such settings multiple differences in environmental exposures, as well as prior exposure to schistosomes, drive differences in both the innate and adaptive immune responses which determine infection susceptibility and vaccine responses (4, 5).

We are therefore working towards the establishment of a controlled human infection model for schistosomiasis in Uganda, where *Schistosoma mansoni* is highly endemic. Almost 30% of the population is estimated to be infected (6) , with half the population at risk (7). As a first step we held a stakeholders' meeting in Uganda in November 2017, and we published the meeting report and resultant road-map for the implementation process (8). A key element of the road-map was to undertake a risk assessment. This document therefore aims to provide an assessment of risks that may arise before, during and after start of a controlled human infection model with *Schistosoma mansoni* (CHI-S) in Uganda.

Male and female schistosomes live in the mesenteric or perivesical veins of their human host, where they mate and produce eggs. These eggs are either released into the environment through faeces and urine or stay within the host tissue where they induce inflammation. When the excreted eggs reach fresh water, they hatch and release miracidia that can then infect a suitable snail host. Infected snails are able to shed larvae, called cercariae, which infect humans. The Leiden University Medical Center (LUMC) CHI-S exposed healthy

naïve volunteers to increasing doses of male cercariae to study the tolerability of such a controlled human infection model. This male-only model avoids the risk of pathology caused by schistosome eggs. To generate the infectious cercariae for a male-only CHI-S, individual laboratory-reared freshwater snails are infected, each with a single miracidium. Clonal replication follows, such that thousands of single-sex cercariae are subsequently shed by the snail. The sex of the cercariae can be determined by PCR, and the appropriate number of cercariae can be prepared for dermal infection. Because snails shed thousands of cercariae over a period of weeks, every time they are exposed to light, it is possible to first perform quality control (QC) testing on every batch (e.g. to assess the viability, sex and bioburden of the cercariae). Following principles set forward in good manufacturing practices (GMP) guidelines, the cercariae and their excipients are produced and tested for consistent quality according to predefined criteria. Only when compliant, is the cercariae batch released for clinical use. To this date, 17 people have been exposed to *S. mansoni* cercariae during CHI-S studies in Leiden.

In terms of the technical aspects of shipping infectious material to Uganda, culturing the infectious material in Uganda and preparing the infectious cercariae, we have considered three options.

Option 1: Shipping of parasites and snails from the Netherlands to Uganda. In this scenario, *S. mansoni* parasites and snails would be shipped from Leiden (The Netherlands) for preparation of the cercariae for human infection in Uganda. From a technical perspective, the easiest approach to rapid implementation of CHI-S in Uganda would be to produce and release the infectious snails in Leiden and subsequently ship them to Uganda. In Uganda, a further snail shedding would be used to generate the infectious cercariae. Alternatively, *S. mansoni* parasites (for example in the form of *S. mansoni* eggs contained in a rodent liver) could be shipped separately from uninfected snails, which would mitigate shipment risks.

The CHI-S model in Leiden uses a schistosome strain which has been genotyped and has been mapped to be of Puerto Rican origin (3). Because this strain has been laboratory adapted and kept in the Leiden facility since 1955, it has the advantage of its known virulence in animals, experience of its effects in the Dutch human volunteers, and its sensitivity to praziquantel. As well, the Leiden model uses *Biomphalaria glabrata* snails which are not indigenous to Uganda (Appendix 1 [Extended data (9)]). Therefore, the ecological risks of

accidental release of schistosomes or snails or into the environment have to be considered.

Option 2: Shipping of parasites from The Netherlands followed by use of local Ugandan snails. This scenario would involve transporting only *S. mansoni* parasites (Puerto-Rican strain), then using local snail species such as *B. choanomphala* (from Lake Victoria) or *B. stanleyi* (from Lake Albert) to produce cercariae in Uganda (10) . Advantages, as in option 1, would be the fact that the parasite strain has been characterized in both animals and humans, which decreases its potential risk for the volunteers. Disadvantages would be possible technical hurdles to be overcome to establish a local snail colony and achieve successful infection with release of infectious *S. mansoni* cercariae. However, expertise in these processes already exists in Uganda (10) , subject to laboratory renovations and staff training to ensure compliance with GMP principles. This option would also be relatively simple to implement.

Option 3: Using local Ugandan parasites and local Ugandan snails. In this scenario the full *S. mansoni* laboratory life cycle would be established in Uganda, using a local snail species and starting with a new *S. mansoni* strain, and a rodent mammalian host. Although the risk of clinically unexpected, unwanted side effects, or of relative resistance to praziquantel treatment, might be higher when using the local strain of *S. mansoni*, the ecological risk would be lowest.

All options require preparation of the cercariae for human infection under strict Quality Assurance and controlled conditions in Uganda with adherence to Good Manufacturing Guidelines. In Leiden, procedures were developed based on GMP principles contained in the European Commission directive 2003/94/EC, with the infectious cercariae considered as an "auxiliary medicinal product". Details of the procedures have been published (3) . These include production in a biosafety level 3 facility, governed by stringent standard operating procedures including for quality control, logging and monitoring; production and counting of infectious cercariae by two independent technologists; and antibiotic treatment and microbiological bioburden testing to ensure that the cercarial product is free of pathogens with potential to harm CHI volunteers. Equivalent procedures and quality control will be needed in Uganda in order to implement CHI-S.

In this document we address risks associated with CHI-S in Uganda on three different levels: i) the introduction of new species (the transport of snails, the

snail culture facilities, the potential for ecological harm as a result of importing snails), ii) the introduction of a new schistosome strain into Uganda, and iii) clinical trial risks common to all options (natural infection during the trial period, and the risks to volunteers resulting from the controlled infection).

RISK ASSESSMENT METHODS

We identified risks and potential approaches to mitigation based on relevant literature, experience from the Leiden CHI-S model, stakeholder discussions, and discussion with experts. The level of risk and effectiveness of proposed controls was determined by consensus between the authors. The inherent risk was defined as the risk before putting controls in place, calculated as the product of the likelihood and impact scores. The residual risk was similarly calculated, based on likelihood and impact scores after controls have been put in place. Mitigating controls could reduce the residual risk score by reducing the likelihood of an event occurring, or by reducing the impact if it should occur. Likelihood was scored as almost certain/common, 5; likely, 4; possible, 3; unlikely, 2; rare, 1. Impact was scored as critical, 5; major, 4; moderate, 3; minor, 2; insignificant 1. Resulting risk scores of 18–25 were considered high, and unacceptable. Resulting risk scores in the range of 9–17 were considered moderate, with further controls desirable if possible, and caution required if implemented at this risk level. Resulting scores of 0–8 were considered low, and usually acceptable.

OPTION 1: SHIPPING OF PARASITES AND SNAILS FROM THE NETHERLANDS TO UGANDA

According to our first idea, infected snails would be shipped. The WHO report 'Guidance on regulations for the Transport of Infectious Substances 2017–2018' (11) provides information on how to adequately transport infectious substances. In accordance with these guidelines, shipment of *S. mansoni* infected snails falls under 'CATEGORY B, INFECTIOUS SUBSTANCES' (UN3373). Shipment of live snails is a time-sensitive undertaking and therefore can only be facilitated by air shipment. Infectious substances cannot be carried on as hand-luggage. Transport of infectious substances are subjected to International Air Transport Association (IATA) requirements. Packaging of Category B substances need to comply with rules set out in the P650 packaging instruction (11) . This involves triple packaging and proper marking and documentation.

Upon arrival in Uganda, it would be crucial for the package to clear customs as quickly as possible so that snails arrive in good condition. In order to achieve this, the customs office should be notified about the arrival of the shipment. In collaboration with the customs officer, all required documentation should be prepared in advance and approval for import of the products should be sought.

Alternatively, snails and *Schistosoma* parasites would be shipped separately. Uninfected snails can be shipped more easily because this shipment does not have to comply with the regulations for the transport of infectious substances. Similar to the previous option, shipment should clear customs as soon as possible. These snails could be kept to reproduce in the Ugandan laboratory to sustain their life cycle.

A second shipment would contain *Schistosoma* parasites. There are two ways in which this material can be transported (still under the 'CATEGORY B, INFECTIOUS SUBSTANCES' (UN3373)):

1. Within a living host such as a *Schistosoma*-infected hamster. These animals can shed *Schistosoma* eggs that can be used to infect the snails.
2. Within a preserved liver sample kept on medium from a *Schistosoma*-infected hamster. This liver sample contains *Schistosoma* eggs. Upon arrival in Uganda, further processing of the sample provides miracidia which can be used to infect the snails. Test shipments should be scheduled to determine the feasibility of such transports and the conditions in which the liver sample should be shipped. From previous experiments in Leiden, the preserved liver sample can be used to infect snails for up to one week after being harvested.

Risks associated with shipping of parasites and snails from the Netherlands to Uganda, and mitigating strategies, are summarized in **Table 1**.

Table 1. Risks associated with shipping of Schistosoma mansoni parasites and Biomphalaria glabrata snails.

Risk	Inherent risk score		Total inherent risk	Controls	Residual risk score		Total risk post control
	Likelihood	Impact			Likelihood	Impact	
Death of snails in transport	Likely	Critical	20	Pilot transport with low numbers of snails to optimize transport conditions	Possible	Critical	15
Delays in customs clearance	Likely	Major	16	Contacting customs officials to discuss required documentations and preparing documents prior to shipment	Possible	Major	12
Spill of infectious materials and non-indigenous snail species	Possible	Major	12	Proper packaging	Unlikely	Moderate	6
Establishment of a *B. glabrata* colony outside laboratory facility	Possible	Critical	15	Proper packaging	Rare	Critical	5

Likelihood was scored as almost certain/common, 5; likely, 4; possible, 3; unlikely, 2; rare, 1. Impact was scored as critical, 5; major, 4; moderate, 3; minor, 2; insignificant 1.

Option 1: snail culture facilities; potential ecological harm

To house the *Biomphalaria glabrata* snails in Uganda, they would need to be kept in strict quarantine. *B. glabrata* are not a naturally occurring snail host in Uganda, and should therefore not spread to the environment. In order to house snails, an incubator, or room temperature, set and monitored at 28˚C is needed. The incubator (if used) door should be fully closed when the laboratory is not in use. Precautionary measures to contain the snails to the facility should be taken and include physical barriers, such as rooms with closed doors and windows. The snail culture basins and water drainage system should be covered with fine mesh to prevent escape (appendix 1 [Extended data (9)]). In addition, access to the laboratory should be controlled and restricted to the research team. The incubator (if used) should preferably be positioned away from the door. Additional security measures could be a double door to create a sluice. Appendix 2 (Extended data (9)) lists precautionary measures that should to be taken when working with schistosomes. Standard operating procedures (SOPs) will be exchanged with LUMC and reviewed to fit the Ugandan facility. These SOPs deal with culture processes as well as the disposal of infectious material.

In case a single snail would accidentally be released into the environment, it is capable of reproducing in the absence of an opposite-sex snail using self-insemination (12). This ability poses an ecological hazard where a single snail could develop into a colony. In addition, snails can be transported over large distances attached to birds and can survive dry conditions for up to two months. This snail itself is not endemic in Uganda, although previously this species has been held at the Vector Control Division of the Ministry of Health for a different project. The consequences of accidental introduction of this new species are difficult to predict, however it may result in the following (Appendix 1 [Extended data (9)]):

1. Interspecific hybridization between *B. glabrata* and local *Biomphalaria* species
2. Uncontrolled spread due to lack of natural enemies, competitors or pathogens
3. Altered *S. mansoni* dynamics, because of potentially higher susceptibility of *B. glabrata* for *S. mansoni* infection

Spread to the environment of *B. glabrata* may go unnoticed, because of its similar morphology to endemic snail species.

Risks associated with culture of *B. glabrata* in Uganda, and mitigating strategies, are summarised in **Table 2**.

Table 2. Risks associated with snail culture facilities.

Risk	Inherent risk score		Total inherent risk	Controls	Residual risk score		Total risk post control
	Likelihood	Impact			Likelihood	Impact	
Spread of *Biomphalaria glabrata* snail to environment	Possible	Critical	15	1) Precautionary measures for snail housing facility including physical barriers and restricted access 2) Use of SOPs regarding disposal of infectious material and non-indigenous snail species	Rare	Critical	5
Establishment of a *B. glabrata* colony outside laboratory facility	Possible	Critical	15	1) Development of containment strategies	Rare	Critical	5

Likelihood was scored as almost certain/common, 5; likely, 4; possible, 3; unlikely, 2; rare, 1. Impact was scored as critical, 5; major, 4; moderate, 3; minor, 2; insignificant 1.

OPTION 2: TRANSPORT OF *S. MANSONI* INFECTIOUS MATERIAL AND USE OF LOCAL SNAIL SPECIES FOR CERCARIAL PRODUCTION

This approach only requires transport of *S. mansoni* infectious material. This would use the second transport approach described in option 1, within a preserved liver sample from a schistosomiasis-infected hamster. The same regulatory guidelines for transporting infectious material apply. With regard to Ugandan snail species, there is variability between snail species in susceptibility to *S. mansoni* infection; however, there is experience of conducting infection of local species at the Vector Control Division (10) , so this is expected to be feasible. A major advantage of this approach is that the potential ecological and genetic risks related to introduction of a non-endemic snail species can be avoided.

OPTION 3: RE-ESTABLISHING THE FULL *S. MANSONI* LABORATORY LIFE CYCLE IN UGANDA, USING A LOCAL SNAIL SPECIES AND *S. MANSONI* STRAIN

The alternative to shipping infectious material and snails from The Netherlands is to re-establish the full laboratory life cycle of *S. mansoni* using Ugandan snail species and Ugandan isolates of *S. mansoni*. The life-cycle has been maintained in the past at the Vector Control Division of the Ministry of Health, but is not currently available. The advantages of using a Ugandan life cycle include reducing the environmental risk associated with non-endemic snail species and schistosome strains. In addition, this model would be most representative of the field infections in Uganda. Similar to option 2, although susceptibility to *S mansoni* infection varies between snail species, we do not expect this to be an issue, because the Vector Control Division has experience in infecting local species. There are however several challenges with using Ugandan snails and isolates. With regard to the new schistosome laboratory strain, the characteristics of this would be unknown in terms of virulence and susceptibility to praziquantel treatment. Determining these characteristics would not be simple, since validated tests for schistosome resistance are currently not available. In addition, the new isolate would not be clonal and variability within the newly collected schistosome population might result in variable responses in the host, and to drug treatment. An inbred Ugandan strain could be achieved by crossing clonal males and clonal females to produce a single F1 generation and subsequently cloning the offspring through snails followed by another crossing. This procedure would need to be repeated several times to

be able to generate a reasonably monomorphic strain. This process would be laborious and time-consuming and might also result in quite atypical parasites, not necessarily representative of the Ugandan population of schistosomes in general. Ugandan populations have been exposed to regular praziquantel treatment for over a decade, so there is a risk that the initial isolates would include individuals with relative praziquantel resistance (13) and could not be established with certainty in the initial stages of the above process. Starting with a more diverse selection of cercariae would generate a more representative laboratory population of Ugandan schistosomes, but would mean that the characteristics of any particular clone (notably pathogenicity or praziquantel resistance) selected for CHI-S would be unpredictable.

Options 1, 2 and 3 all require the establishment of facilities in Uganda for production of the infectious cercariae under GMP principles, in order to ensure high quality, reproducible infectious doses. Option 3 requires also the establishment of suitable, specific pathogen free animal facilities to house the rodents (hamsters or mice) that will provide the mammalian hosts in the laboratory life cycle. Risks associated with these elements are also considered here (**Table 3**).

Table 3. Risks associated with re-establishing Uganda Schistosoma mansoni life cycle.

Risk	Inherent risk score		Total inherent risk	Controls	Residual risk score		Total risk post control
	Likelihood	Impact			Likelihood	Impact	
New isolates of *S. mansoni* from the Ugandan population might exhibit variable praziquantel susceptibility, or praziquantel resistance	Possible	Critical	15	1) Test new isolates for praziquantel susceptibility *in vitro* and in an animal model before use in CHI	Unlikely	Critical	10
New isolates of *S. mansoni* from the Ugandan population might exhibit unexpected virulence	Possible	Critical	15	1) Test new isolates for relative virulence in an animal model before use in CHI	Unlikely	Critical	10
Production processes based on GMP principles for single-sex infectious cercariae not established in Uganda	Possible	Critical	15	1) Development of appropriate animal and snail facilities 2) Training of Ugandan staff 3) Monitoring and review by experienced LUMC collaborators 4) Monitoring and review by Ugandan regulators	Rare	Critical	5

Likelihood was scored as almost certain/common, 5; likely, 4; possible, 3; unlikely, 2; rare, 1. Impact was scored as critical, 5; major, 4; moderate, 3; minor, 2; insignificant 1.

NATURAL INFECTION DURING TRIAL PERIOD

The single-sex *S. mansoni* challenge has been designed to prevent the occurrence of egg-associated morbidity. In the current model, volunteers participating in the trial will be infected using only male cercariae which penetrate the skin and result in patent infection. In future, a single-sex female cercariae model may also be used to infect volunteers. The sex of the male cercariae can be determined using a specifically designed multiplex real-time PCR which has been described elsewhere (3). Once infected, individuals should avoid any exposure to contaminated water. If a subject were to be naturally infected over the course of the study, this might lead to mixed, male and female, infections, with mating of the schistosomes resulting in egg production that causes morbidity. If the Puerto Rican strain used in Leiden is imported for use in Uganda, mating and (if adequate sanitation is not used) excretion of eggs into the environment could alter the genetic make-up of Ugandan schistosome populations, with unknown consequences. However, given the fact that the Puerto Rican strain has been kept in rodents for >60 years, it seems likely that fitness in humans will be, if anything, lower than Ugandan human strains. Moreover, given that the Puerto-Rican strain is relatively inbred after prolonged passage in the laboratory, and was shown to be praziquantel-sensitive in the CHI-S, hybridisation with Ugandan schistosome populations is unlikely to result in increased praziquantel resistance or virulence.

The chance of natural infection can be limited by choosing a study population which does not come into contact with freshwater. However, this would over-restrict recruitment from the true target population, which is people at risk of

S. mansoni infection. Options to minimise this risk among volunteers from the preferred target population include the following:

1. The feasibility of avoiding fresh water may be surveyed using questionnaires in a pilot study at the field site and the information used to select volunteers least at risk of re-exposure, and to make provisions to support volunteers to avoid re-exposure.
2. While selecting subjects, the investigator may ask whether the subject is likely to spend time in, or to travel to, areas where the risk of contracting a natural infection is high. If so, once again it should be stressed that contact with fresh water should be avoided; volunteers unlikely to achieve this would be excluded.
3. Apart from providing information to the volunteer and raising awareness of this issue, frequent testing for eggs in stool and urine samples may be performed by microscopy (and PCR). Eggs can be found 5–7 weeks after mixed male and female infection (1). *S. mansoni* eggs in stool would indicate a concomitant natural infection, which would necessitate immediate treatment of the volunteer with praziquantel. However, stool microscopy and PCR is likely to be unreliable given variable egg excretion and the low sensitivity of stool examination for eggs (14).
4. In those trials in which natural infection may be a considerable risk, testing using plasma circulating anodic antigen (CAA) may be conducted weekly from the outset of the trial. Both natural and experimental infections may then be terminated as soon as patent infection has been detected (e.g. at ~7 weeks post controlled human infection, when CAA levels > 1pg/mL). Early abrogation of the infection will prevent mating and egg laying. There would be modest drawbacks to the resulting data, because it would not be possible to study the dynamics of antigen excretion over time and quantitation of infection would be less accurate.
5. Alternatively, volunteers may be displaced to a non-endemic region for the study duration. However, the prolonged, seven to 12-week "admission" required for the CHI-S would be a major burden and inconvenience, as opposed to the relatively short-duration (24 days) for malaria CHI studies where such approach has been employed (15). The possibility of volunteers absconding during the study, given the long duration, might be significant, abrogating the value of such an approach. Additionally, this would have cost implications, in terms of providing suitable accommodation and compensation for loss of income.

Risks associated with natural infection during the CHI-S, and mitigating strategies, are summarised in **Table 4**.

Table 4. Risks associated with natural infection during trial period.

Risk	Inherent risk score		Total inherent risk	Controls	Residual risk score		Total risk post control
	Likelihood	Impact			Likelihood	Impact	
Mixed sex infection in trial volunteers	Likely	Moderate *	12	1) Avoidance of fresh water bodies during trial period 2) Pilot survey to establish feasibility of fresh water avoidance 3) Selection of trial volunteers with low risk of contracting natural infection 4) Abrogation of infection as soon as the trial endpoint has been reached (e.g. CAA> 1 pg/mL) 5) Displacement of volunteers to non-endemic setting with excellent water and sanitation facilities	Rare	Moderate	3
Mixed sex infection in trial volunteers leading to release of Puerto Rican strain into environment	Likely	Moderate	12	1) Full clearance of infections before trial starts 2) Continuous screening for egg production 3) Abrogation of infection as soon as the trial endpoint has been reached (e.g. CAA> 1 pg/mL) 4) Displacement of volunteers to non-endemic setting with excellent water and sanitation facilities.	Rare	Moderate	3

Likelihood was scored as almost certain/common, 5; likely, 4; possible, 3; unlikely, 2; rare, 1. Impact was scored as critical, 5; major, 4; moderate, 3; minor, 2; insignificant 1.

[i] * The impact of natural co-infection on morbidity is classed as moderate (rather than major or critical) since volunteers who acquire such an infection would presumably be at risk of mixed-sex natural infections as a result of their usual behaviours and occupation. The risk of egg-related morbidity due to the presence of male worms from the CHI-S would therefore add little to the risk resulting from exposure to natural infection. CAA - circulating anodic antigen

RISKS TO VOLUNTEERS RESULTING FROM THE CONTROLLED HUMAN INFECTION

Controlled infection with *S. mansoni* has been successfully performed in 17 Dutch volunteers. Although the single sex infection does not cause egg-related morbidity in volunteers, it may cause symptoms in response to the infection. These include dermatitis due to the percutaneous penetration of the cercariae and an acute schistosomiasis as a consequence of a systemic hypersensitivity response (16) . Severe acute schistosomiasis syndrome (Katayama fever) may present with symptoms such as fever, fatigue, myalgia, malaise, non-productive cough, eosinophilia and patchy infiltrates on chest radiography. In Leiden, several volunteers reported with systemic symptoms which seemed to be an acute schistosomiasis syndrome, with one volunteer presenting with prolonged symptoms of Katayama fever (16) . In addition, one volunteer presented with peri-orbital oedema which lasted one day, and may have been related to the infection (16) . Such symptoms can be treated symptomatically and all recovered. Both these volunteers had received the highest dose of cercariae (30 cercariae) used in Leiden. The risk of severe symptoms can be minimised by dose escalation in modest increments. The impact can be reduced by careful monitoring, provision of symptomatic relief and abrogation of infection by treatment if necessary. Frequent follow up visits need to be scheduled throughout the trial to discuss adverse events and conduct clinical assessments of the study volunteers. Safety laboratory tests need to be routinely performed. Volunteers can also experience side effects related to the praziquantel treatment. Common side effects include nausea, dizziness, and fatigue. Volunteers can be reassured that these symptoms are well recognised and transient. Their severity can be reduced by taking praziquantel after food. Symptomatic relief can be provided when required.

The 2017 stakeholders' meeting identified community engagement to ensure proper understanding of the CHI-S as an essential basis for ethical conduct of a CHI study. CHI is a novel concept in Uganda, where CHI have not been

undertaken in the past and understanding of medical research, in general, is at a low level. The idea of a "medical" procedure being undertaken which is expected to cause symptoms, and undertaken for the greater, rather than an individual, good needs careful explanation. Rumours and misunderstandings have the potential to critically affect the work, and to have an adverse effect also on other institutional research activities. Engagement with national and community leaders, work with community advisory boards who can identify, and help to address, misinformation; effective education of volunteers to a full understanding of the expected effects of the CHI (and reasons for undertaking it) will all be essential to the smooth and safe running of these projects. Experiences from the first malaria CHI in Kenya give helpful guidance as to which issues are particularly relevant to participants and may require careful explanation (17).

Volunteers will receive remuneration for participating in the trial to reimburse for expenses and compensate for time and burden of participation. Careful consideration will need to be given to determine the exact amount of the remuneration to avoid coercion. Recent remuneration guidelines from Malawi can help to calculate the amount (18). In addition, formative research is currently being undertaken to explore within the target community what remuneration would be considered appropriate and acceptable.

Risks related to volunteers and communities during the CHI-S, and mitigating strategies, are summarised in **Table 5**.

Table 5. Risks associated with controlled human infection with Schistosoma mansoni.

Risk	Inherent risk score		Total inherent risk	Controls	Residual risk score		Total risk post control
	Likelihood	Impact			Likelihood	Impact	
Symptoms related to infection	Common	Major	20	1) Slow dose escalation in modest increments 2) Frequent follow up visits and collection of adverse events. 3) Clinical assessment and routine safety lab. 4) Symptomatic treatment with corticosteroids or abrogating infection with praziquantel (which kills adult worms) if needed. 5) Abrogate infection with artesunate (which kills immature forms)	Common	Moderate	15
Symptoms related to treatment with praziquantel	Common	Moderate	15	1) Take praziquantel with food 2) Clinical assessment, reassurance, symptomatic relief if needed	Common	Minor	10
Misunderstanding of the nature of CHI-S studies	Likely	Critical	20	1) Education of community leaders, opinion makers and regulators 2) Work with community advisory board 3) Education of potential volunteers using tested materials 4) Informed consent verified with tests of comprehension	Possible	Major	12
Inappropriate remuneration leading to coerced participation	Possible	Moderate	9	1) Formative research to determine appropriate remuneration	Unlikely	Moderate	3

Likelihood was scored as almost certain/common, 5; likely, 4; possible, 3; unlikely, 2; rare, 1. Impact was scored as critical, 5; major, 4; moderate, 3; minor, 2; insignificant 1.

DISCUSSION

In this document we have reflected on the potential risks involved in establishing a controlled human infection model for schistosomiasis in Uganda. The opinions expressed and risk scores allocated have been arrived at by discussion between the authors and are therefore subjective. In submitting this document to open peer review through the African Academy of Sciences Open Research Platform we welcome discussion of these issues.

Based on the assessments made, our own reflections and proposed plans are as follows.

First, we have decided not to pursue the option of importing *B. glabrata* snails from the Netherlands to Uganda. Although the proposed controls were estimated to reduce the risk or establishing a colony outside the laboratory to low, it seems unnecessary to incur them. Since snail species endemic to Uganda are susceptible to *S. mansoni* infection we expect that option 2 will work.

Second, we propose to further pursue the option of using the Puerto Rican laboratory strain of *S. mansoni* in the CHI-S in Uganda. We consider that the recognised virulence and praziquantel susceptibility profile of this strain makes it the safest option for CHI-S and have decided to have safety prevail over the ecological risk. The long-term in-breeding of the laboratory strain is an asset in this regard, making the characteristics of each clone of male cercariae reasonably predictable, and the strain possibly less fit as compared to circulating Ugandan strains. We also believe that the ecological risk of possible spread of the Puerto Rican strain of Sm will be minimized with the proposed measures.

To generate infectious cercariae for human infection and challenge studies following the principles of GMP it will be essential to establish a suitably controlled snail facility in Uganda. For sustainability (to avoid the need of repeated shipping of infectious material from the Netherlands) it will also be necessary to establish a specific pathogen free animal facility to house the mammalian host and complete the laboratory life cycle.

With regard to the selection of volunteers, and avoidance of natural infection during the CHI-S, current activities include engagement with relevant Ugandan communities which are potential settings for recruitment of volunteers. As part of the engagement, options for avoidance are being explored. Our current view is that careful volunteer selection, close follow up and immediate abrogation

of infection (on detection of CAA) will be preferable to 12-week “admissions”; but views from the communities will influence our future approach.

Controlled human infections with known pathogens inevitably involve risks and possibly the burden of symptoms. Available mitigations in several examples reduced our risk scores only to moderate, rather than low: for example, symptomatic treatment and early abrogation of infection cannot reduce the likelihood of symptoms below common, but can reduce the impact of the symptoms. Such areas emphasise the need for caution – for example, small group sizes and carefully monitored dose-escalation approaches.

We realize that symptoms may be different among Ugandan volunteers than among Dutch volunteers. Particularly, Katayama fever is considered less likely to occur in subjects from endemic, compared to subjects from non-endemic settings (1) . Nevertheless, we shall provide full information to potential volunteers about symptoms predicted from the literature, and those which occurred previously in the Dutch volunteers. We are currently piloting educational materials, volunteer information sheets, and tests of comprehension in order to ensure that Ugandan volunteers can be enrolled with genuine understanding and fully informed consent. As well, we shall work with community leaders and advisors to ensure optimal understanding of the work, and to mitigate the impact of rumours about the work which are likely to arise.

We conclude that, with careful risk management, CHI-S can be safely implemented in Uganda with a view to accelerating vaccine development against this important communicable disease.

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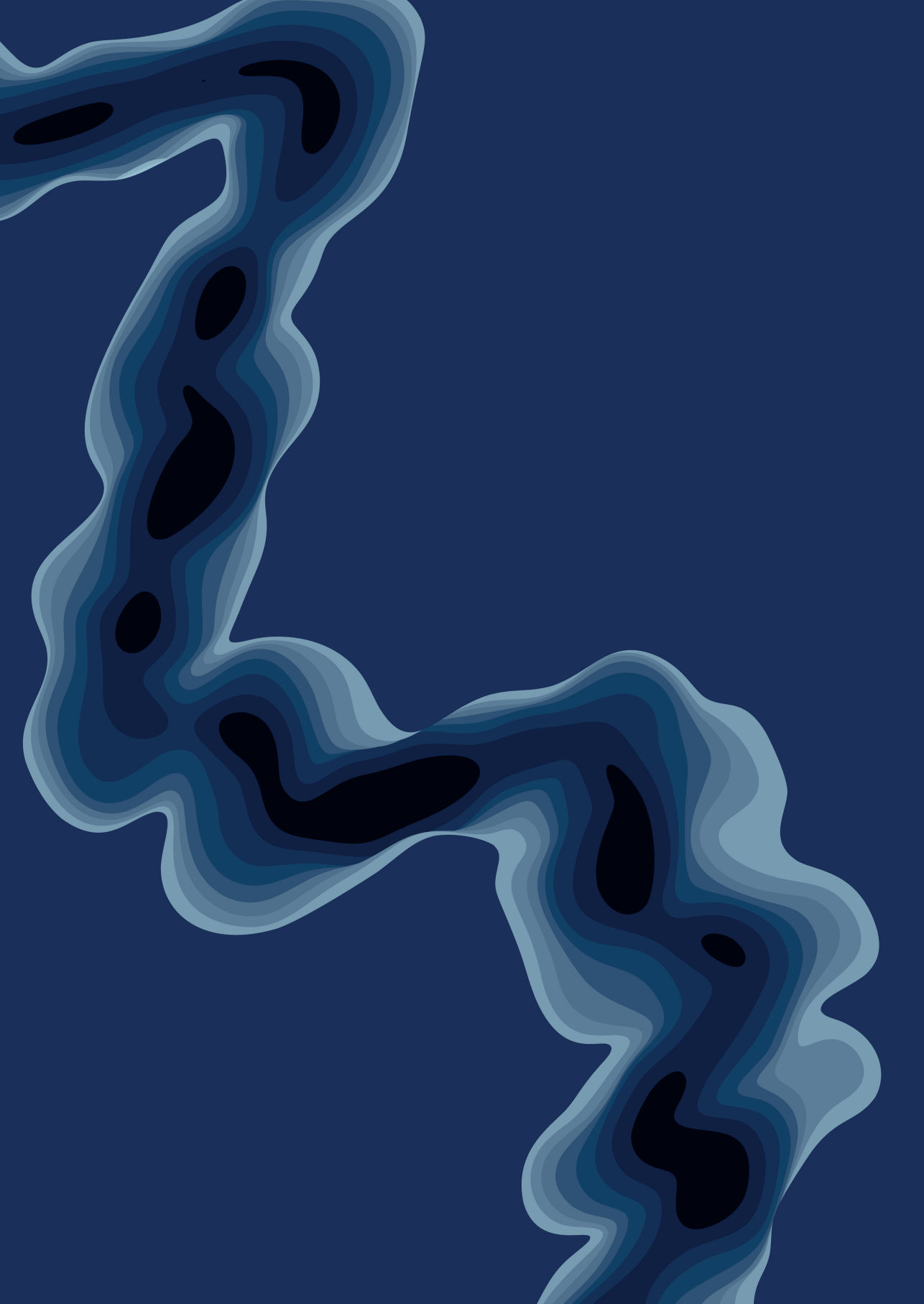

Chapter 5

Clinical tolerance but no protective efficacy in a placebo-controlled trial of repeated controlled schistosome infection

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ABSTRACT

Background: Partial protective immunity to schistosomiasis develops over time, following repeated praziquantel treatment. Moreover, animals develop protective immunity after repeated immunisation with irradiated cercariae. Here, we evaluated development of natural immunity through consecutive exposure-treatment cycles with *Schistosoma mansoni* (*Sm*) in healthy, *Schistosoma*-naïve participants using single-sex controlled human *Sm* infection.

Methods: Twenty-four participants were randomised double-blind (1:1) to either the reinfection group, which received three exposures (week 0,9,18) to 20 male cercariae or the infection control group, which received two mock exposures with water (week 0,9) prior to cercariae exposure (week 18). Participants were treated with praziquantel (or placebo) at week 8, 17 and 30. Attack rates after the final exposure (week 19-30) using serum circulating anodic antigen (CAA) positivity were compared between groups. Adverse events were collected for safety.

Results: Twenty-three participants completed follow-up. No protective efficacy was seen, given 82% (9/11) attack rate after the final exposure in the reinfection group and 92% (11/12) in the infection control group (protective efficacy 11%; 95% CI -24% to 35%; p =0.5). Related adverse events were higher after the first infection (45%), compared to the second (27%) and third infection (28%). Severe acute schistosomiasis was observed after the first infections only (2/12 in reinfection group and 2/12 in infection control group).

Conclusion: Repeated *Schistosoma* exposure and treatment cycles resulted in apparent clinical tolerance, with fewer symptoms reported with subsequent infections, but did not result in protection against reinfection.

Trial registration: ClinicalTrials.gov NCT05085470.

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INTRODUCTION

Schistosomiasis, an infection with *Schistosoma* worms, causes considerable disease burden with over 200 million people infected and another 800 million at-risk of infection worldwide (1). While mass drug administration with praziquantel (PZQ) is widely used to reduce the infectious burden, progress in disease control has stalled in certain areas, highlighting the need for additional control strategies such as vaccines. Vaccine research is encouraged by data suggesting some level of immunity, but not full protection i.e. sterile protection, to *Schistosoma* (re)infection is acquired after multiple infections. This includes epidemiological data from *Schistosoma*-endemic areas that show an age-dependent decrease in infection burden most likely due to partially decreased susceptibility to infection over time (2), as well as promising results of immunisation studies with irradiated cercariae resulting in 70-80% worm burden reduction in rodent and non-human primate models (3). Despite such studies, our knowledge of what immune mechanisms result in (natural) immunity or, in other words, partial protection from infection remain limited and correlates of protection are not well defined and differ between studies (4-7). Previously, we established a controlled human infection model with schistosomes (CHI-S) and demonstrated that single-sex exposure to 20 male *Schistosoma mansoni* (*Sm*) cercariae resulted in detectable infection in 82% (9 out of 11) of individuals based on serum circulating anodic antigen (CAA) detection and resulted in few severe side effects. Moreover, CHI-S led to induction of high levels of schistosome-specific IgG1, which in animal models have been associated with protection against reinfection (7). We therefore used this CHI-S model to investigate (protective) immune responses to repeated exposure and treatment cycles, to measure the development of protective immunity in humans and investigate the safety of (repeated) exposure to male cercariae.

RESULTS

Study population

In total, 25 individuals were screened for eligibility, of which one was excluded based on inability to attend all study visits (**Figure 1**). Twenty-four participants were randomly allocated to the reinfection (n=12) or infection control group (n=12). The reinfection group was exposed to 20 *Sm* cercariae three times (week 0, 9, and 18), while the infection control group was only exposed once (week 18) and received two mock exposures (week 0 and 9). Treatment with PZQ 60

mg/kg (or placebo tablets for infection controls) was given 8 weeks after the first and second (mock) exposure and 12 weeks after the third exposure for all participants. One participant in the reinfection group was lost to follow-up shortly after the third exposure and was given PZQ treatment to clear the infection.

The median age of participants was 23 years old (range 18-44), 13 were female (54.2%) and the median BMI was 24.7 kg/m^2 (range 19·3-31·4) at baseline (**Table 1).** To monitor potential failed skin invasion we performed microscopy on rinse water after each *Schistosoma* exposure, finding very few remaining whole cercariae (range 0-2), or heads (range 0-3) (**Supplementary Table S1**).

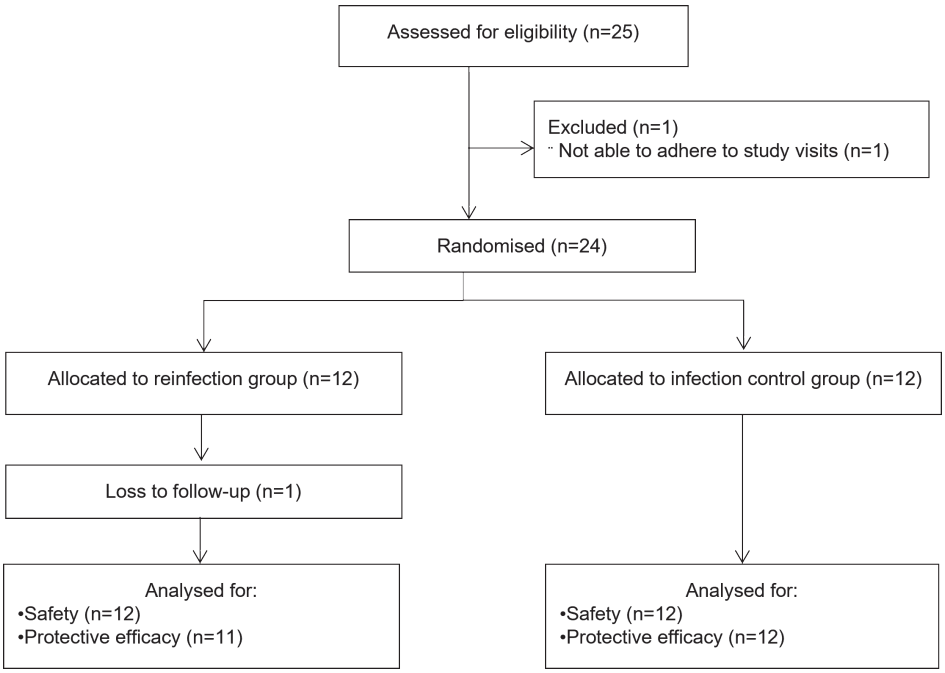

Figure 1: Consort flow for study participants.

Table 1. Baseline characteristics of study participants.

	All (N=24)	Infection control (N=12)	Reinfection (N=12)
Sex			
Male	11 (45.8%)	6 (50.0%)	5 (41.7%)
Female	13 (54.2%)	6 (50.0%)	7 (58.3%)
Age (years)			
Mean (SD)	26.4 (8.11)	24.0 (5.85)	28.8 (9.51)
Median [Min, Max]	23.0 [18.0, 44.0]	23.0 [19.0, 41.0]	23·5 [18.0, 44.0]
BMI (kg/m^2)			
Mean (SD)	24.7 (3.24)	24.1 (2.42)	25.3 (3.92)
Median [Min, Max]	24.4 [19.3, 31.4]	24.4 [20.0, 29.2]	25.5 [19.3, 31.4]

Safety

Adverse events (AE) data was analysed for all 24 participants. No serious adverse events were reported. Over the course of the study, 246 related AEs were reported, of which 143 (58%), 66 (27%), and 37 (15%) were categorised as mild, moderate, and severe, respectively. Of these, 75% (n=185) were associated with *Schistosoma* exposure and 24% (n=58) were common side effects of PZQ. The reinfection group reported 114 AEs related to *Schistosoma* exposure (**Table 2**), with the highest number reported after the first exposure (n=51, 45%). After the second and third exposure comparable numbers of AEs were reported (exposure 2: n=31, 27%; exposure 3: n=32, 28%). In the infection control group, most AEs related to *Schistosoma* exposure were reported after the third exposure (n=45, 63%), although notably a considerable number of AEs were observed after the two initial mock exposures, suggesting a relatively high background incidence of these AEs (exposure 1: n=8, 11%; exposure 2: n=18, 25%).

The risk of PZQ-related AEs was similar after each treatment in the reinfection group (**Supplementary Table S2**) and only very few AEs were reported after treatment with placebo in the infection control group (**Supplementary Table S3**).

Symptoms of *Schistosoma* exposure included local skin reactions as well as systemic responses (acute schistosomiasis, AS) starting after three weeks. Systemic symptoms lasted a median one day (IQR: <1 – 4 days). Clustering of symptoms was observed in some participants, suggestive of AS (**Supplementary Figure S1**). Severe AS (i.e. interfering with daily activities) was observed in four participants and all occurred after their first (true)

exposure, two in the infection control group and two in the reinfection group **(Supplementary Table S4)**. Three were treated with prednisolone 30mg for five days, with subsequent tapering of the dose (20mg, 10mg, to 5mg over the course of a week) to alleviate symptoms. Participants with severe AS after the first exposure in the reinfection group reported no (n=1) or milder (n=1, moderate) AEs after subsequent exposures. Eosinophil levels peaked in the reinfection group after the third exposure **(Figure 2A).** No clinically relevant changes in liver function tests were observed.

Table 2. Number of related AEs reported after each (re)exposure to Sm cercariae.

	Reinfection group, n (%)	**Infection control group, n (%)**
Exposure 1, week 0-8	51 (45%)	8 (11%)*
Exposure 2, week 9-17	31 (27%)	18 (25%)*
Exposure 3, week 18-30	32 (28%)	45 (63%)
Total	114 (100%)	71 (100%)

* mock exposure with water

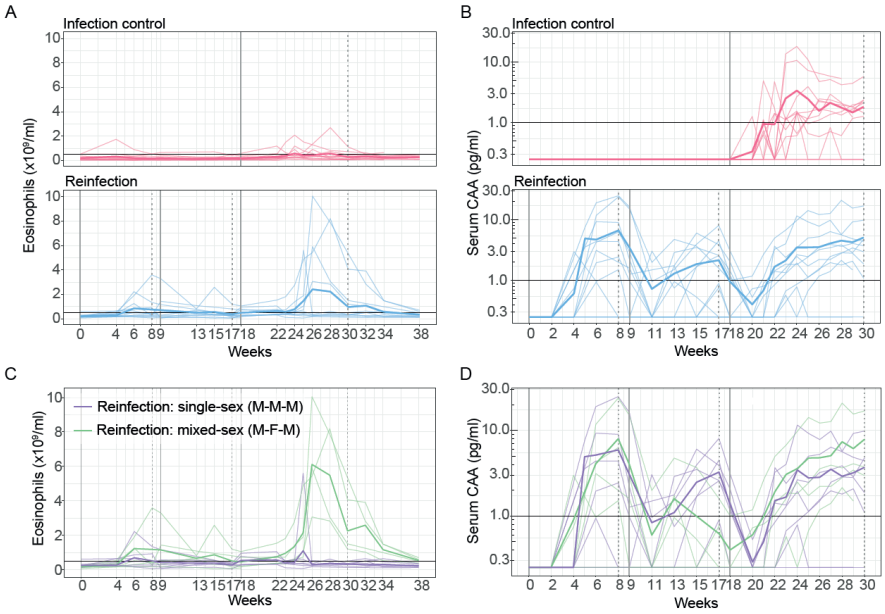

Figure 2: Eosinophil counts and CAA levels after (re)exposure to Sm cercariae. Plots show the changes over time in eosinophils (A) and CAA (B) in infection control (pink, n=12) and reinfection (blue, n=12) participants. Eosinophils (C) and CAA (D) in the reinfection group is then plotted stratified on whether single-sex (M-M-M) exposure (purple, n= 7) or accidental mixed-sex (M-F-M) exposure occurred. Individual participant data is plotted, thicker lines show the group means. The horizontal black line shows the cut-off for abnormal counts (≥0.5 *10^9/mL for eosinophils; ≥1.0 pg/mL for CAA). The solid, grey vertical line shows Sm exposure weeks, while the grey, black vertical line shows when PZQ treatment was given.

Protective efficacy

The attack rate based on CAA positivity after the third exposure in the reinfection group was 82% (9/11) and 92% (11/12) in the infection control group, corresponding to a protective efficacy of 11% with a wide 95% confidence interval that included zero (-24% to 35%), indicating no protection (p=0.5). The proportion of CAA positive participants in the reinfection group after the first and second exposures was 64% (7/11) for both exposures. CAA levels over time did not decrease with subsequent exposures in the reinfection group (**Figure 2B)**. There was no association between severe acute schistosomiasis and CAA levels **(Supplemental Figure S2).** After treatment following the third exposure, three participants received additional PZQ treatment, because of persistent CAA positivity six and/or eight weeks after. Complete clearance of infection, i.e. negative CAA, was achieved in all participants and confirmed at a final visit one year after.

Accidental exposure to female cercariae and potential egg production

Schistosoma PCR on faeces were all negative after the first and second exposure, however after the third exposure, one participant showed a positive result (CT ~32) indicating presence of *Schistosoma* DNA and egg-production, which was later confirmed by microscopy. The number of eggs found was low (6 eggs in three separate Ridley x 6 slides). All procedures for production of challenge material were rechecked and no irregularities in study processes found. Upon molecular retesting of all stored cercariae used for infection, we discovered that five participants, during the second exposure, were accidentally exposed to 20 female, instead of male cercariae due to sample mislabelling. We hypothesise that persistent single-sex females, which are more resistant to treatment with PZQ (8), after the second infection-treatment cycle in these individuals could have led to a patent egg-producing male-female worm pair after third infection. Procedures were adapted and a second molecular confirmation step was implemented to avoid such incidents in the future.

In post-hoc analyses, participants with mixed-sex (male-female-male (M-F-M)) exposure had higher peak eosinophil counts after the third exposure compared to those with single-sex male (M-M-M) exposure (**Figure 2C) ,** but adverse events and CAA positivity/kinetics did not seem to differ between the two groups (**Figure 2D**). Of the three participants requiring additional PZQ treatment, two were infection controls and one was a reinfection participant who was only exposed to male cercariae.

Antibody, chemokine, and cytokine responses

M-F-M exposure appeared to influence the (egg-specific) antibody and cytokine responses and are therefore presented separately. Within 8 weeks after the initial exposure to cercariae, 21 (out of 23) participants had seroconverted for worm-specific IgM **(Figure 3A).** One seroconverted later at week 18, while the other remained negative. IgG and IgG1 antibodies against adult-worm antigen increased after exposure in all but one participant. Peak levels in the reinfection group appeared to increase with subsequent exposures, suggesting boosting (**Figure 3B&C**). Increases in IgG against soluble egg antigen (SEA) were observed in most participants, as previously also observed in male-only exposure possibly due to antibody cross-reactivity between cercariae and eggs (9), however those exposed to M-F-M had higher peak values than those only exposed to M-M-M cercariae **(Figure 3D).**

Serum cytokines and chemokines show similar kinetics after the first exposure in both reinfection and infection controls (**Figure 3E-J**) as none of these mean

cytokine/chemokine levels differed between the groups 4 weeks after primary exposure. We observed some evidence that levels of CCL4 were lower at week 22 (4 weeks after third exposure) compared to week 4 (mean difference -70.3, 95%CI: -129.7; -11.3, p =0.04). Although visually, CXCL10 and TNF levels also appear lower after the third infection, we were unable to detect a statistically significant difference, potentially due to the small sample size. After the third exposure, in the reinfection group CCL23 (p<0.001), CCL4 (p=0.05), and TNF (p<0.001) were higher in the M-F-M exposed compared to the M-M-M exposed. No association was observed between severe acute schistosomiasis symptoms and circulating cytokines or chemokines (**Supplementary Figure S3).**

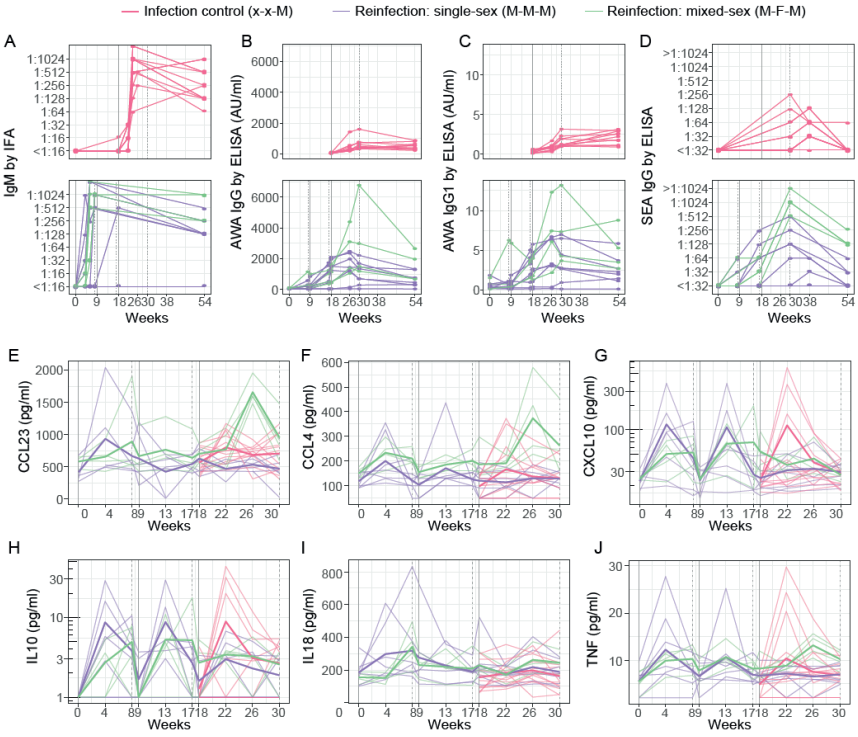

Figure 3: Antibody, chemokine, and cytokine responses after (re)exposure to Sm cercariae. Plots show the individual changes in antibody levels in worm-specific IgM (A), AWA-specific IgG (B), AWA-specific IgG1 (C), and SEA IgG (D). For CCL23 (E), CCL4 (F), CXCL10 (G), IL-10 (H), IL18 (I), and TNF (J) individual participant data and group means (thicker lines) are plotted. Data is stratified for infection controls (pink, n=12), reinfection single-sex (M-M-M) exposure (purple, n=7), and reinfection accidental mixed sex (M-F-M) exposure (green, n=5). The solid, grey vertical line shows Sm exposure weeks (0,9,18), while the dotted, grey vertical line shows when PZQ treatment was given (8,17,30). AWA = adult worm antigen; SEA = soluble egg antigen

DISCUSSION

In this study, we demonstrate that repeated controlled exposure to *Sm* cercariae does not lead to protection against reinfection, but induces tolerance to clinical symptoms already after the first infection with fewer AEs being reported after subsequent infections.

In line with previous CHI-S, local skin reactions (rash and itch) and systemic symptoms of acute schistosomiasis (AS) were commonly observed albeit of short duration, with severe AS reported in four of 24 individuals after the first exposure. This risk of severe AS after primary exposure is both consistent between the reinfection and infection control group and across previous studies (8, 9). The risk of AS decreased with subsequent exposures, which may explain why AS is infrequently reported in endemic populations (10), where exposure to *Schistosoma* antigens is thought to start at an early age, potentially even *in utero* (11), and occurring further throughout life. In our earlier work we have shown severe acute schistosomiasis to be accompanied by a Th1 biased inflammatory response at week 4 (12), but no relationship between CAA and symptoms (8, 9), which was confirmed in the current study. Clinical tolerance is likely to be accompanied by regulatory responses but further research will be needed to delineate the details of the underlying mechanisms.

Different to earlier CHI-S studies, here we included an infection control group that received mock infections with water. Both participants and investigators were masked to group allocation, resulting in a large number of adverse events classified as potentially related to infection with *Schistosoma*, even after water exposure. This demonstrates that AS symptoms, e.g. abdominal symptoms or headache, are aspecific and have a high incidence in the general population, making AS diagnosis challenging. While individual symptoms are aspecific, our data indicates that particularly clustering of symptoms 4-5 weeks post-challenge are highly suggestive of AS. By looking at the difference in risk of symptoms between those exposed to *Schistosoma* and water, we can now more reliably assess the safety of CHI-S. For future studies looking to establish safety of a novel controlled human infection model, the inclusion of an infection control group may be considered, especially if the expected symptoms are aspecific and common.

Contrary to our hypothesis, we did not observe any evidence for sterile protection based on serum CAA levels after two exposure and treatment cycles. Moreover, the CAA kinetics following the second and third exposure did not show any sign of partial protection despite IgG1 boosting, as peak CAA values

did not decrease with consecutive exposures. Our current understanding of resistance to reinfection in humans comes from epidemiological studies in endemic settings, that suggest immunity can develop as a result of worm death and subsequent antigen release, as observed in occupationally exposed adults in endemic settings (13). Worm-specific IgG responses are associated with protection in animal immunisation studies with irradiated cercariae (14), and with protection in endemic settings (15). Although some individual studies in endemic settings have suggested that higher levels of worm-specific IgE levels are protective, this could not be confirmed after meta-analysis (5). Apart from the infectious dose, which is much higher in animal studies (>1000 cercariae) and in endemic settings, the apparent discrepancy between these studies and our findings could be explained by the quality and specificity of the IgG response. Perhaps the anti-worm IgG responses we observed are not against specific protective antigens on the worm, or not reach a higher enough titre, two factors previously shown to be critical for protection (16, 17). Moreover, antibody functionality may also be shaped by the number of cumulative exposures, which in endemic settings is higher than in our study.

Several participants were accidentally exposed to male-female-male (M-F-M) cercariae, of which we confirmed egg production in one participant, suggesting that 1) female worms are not fully cured with PZQ 60 mg/kg; and 2) surviving female worms are able to pair with incoming male worms. Unlike female-only infection where decreased susceptibility to PZQ is observed (8), the potential resulting mixed-sex and single-sex male infections responded well to PZQ, as only few participants (3 out of 23) required a second dose of PZQ before being fully cured. Cure rates after initial treatment with PZQ 60 mg/kg were also higher compared to our previous male-only CHI-S study in which 6 (out of 14) participants required an additional dose after being initially treated with PZQ 40 mg/kg (9).

CAA levels in those exposed to M-M-M and M-F-M cercariae did not differ, however the composition of single vs. paired worms cannot be determined. We noted several differences between potentially mixed-sex vs single sex infected participants in the reinfection group. From our data, it seems that potential egg production is accompanied by higher eosinophil, CCL23, CCL4, and TNF levels, as well as higher IgG antibody titres against soluble egg antigen. An increasing dominance of type-2 responses after egg production is well described (18, 19), and is evidenced here by the increase in eosinophils and CCL23, a chemokine constitutively produced by eosinophils during type-2 inflammation (20, 21). Notably, the initial response to potential egg production

is also characterised by the pro-inflammatory cytokines CCL4 and TNF, as previously reported in murine systems (22-24).

Although there are clear limitations of the CHI-S model in its comparability to natural infection, the fact that we did not find any protection suggests that the immune regulatory potential by schistosomes may be much stronger than we originally envisioned. However, we note several methodological choices which may have affected the protection outcome. Compared to irradiated schistosomes, our strategy of pzq treatment abrogates infection at a later timepoint maybe allowing for more regulatory responses to develop. Additionally, the use of schistosomes of one sex only may also limit the induction of immunity as well as the low number of schistosomes for immunisation and the limited number of immunisation. To further investigate natural immunity, we are looking forward to CHI-S studies in pre-exposed individuals which will answer these questions. It is also good to note that although we observe clinical tolerance, the study was not primarily powered to detect differences in AE incidence.

All together this study shows the rapid induction of clinical tolerance to schistosomes and lack of protective immune responses despite induction of antibodies and boosting thereof. An in-depth study of the antigen specificity of these responses, the cellular immune environment, and egg-driven immune responses, can not only boost our understanding of schistosome immune regulation, but also provide a starting point to downselect vaccine targets.

METHODS

Study design and participants

This double-blind, placebo-controlled randomised trial was performed at the Leiden University Medical Center, The Netherlands between November 2021 and September 2022.

Healthy participants aged 18-45 without prior (suspected) exposure to schistosomes and without travel plans to *Schistosoma*-endemic regions during the study period were recruited from Leiden and surrounding area through advertising. We excluded participants with a history or evidence of any (pre-existing) illness that could compromise the health of the individual participant during the study or influence interpretation of study results. Moreover,

participants with a known hypersensitivity to or contra-indications to the rescue medication (PZQ, artesunate, or lumefantrine) were also excluded.

Sex as a biological variable

Data on participant's sex was self-reported and used for descriptive purposes and not for analyses. Cercarial sex (male or female) was determined using molecular techniques as described elsewhere.

Randomisation and masking

Participants were randomised to the reinfection or infection control group in a 1:1 ratio using a randomisation list. Randomisation was performed by a researcher independent of the study team. The participants and study team were blinded to group allocation.

Study procedures

The reinfection group was exposed to 20 *Sm* cercariae three times (week 0, 9, and 18), while the infection control group was only exposed once (week 18) and received two mock exposures (week 0 and 9). Single-sex cercariae were produced as described previously (9, 25). In brief, snails were infected with a single *Sm* miracidium resulting in a monosexual infection. After five weeks, infected snails started shedding cercariae that are either male or female. Sex of these cercariae was determined using molecular techniques. These cercariae were then applied to the participant's forearm in 0.5 mL mineral water for 30 minutes to mimic the natural route of infection. Next, the rinse water was checked for remaining cercarial heads and/or tails by microscopy by a lab technician, independent from the clinical team. After each (mock) exposure participants were followed up frequently for adverse event and sample collection to determine infection status. Treatment with PZQ 60 mg/kg (or placebo tablets for infection controls) was given 8 weeks after the first and second (mock) exposure. All participants were treated with PZQ 60 mg/kg 12 weeks after the third exposure and monitored afterwards for treatment success. Treatment was repeated in persistent infections (CAA ≥1.0 pg/mL).

Outcomes

The primary outcomes were 1) the protective efficacy of repeated exposure to male *Sm* measured as the difference in frequency of serum CAA positivity (≥1.0 pg/mL) between the reinfection and infection control group after the third exposure; and 2) the frequency and severity of adverse events after (repeated) exposure to male *Sm* cercariae.

To determine infection status, worm-derived CAA was measured in 0.5 mL serum using the upconverting reporter particle lateral flow assay (UCP-LF CAA) as described previously (9, 26). Participants were considered infected if they had at least one CAA value ≥1.0 pg/ml before PZQ treatment. CAA values below the lower limit of detection of the assay (<0.5 pg/ml) were set to 0.25 pg/ml. CAA was measured retrospectively on serum samples after treatment of the third exposure in order to prevent deblinding.

To determine the safety of (repeated) exposures, adverse events were collected and blood tests were performed. Adverse events were graded for severity and relatedness. Severity was assigned in three levels: symptoms that do not interfere with daily activities (mild); symptoms that interfere or limit daily activities (moderate); and symptoms that result in absenteeism or requires bed rest (severe). Relatedness of adverse events were assessed based on clinical judgement taking into account chronology, timing of event, and alternative diagnoses. In addition, we ascribed these related adverse events to either schistosome exposure, drug treatment, or study procedure (e.g. blood draws). We differentiated local (immediate) exposure site symptoms (rash, itch) and symptoms of AS. AS symptoms included (a combination of) fever, urticaria and angioedema, night sweats, myalgia, arthralgia, dry cough, diarrhoea, abdominal pain, and headache occurring between 2-12 weeks after exposure without other clear cause. Safety blood tests included eosinophil counts and liver enzyme assessment. Faecal samples were assessed for *Schistosoma* DNA by PCR after each exposure, before treatment (27). In addition, we measured worm-specific IgM (IFA) and soluble egg antigen-specific IgG (ELISA) antibodies in serum using our in-house diagnostic assays (9, 28). Adult worm antigen (AWA)-specific IgG and IgG1 were measured using ELISA. 96-well half-area high bind Microplates (Corning) were coated overnight at 4 °C with 25 µg/ml of AWA, prepared as described previously,(29) in 0.1 M sodium carbonate buffer (pH 9.6). Plates were washed 3 times with washing buffer (0.05% Tween in PBS) and blocked with 5% skimmed milk in PBS for 2 h at room temperature. Plasma samples were serially diluted 2.5x in 0.5% skimmed milk (1:100 to 1:12500). After 3 washes, diluted plasma samples were added to the plate and incubated at room temperature for 2 h. After 5 washes plates were incubated with goat-anti-human IgG (1:5000) or mouse-anti-human IgG1 (1:300, Thermofisher) conjugated with horseradish peroxidase (in 0.5% skim milk, 0·05% EDTA in PBS) for 1 h at room temperature. After 6 washes, TMB (3,3',5,5'-Tetramethylbenzidine) substrate was added. The reaction was stopped with 10% sulfuric acid after colour development. Plates were read at 450 nm, with 570 nm used as a reference measurement and subtracted.

Measurements were normalized to a standard curve consisting of polyclonal IgG (Merck) and expressed as AU ml^{-1}.

We used a custom Luminex kit to measure CCL4, CXCL10, IL5, IL13, TNF, CCL23, IFNγ, IL10, and IL18 (Bio-techne). Cytokines were included in the analysis if over 40% of samples were above the lower limit of detection. Three cytokines were excluded from analysis - IL5, IL13, IFNγ - which were detectable in less than 5% of all samples.

Statistical analyses

Based on the previously determined attack rate (AR) of 82% after exposure to 20 male cercariae,(9) we calculated that we would require 11 participants in each group to detect a 70% relative reduction in CAA positivity with 80% power and (two-sided) α = 0·05 significance level. The effect size is based on earlier studies in non-human primates which showed that immunisation with irradiated cercariae led to a 70-80% reduction in worm burden (30, 31). To account for loss to follow-up, we aimed to include 24 participants, 12 in each group. The adverse event data was analysed in the intention-to-treat group (n=24), protective efficacy was analysed in the per-protocol group (n=23) consisting of participants who completed follow-up until week 30 and calculated similarly to vaccine efficacy estimates (1-RR or $1 - {}^{AR_{reinfection}}/_{AR_{infection\ controls}}$) with corresponding 95% confidence intervals. Data analyses and visualisation was performed using R (v4.3) and R studio (v2023.06.1). Cytokine levels between infection controls and reinfection participants were compared using unpaired t-tests, while differences in cytokine levels 4 weeks after first and third exposure in the reinfection group were assessed using linear mixed models with participant as a random effect and time in weeks as a fixed effect (as a factor) using packages lme4 (version 1.1–35) and lmerTest (version 3.1–3).

Study approval

Ethics approval was obtained from the local ethics review committee (METC LDD, P21.070) and registered prospectively on clinicaltrials.gov (NCT05085470). The study was conducted in accordance with the ICH guidelines for Good Clinical Practice and Declaration of Helsinki. Prior to any study procedure, informed consent was obtained from all participants.

DECLARATIONS

Data availability

Individual data underlying the figures presented in this manuscript are available in the "Supporting data file". After publication, all data will undergo FAIRification and will be made available anonymised through a LUMC-based fair data point which will be made accessible through data visiting.

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Author contributions

MR acquired funding. JK, MR prepared the research protocol. JK, EH, MR, CH, MY were involved in study design. EH, JS, MC, EvdS, IvA, AvD were involved in production and release of cercariae. JK, EH, JJ, OL, GR, SH generated the data. EB, LW, LvL, GvD, PC were involved in the infection endpoint measurements and interpretation. JK, JJ were involved in data curation, project administration. JK, EH performed the data analyses and prepared the first draft. All authors have read and approved the final version of the manuscript.

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SUPPLEMENTARY DATA

Supplementary Table S1. Microscopy counts of cercariae in rinse water after exposure.

		Reinfection group (n=12), median (range)	Infection control group (n=12) , median (range)
Exposure 1 Week 0	Heads	0.5 (0-1)	0 (0-0)*
	Tails	8.5 (2-15)	0 (0-0)*
	Whole cercariae	0 (0-2)	0 (0-0)*
Exposure 2 Week 9	Heads	0 (0-2)	0 (0-0)*
	Tails	5 (1-8)	0 (0-0)*
	Whole cercariae	0.5 (0-1)	0 (0-0)*
Exposure 3 Week 18	Heads	0 (0-3)	0 (0-2)
	Tails	7 (5-14)	4 (1-8)
	Whole cercariae	0 (0-2)	0.5 (0-2)

* Mock exposures with water.

Supplementary Table S2. Risk of PZQ-related symptoms after treatment.

	Reinfection (n=12)			Infection control (n=12)		
	Treatment 1	Treatment 2	Treatment 3	Treatment 1*	Treatment 2*	Treatment 3
Any PZQ symptom#						
Mild	2 (17%)	2 (17%)	3 (25%)	1 (8%)	0	3 (25%)
Moderate	2 (17%)	4 (33%)	2 (17%)	0	0	1 (8%)
Severe	4 (33%)	0	1 (8%)	0	0	1 (8%)
Abdominal Pain						
Mild	0	0	0	0	0	0
Moderate	0	0	0	0	0	1 (8%)
Severe	0	0	0	0	0	0
Nausea						
Mild	2 (17%)	1 (8%)	2 (17%)	1(8%)	0	3 (25%)
Moderate	1 (8%)	2 (17%)	0	0	0	0
Severe	2 (17%)	0	0	0	0	1(8%)
Heartburn						
Mild	0	0	0	0	0	0
Moderate	0	0	0	0	0	0
Severe	0	0	0	0	0	1(8%)
Dizziness						
Mild	2	2 (17%)	3 (25%)	0	0	1(8%)
Moderate	1(8%)	2 (17%)	2 (17%)	0	0	1(8%)
Severe	4	0	0	0	0	0
Disturbance of smell or taste						
Mild	0	0	1(8%)	0	0	0
Moderate	0	0	0	0	0	0
Severe	0	0	0	0	0	0
Fever						
Mild	0	0	0	0	0	0
Moderate	0	0	0	0	0	0
Severe	0	0	1(8%)	0	0	0
Headache						
Mild	0	0	1(8%)	0	0	0
Moderate	0	0	0	0	0	0
Severe	0	0	0	0	0	0
Malaise/fatigue						
Mild	0	0	0	0	0	0
Moderate	0	4 (33%)	0	0	0	0
Severe	3 (25%)	0	1(8%)	0	0	1(8%)
Loss of appetite						
Mild	0	0	0	0	0	0
Moderate	0	1(8%)	0	0	0	0
Severe	1(8%)	0	0	0	0	0

* Treatment with placebo
# Only maximum severity counted
PZQ: praziquantel

Supplementary Table S3. Number of related AEs reported after each PZQ treatment.

	Reinfection group, n (%)	Infection control group, n (%)
Treatment 1, week 8	17 (42%)	3 (18%)*
Treatment 2, week 17	13 (31%)	0*
Treatment 3, week 30	14 (27%)	14 (82%)
Total	41 (100%)	17 (100%)

* treatment with placebo
PZQ = praziquantel

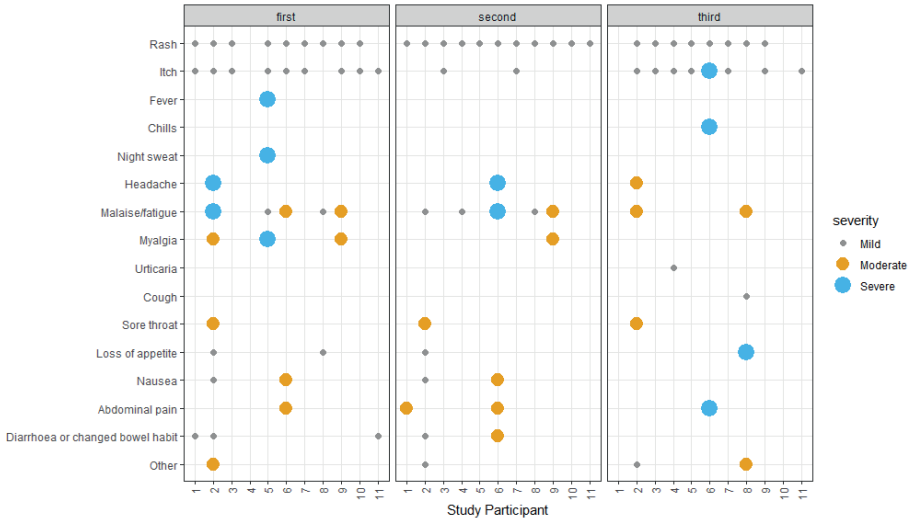
Supplementary Table S4. Risk of Schistosoma-related symptoms after (re)infection.

	Reinfection (n=12)			Infection control (n=12)		
	Exposure 1	**Exposure 2**	**Exposure 3**	**Exposure 1***	**Exposure 2***	**Exposure 3**
Any local symptom#						
Mild	10 (83%)	11 (92%)	8 (67%)	0	4 (33%)	7 (58%)
Moderate	0	0	0	0	1 (8%)	0
Severe	0	0	1 (8%)	0	0	0
Rash						
Mild	9 (75%)	11 (92%)	8 (67%)	0	3 (25%)	7 (58%)
Moderate	0	0	0	0	0	0
Severe	0	0	0	0	0	0
Itch						
Mild	9 (75%)	2 (17%)	7 (58%)	0	3 (25%)	5 (42%)
Moderate	0	0	0	0	1 (8%)	0
Severe	0	0	1 (8%)	0	0	0
Any systemic symptom#	3 (25%)	2 (17%)	1 (8%)	0	1 (8%)	2 (17%)
Mild	2 (17%)	3 (25%)	1 (8%)	1 (8%)	2 (17%)	2 (17%)
Moderate	2 (17%)	1 (8%)	2 (17%)	1 (8%)	1 (8%)	2 (17%)
Severe						
Acute schistosomiasis						
Mild	0	0	0	0	0	1 (8%)
Moderate	1 (8%)	2 (17%)	1 (8%)	0	0	1 (8%)
Severe	2 (17%)	0	0	0	0	2 (17%)
Fever						
Mild	0	0	0	0	1 (8%)	1 (8%)
Moderate	0	0	0	0	1 (8%)	0
Severe	1 (8%)	0	0	0	0	2 (17%)
Chills						
Mild	0	0	0	0	0	1 (8%)
Moderate	0	0	0	0	0	0
Severe	0	0	1 (8%)	0	0	0
Night sweats						
Mild	0	0	0	0	0	0
Moderate	0	0	0	0	0	0
Severe	1 (8%)	0	0	0	0	0
Headache						
Mild	0	0	0	0	0	1 (8%)
Moderate	0	0	1 (8%)	1 (8%)	0	1 (8%)
Severe	1 (8%)	1 (8%)	0	0	0	2 (17%)
Malaise/fatigue						
Mild	2 (17%)	3 (25%)	0	1 (8%)	0	1 (8%)
Moderate	2 (17%)	1 (8%)	2 (17%)	0	1 (8%)	2 (17%)
Severe	1 (8%)	1 (8%)	0	0	0	1 (8%)
Myalgia						
Mild	0	0	0	0	0	1 (8%)
Moderate	2 (17%)	1 (8%)	0	0	0	0
Severe	1 (8%)	0	0	0	0	1 (8%)

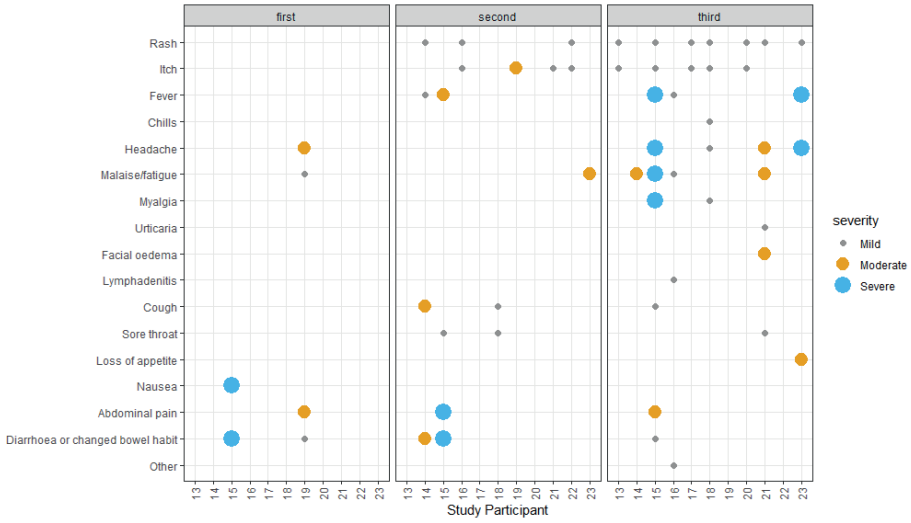
	Reinfection (n=12)			Infection control (n=12)		
	Exposure 1	**Exposure 2**	**Exposure 3**	**Exposure 1***	**Exposure 2***	**Exposure 3**
Urticaria						
Mild	0	0	1 (8%)	0	0	1 (8%)
Moderate	0	0	0	0	0	0
Severe	0	0	0	0	0	0
Facial oedema						
Mild	0	0	0	0	0	0
Moderate	0	0	0	0	0	1 (8%)
Severe	0	0	0	0	0	0
Lymphadenitis						
Mild	0	0	0	0	0	1 (8%)
Moderate	0	0	0	0	0	0
Severe	0	0	0	0	0	0
Cough						
Mild	0	0	1 (8%)	0	1 (8%)	1 (8%)
Moderate	0	0	0	0	1 (8%)	0
Severe	0	0	0	0	0	0
Sore throat						
Mild	0	0	0	0	2 (17%)	1 (8%)
Moderate	1 (8%)	1 (8%)	1 (8%)	0	0	0
Severe	0	0	0	0	0	0
Loss of appetite						
Mild	2 (17%)	1 (8%)	0	0	0	0
Moderate	0	0	0	0	0	1 (8%)
Severe	0	0	1 (8%)	0	0	0
Nausea						
Mild	1 (8%)	1 (8%)	0	0	0	0
Moderate	1 (8%)	1 (8%)	0	0	0	0
Severe	0	0	0	1 (8%)	0	0
Abdominal pain						
Mild	0	0	0	0	0	0
Moderate	1 (8%)	2 (17%)	0	1 (8%)	0	1 (8%)
Severe	0	0	1 (8%)	0	1 (8%)	0
Diarrhoea or changed bowel habit						
Mild	3 (25%)	1 (8%)	0	1 (8%)	0	1 (8%)
Moderate	0	1 (8%)	0	0	1 (8%)	0
Severe	0	0	0	1 (8%)	1 (8%)	0
Other						
Mild	0	1 (8%)	1 (8%)	0	0	1 (8%)
Moderate	1 (8%)	0	1 (8%)	0	0	0
Severe	0	0	0	0	0	0

* Mock exposure with water
# Only maximum severity counted

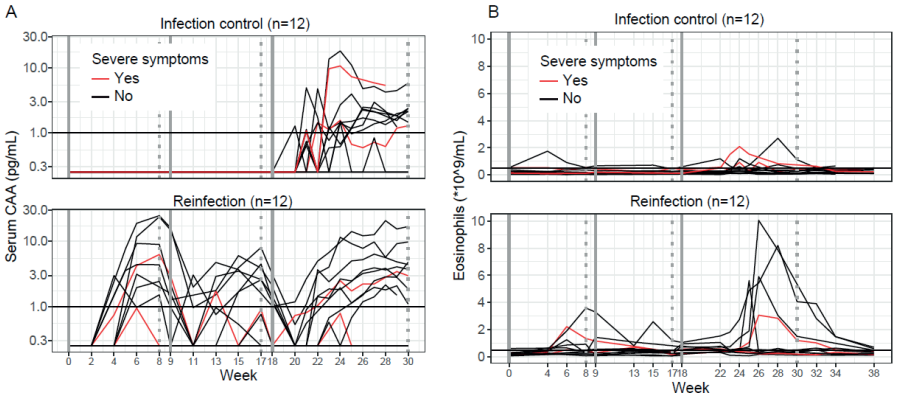
A. Reinfection group



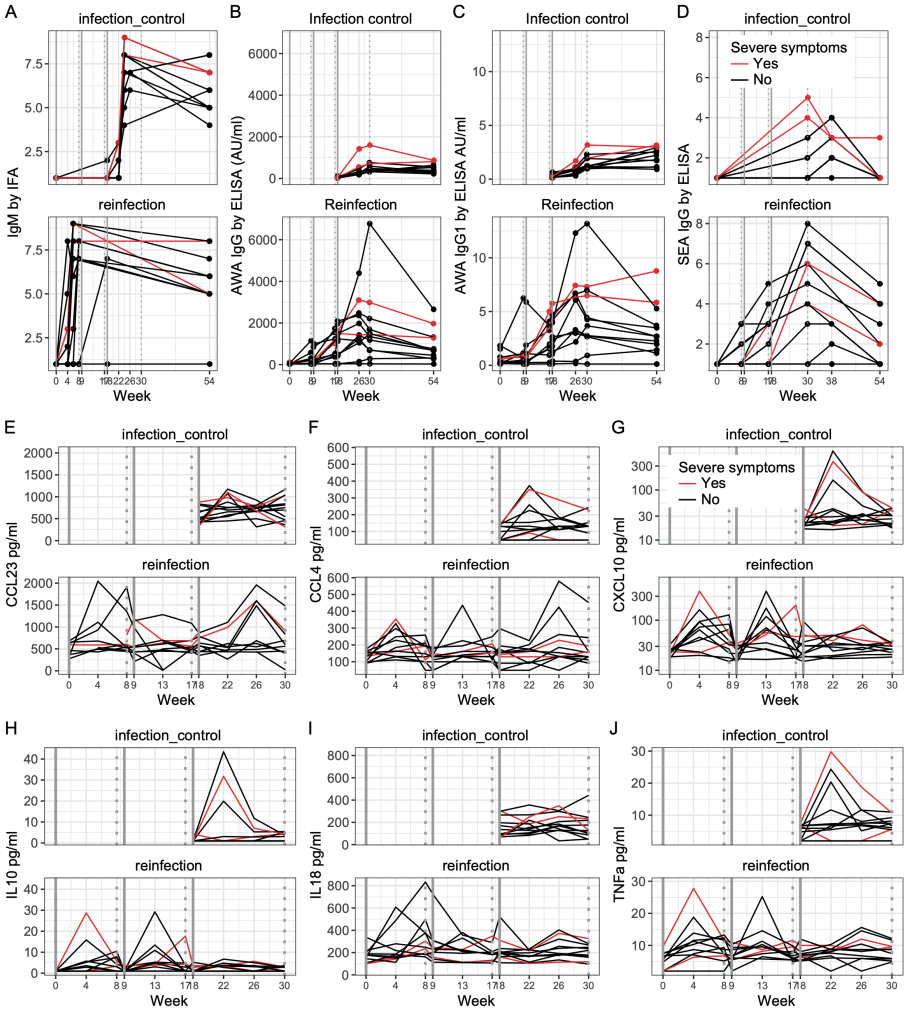
B. Infection control group



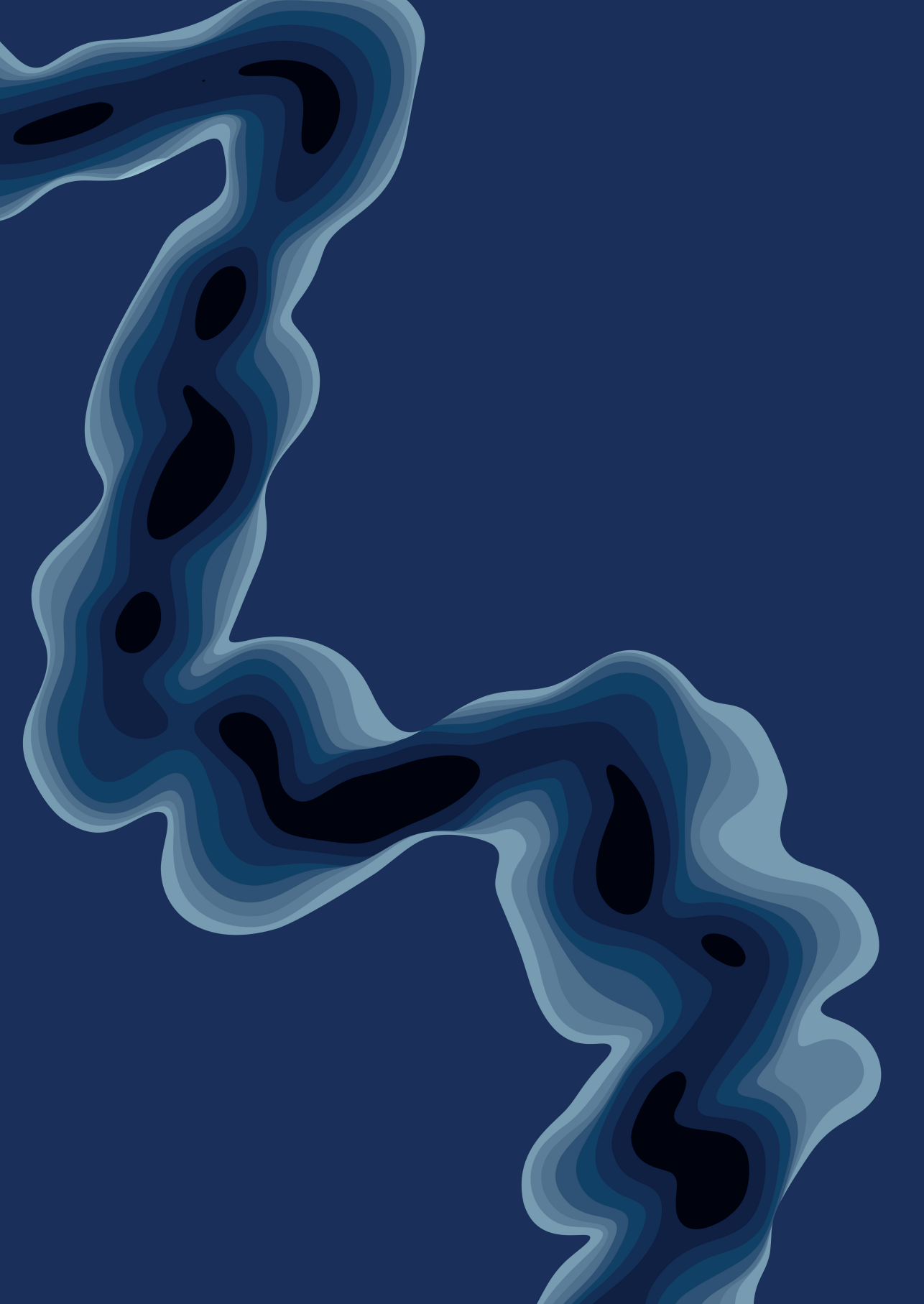
Supplementary Figure S1. Severity and type of adverse events after Schistosoma (re) exposure stratified by group and exposure. Graphs shows the incidence of local and systemic Schistosoma-related adverse events. It shows clustering of symptoms in particular participants, suggestive of acute schistosomiasis (in red boxed). Only the maximum severity for each adverse event after each exposure is plotted. Note that participants 12 and 24 did not report related adverse events and are therefore not shown in the graph.



Supplementary Figure S2 CAA levels and eosinophil counts after (re)exposure to Sm cercariae, stratified by severe acute schistosomiasis symptoms. Plots show the changes over time in CAA (A) and eosinophils (B) in infection control (n=12) and reinfection (n=12) participants stratified by severe acute schistosomiasis symptoms (yes, n=4). Individual participant data is plotted. The horizontal black line shows the cut-off for abnormal counts (≥0.5 *10^9/mL for eosinophils; ≥1.0 pg/mL for CAA). The solid, grey vertical line shows Sm exposure weeks, while the grey, black vertical line shows when PZQ treatment was given.



Supplemental Figure S3: Antibody, chemokine, and cytokine responses after (re)exposure to Sm cercariae, stratified by severe acute schistosomiasis symptoms. Plots show the individual changes in antibody levels in worm-specific IgM (A), AWA-specific IgG (B), AWA-specific IgG1 (C), and SEA IgG (D). For CCL23 (E), CCL4 (F), CXCL10 (G), IL-10 (H), IL18 (I), and TNF (J) individual participant data are plotted. Data is stratified for severe acute schistosomiasis symptoms (red, yes, n=4). The solid, grey vertical line shows Sm exposure weeks (0,9,18), while the dotted, grey vertical line shows when PZQ treatment was given (8,17,30). AWA = adult worm antigen; SEA = soluble egg antigen



Chapter 6

Safety and preliminary efficacy of Sm-p80 + GLA-SE (SchistoShield®) vaccine against controlled human schistosome infection in healthy, *Schistosoma*-naïve adults: protocol for a double-blind, placebo-controlled randomised controlled human infection study

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ABSTRACT

Background: Schistosomiasis continues to affect health worldwide, despite ongoing efforts to control disease through mass drug administration with praziquantel. So far, no vaccine has been licensed, but three candidates, including Sm-p80 adjuvanted with GLA-SE (SchistoShield®), are currently in clinical testing. Controlled human infection studies have the potential to speed up vaccine development as they can be used to quickly provide preliminary estimates of vaccine efficacy in a small group of participants. Recently, a controlled human infection with schistosomes (CHI-S) model has been established using single-sex cercariae that do not produce eggs and therefore prevents egg-associated morbidity in study participants. This study will evaluate the safety, immunogenicity, and preliminary vaccine efficacy of the Sm-p80 + GLA-SE vaccine candidate using CHI-S.

Methods/design: This is a double-blind, placebo-controlled randomised trial in which 48 *Schistosoma*-naïve participants (18-45 years old) will be enrolled and randomised in a 1:1 ratio to receive either three immunisations with 30 µg Sm-p80 + 5 µg GLA-SE or placebo (week 0,4,8). Subsequently, all participants will be challenged with 20 male *Schistosoma mansoni (Sm)* cercariae at week 12 and treated with praziquantel at week 24 to cure infection. At each visit, adverse events will be recorded and participants will undergo a blood draw by venepuncture. They will keep a diary to record adverse events for 24 weeks. The primary outcome is the protective efficacy of Sm-p80 + GLA-SE to male *Sm* cercariae measured by the difference in frequency of serum circulating anodic antigen positivity (≥1.0 pg/mL) after CHI-S between the vaccine and placebo group.

Discussion: The CHI-S allows for efficient evaluation of schistosomiasis candidates and can provide early efficacy data crucial for further vaccine development.

Trial registration: NCT05999825 (clinicaltrials.gov)

Keywords: Controlled human *Schistosoma* infection, Sm-p80, *Schistosoma* vaccine

BACKGROUND

Schistosomiasis is a parasitic disease of global importance that affects around 150 million people mainly living in (sub)tropical regions (1). Control of disease relies on mass drug administration with praziquantel of at-risk populations, however sustained transmission and frequent reinfection hamper elimination efforts. So far, there is no effective licensed vaccine against schistosomiasis. Only three candidates are currently in clinical testing (2). One of these candidates is SchistoShield® consisting of Sm-p80, a recombinant *Schistosoma .mansoni (Sm)* calpain protein produced in *Escherichia coli*, and Glucopyranosyl Lipid Adjuvant (GLA) formulated in a stable emulsion (GLA-SE) as an immunological adjuvant. Preclinical studies in mice and baboons with Sm-p80 + GLA-SE show ~60% reduction in worm burdens after immunisation (3, 4). Phase 1 studies to investigate the safety and immunogenicity of Sm-p80 + GLA-SE in *Schistosoma*-naïve adults (United States, NCT05292391) have recently been completed and demonstrated excellent safety and immunogenicity (unpublished data). Subsequently, phase Ib studies in populations with prior exposure (Burkina Faso and Madagascar, NCT05762393) have recently commenced. Further studies are now required to assess the (preliminary) efficacy of this vaccine. Controlled human infection studies have the potential to speed up vaccine development as they can be used to quickly provide preliminary estimates of vaccine efficacy in a small group of participants (5). Previously, we have developed a controlled human infection with *Sm*(CHI-S) model using single-sex cercariae that do not produce eggs and preventing egg-associated morbidity in study participants. Infection with 20 male cercariae has been found to be safe and well-tolerated in previously unexposed individuals from a non-endemic setting (6). In this double-blind, placebo-controlled randomised vaccine study, we will investigate the safety, immunogenicity, and preliminary efficacy of the Sm-p80 + GLA-SE vaccine against schistosomiasis in healthy, *Schistosoma*-naïve participants.

METHODS: PARTICIPANTS, INTERVENTIONS AND OUTCOMES

Participants

This study will take place at Leiden University Medical Centre (LUMC), The Netherlands. The study population will consist of healthy adults aged 18-45 years without previous exposure to *Schistosoma*. The full in- and exclusion criteria are listed in **Table 1**. Prospective participants will be recruited through (online) advertisements and social media and sent the subject information

sheet (SIS) which contains all relevant study related information prior to the screening visit. Informed consent will be obtained by the clinical investigators prior to any study procedures.

Table 1. In- and exclusion criteria

Inclusion criteria	Exclusion criteria
• Subject is aged ≥ 18 and ≤ 45 years and in good health. • Subject has adequate understanding of the procedures of the study and agrees to abide strictly thereby. • Subject is able to communicate well with the investigator, is available to attend all study visits. • Subject will not travel to *Schistosoma*-endemic countries up until treatment at week 24. • Subject agrees to refrain from blood and plasma donation to blood banks or for other purposes throughout the study period. • For female subjects: subject agrees to use adequate contraception and not to breastfeed for the duration of study. • Subject has signed informed consent.	• Any history, or evidence at screening, of clinically significant symptoms, physical signs or abnormal laboratory values suggestive of systemic conditions, such as cardiovascular, pulmonary, renal, hepatic, neurological, dermatological, endocrine, malignant, haematological, infectious, immune-deficient, (severe) psychiatric and other disorders, which could compromise the health of the participant during the study or interfere with the interpretation of the study results. • The chronic use of any drug known to interact with praziquantel, artesunate or lumefantrine metabolism. Because lumefantrine may cause extension of QT-time, chronic use of drugs with effect on QT interval will result in exclusion from study participation. • Any planned vaccination within 28 days before the start of the trial until the end of the immunisation phase (week 12), with the exception of SARS-CoV-2 vaccines or influenza vaccines. • For female subjects: positive serum pregnancy test on the day before first immunisation. • Any history of schistosomiasis or treatment for schistosomiasis. • Positive serology for schistosomiasis or elevated serum circulating anodic antigen at screening. • Known hypersensitivity to or contra-indications (including co-medication) for use of praziquantel, artesunate or lumefantrine. • Being an employee or student of the department of Parasitology or Infectious Diseases of the LUMC.

Interventions

Enrolled participants will be randomised to the vaccine or placebo arm. Participants in the vaccine arm will be immunised with 30 µg Sm-p80 + 5 µg GLA-SE at days 0, 28, and 56, while the placebo arm will receive mock immunisations with saline at these time points. Vaccines and placebo will be administered intramuscularly in the deltoid muscle.

At week 12 all participants will be exposed to 20 male *Sm* cercariae. *Sm* cercariae will be produced as previously described (7). In short, *Biomphalaria glabrata* snails will first exposed to a single *Sm* miracidium. After five weeks, infected

snails will start shedding cercariae that are either male or female. Cercarial sex is determined using the W1 repeat PCR and only male-shedding snails will be used for producing the challenge inoculum. The controlled human schistosome infection is achieved by pipetting 20 male cercariae in 0.5 mL of water onto the skin. After 30 minutes, the water will be removed and checked for cercarial heads and tails, or whole cercariae. After exposure, participants will regularly visit the study centre for follow-up visits and will be given treatment at week 24 with praziquantel at 60 mg/kg dose to cure infection.

Outcomes

The primary outcome of this study is the protective efficacy of Sm-p80 + GLA-SE to male *Sm* cercariae as measured using the difference in frequency of serum circulating anodic antigen (CAA) positivity (≥1.0 pg/mL) at any time between 2-12 weeks after CHI-S as compared between the vaccine and placebo group. The secondary outcomes include safety and immunogenicity, measured by the frequency and severity of adverse events and anti-Sm-p80 IgG antibody titres after (repeated) immunisation with Sm-p80 + GLA-SE. In addition, exploratory outcomes include the comparison of time to positive serum CAA, peak serum CAA concentrations, and peak eosinophil counts after CHI-S, as well as comparisons of antibody and cellular responses against *Sm* antigens between the two study arms and CAA positive and CAA negative participants.

STUDY PROCEDURES

Recruitment of participants: Advertisements will be placed in prominent places in public spaces as well as on social media, on the intranet, on the website of the LUMC department of parasitology and infectious diseases and www.vaccinonderzoek.nl. When a potential participant shows interest in participating in the study, detailed information in a subject information sheet will be e-mailed to them. There will be at least 48 hours between this e-mail and the screening visit to make sure the potential participant has time to think about participation and discuss this with friends and relatives or with the independent expert assigned to this study.

Screening visit: During the screening visit, participants will first be given a summary of the study and the opportunity to address any study-related questions after which they will be asked to sign the informed consent form if they still want to participate. All participants must consent to HIV, hepatitis B, hepatitis C serological screening, urine toxicology and for females a

pregnancy test at screening. Subsequently participants are asked to complete an application form which includes a questionnaire regarding their health. The questionnaire answers will be discussed and in- and exclusion criteria will be checked. The possibility of withdrawal from the infection study, at any time and without any declaration of the reason, as well as the resulting necessary follow-up visits for safety will be pointed out to the participants. All participants will be asked to supply a phone number of a person who may be contacted in case of emergency. A physical examination will be performed and vital signs (tympanic temperature, blood pressure, and pulse) will be measured.

Immunisation with Sm-p80 + GLA-SE or placebo (weeks 0, 4, and 8): One day before immunisation, participants will visit the clinical trial centre for a final check of in- and exclusion criteria, a focussed physical exam and vital signs may be performed if indicated. Whether participants are subsequently immunised with vaccine or placebo depends on which group they are randomised to. Blood will be drawn for safety checks. All female participants will undergo a pregnancy test on screening and on the day before immunisation. After immunisation, participants will be observed for 30 minutes for potential allergic reactions.

Post-immunisation visits: After each immunisation, participants will visit the trial centre twice for regular check-ups, 3 and 7 days after immunisation. At these visits, adverse events (AEs) will be recorded and participants will undergo a blood draw by venepuncture. At every physical visit the tympanic temperature will be checked. A focused physical examination will be performed if deemed necessary by the trial physician. Participants will be requested to note adverse events in symptom diaries that will be provided.

Infection with male *Sm* cercariae (CHI-S week 12): One day before exposure to male *Sm* cercariae, participants will visit the clinical trial centre for a final check of in- and exclusion criteria, a focussed physical exam and vital signs may be performed if indicated. Blood will be drawn for baseline (pre-infection) assessments and safety laboratory test results will be checked. All female participants will undergo a pregnancy test on the day before infection. Exposure to male *Sm* cercariae will be performed at the LUMC according to previously established protocols at 4 weeks after the last immunisation, i.e.. week 12 of the study. Male cercariae will be allowed to penetrate the skin of human participants by applying 0.5 mL of Bar-le-duc water containing 20 *Sm* male cercariae on the skin for 30 minutes. Participants will be observed for at least 30 minutes after the exposure.

Post-infection follow up visits: Starting at week 14 (2 weeks after CHI-S), participants will have weekly follow-up visits until week 24. Afterwards, the frequency of visits will decrease to once every month until week 36. At all follow up visits, AEs will be recorded and participants will undergo a blood draw by venepuncture. Tympanic temperature will be checked weekly until week 24. A focused physical examination will be performed if deemed necessary by the trial physician. Participants will be instructed to report to the clinical trial physician in case of any grade 2 (moderate) or grade 3 (severe) adverse events to ensure early detection and possible treatment of symptoms of acute schistosomiasis symptoms between week 14-24. A clinical trial physician will be available by mobile phone 24/7 during the entire study period. Additional diagnostics (including serum CAA tests) can be performed on discretion of the trial physician at any time if it is deemed necessary for the safety of the study participants. Participants will provide a stool sample at week 24 (before treatment) which will be tested with PCR to rule out egg production.

Treatment with praziquantel: Participants will be treated with 60 mg/kg praziquantel after exposure to cercariae at week 24 (12 weeks after CHI-S). The treatment will consist of a weight-based number of praziquantel 600 mg tablets. Treatment will be evaluated at week 28 and 32 and if CAA remains detectable (≥1.0 pg/mL) or indeterminate (between 0.5- 1.0 pg/mL) another round of treatment with praziquantel will be administered.

Safety laboratory evaluation: The safety blood sampling schedule is depicted in **Table 2**. Safety analyses includecomplete blood count (including automated differential count of white blood cells), erythrocyte sedimentation rate, creatinine, blood urea nitrogen, sodium, potassium, bilirubin, alkaline phosphatase, gamma-glutamyl transferase, aspartate transaminase, and alanine transaminase ,. Biological safety parameters will be measured on plasma or serum samples at the central clinical chemistry laboratory of the LUMC. Assessment of successful schistosomiasis infection will be performed by serum CAA measurements. A PCR analysis of faecal samples to check the presence of eggs will be performed at week 24.

SAMPLE SIZE CALCULATION

Based on the combined attack rate of around 85% (29 out of 34) after challenge with 20 male cercariae in our earlier studies (6), we calculated that we would require 19 participants in each group to detect a 50% relative reduction in CAA positivity with 80% power and (two-sided) $\alpha = 0.05$ significance level. This effect size reflects a clinically relevant vaccine efficacy estimate as per WHO Scientific Working Group on Schistosomiasis in 1999. More recently, a 70% vaccine efficacy was proposed in a consensus-based preferred product characteristics for a schistosomiasis vaccine, which we would also be able to reliably detect with this sample size (8). Moreover, in baboon vaccine studies a 43% reduction in male worms was observed in Sm-p80 + GLA-SE immunised animals (4). To account for attrition, we are including 24 people per group, bringing the total to 48 participants.

Table 2. Overview of study procedures

Part A: Immunisation phase

Visit no.		1	2	3	4	5	6	7	8	9	10	11	12
Visit name	scr	I1-1d	I1	I1+3d	I1+7d	I2-1d	I2	I2+3d	I2+7d	I3-1d	I3	I3+3d	I3+7d
Week	NA	0	0	0	1	4	4	4	5	8	8	8	9
Day	NA	-1	0	3	7	27	28	31	35	55	56	59	63
Deviation (days)	NA	1	0	1	2	1	0	1	2	1	0	1	2
Obtain informed (re)consent	X	X				X				X			
Temperature	X	X	X	X	X	X	X	X	X	X	X	X	X
Clinical evaluation	X	X		X	X	X		X	X	X		X	X
Immunisation			X[3]				X[3]				X[3]		
Safety tests	X[1,2]	X[2]				X[2]				X[2]			
Serum pregnancy test for female participants		X				X				X			
UCP CAA (serum)	X												
Schistosomiasis serology: worm IFA, egg ELISA	X												
Immunological assays: antibodies		X		X	X	X		X	X	X		X	X
Immunological assays: cellular		X		X[5]	X[5]					X		X[5]	X[5]
Transcriptomics		X		X	X	X		X	X	X		X	X

1: HIV, HBV, HCV, urine cocaine and amphetamines
2: automated CBC, ESR, creatinine, BUN, sodium, potassium, bilirubin, APT, γGT, AST, ALT, glucose
3: vaccination with Sm-p80/GLA-SE or water for injection depending on randomisation group
4: APT, γGT, AST, ALT
5: including leukocyte differentiation to normalise PBMCs

Part B: Challenge phase

Visit no.	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29
Visit	C-1d	C	C+2w	C+3w	C+4w	C+5w	C+6w	C+7w	C+8w	C+9w	C+10w	C+11w	C+12w	C+16w	C+20w	C+24w	C+40w
Week	12	12	14	15	16	17	18	19	20	21	22	23	24	28	32	36	52
Day	83	84	98	105	112	119	126	133	140	147	154	161	168	196	224	252	364
Deviation (days)	1	0	3	3	3	3	3	3	3	3	3	3	3	7	7	7	14
Obtain reconsent	X																
Temperature	X	X	X	X	X	X	X	X	X	X	X	X	X				
Clinical evaluation	X		X	X	X	X	X	X	X	X	X	X	X	X	X	X	
Exposure to cercariae		X															
Praziquantel (60 mg/kg)													X				
Eosinophils					X	X	X	X	X	X	X	X	X	X	X	X	X
Safety tests	X[2]								X[4]								
Serum pregnancy test for female participants	X																
UCP CAA (serum)	X		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Schistosomiasis serology: worm IFA, egg ELISA, *in vitro* killing	X				X				X						X		X
Schistosomiasis PCR eggs (faeces)													X				
Immunological assays (antibody and cellular)	X		X[5]		X[5]				X[5]				X[5]				X[5]
Transcriptomics	X		X		X				X				X				X

2: automated CBC, ESR, creatinine, BUN, sodium, potassium, bilirubin, APT, γGT, AST, ALT, glucose
3: vaccination with Sm-p80/GLA-SE or water for injection depending on randomisation group
4: AF, γGT, AST, ALT
5: including leukocyte differentiation to normalise PBMCs

ASSIGNMENT OF INTERVENTIONS: ALLOCATION AND BLINDING

Group allocation

Participants will be allocated to the intervention group (vaccine) or placebo group (infection control) at random according to an independently prepared randomisation list with a 1:1 ratio, stratified per cohort. Group allocation will be defined by the randomisation number which will be linked to the subject identification code at the first immunisation. Based on this randomisation number, the pharmacist will prepare a syringe with vaccine or placebo for administration according to the list and provide this to the clinical team without disclosing contents of the syringe. The clinical team will check randomisation number and subject identification code before administering the product, unaware of its contents, therefore blinding is secured throughout the process.

Blinding

Participants, trial investigators and primary outcome assessors will be blinded to the identity of the study groups for the whole duration of the trial (until week 24). Earlier unblinding will only occur in case of emergencies, by one of the investigators after discussion with the data safety monitoring committee (DSMB), or by the DSMB itself in case of an adverse event requiring emergency treatment.

DATA COLLECTION AND MANAGEMENT

All data collected by the investigator will be reported in real time using electronic case report forms (eCRFs, Castoredc). At the inclusion visit participants will be issued a paper diary for symptoms. They will be asked to record signs, symptoms and medication use. These participant diaries are a tool to capture symptoms accurately. The diaries will be reviewed at every visit and used as a starting point to discuss possible AEs and/or medication use. Given the two distinct phases of this study (immunisation and CHI-S) characterised by specific signs and symptoms, we separately defined solicited AEs (**Table 3**).

Table 3. Overview of solicited AE during immunisation and CHI-S phase

Immunisation phase (week 0-12)		**CHI-S phase (week 12-24)**	
Local AEs:	Systemic AEs:	Local AEs:	Systemic AEs:
• Pruritis	• Fever	• Pruritis	• Fever
• Erythema	• Chills	• Rash	• Urticaria
• Induration/swelling	• Fatigue		• Headache
• Pain	• Malaise		• Fatigue
• Tenderness	• Myalgia		• Malaise
	• Arthralgia		• Arthralgia
	• Headache		• Night sweats
	• Nausea		• Back pain
	• Vomiting		• Anorexia
			• Nausea
			• Vomiting
			• Abdominal pain
			• Diarrhoea

Symptoms, signs, and lab abnormalities will generally be ranked as (1) mild, (2) moderate, (3) severe, or serious (4) depending on their intensity according to the following scale:

- Mild (grade 1): awareness of symptoms that are easily tolerated and do not interfere with usual daily activity;
- Moderate (grade 2): discomfort that interferes with or limits usual daily activity;
- Severe (grade 3): disabling, with subsequent inability to perform usual daily activity, resulting in absence or required bed rest;
- Serious (grade 4): requiring emergency treatment, life threatening.

For each AE its relationship to study procedures will be assessed. The investigators will use clinical judgment to determine the relationship. Alternative causes, such as natural history of the underlying diseases, concomitant therapy, other risk factors and the temporal relationship of the event to the trial intervention will be considered and investigated.

All clinical trial data, blood samples, or other participant material will be labelled with the participant study identification number. This is a unique code for each participant which does not contain any personal identifiers. When processing the data only the unique code will be used. Samples will be stored in a designated -80°C freezers and liquid nitrogen tank. Access to the rooms is restricted and door movements and temperature will be logged. Samples will be stored for at least 25 years.

STATISTICAL METHODS

The protective efficacy of three times immunisation with Sm-p80 + GLA-SE against *Schistosoma mansoni* infection will be assessed using probabilities of CAA positivity 2-12 weeks after CHI-S for both groups, and will be calculated by [(% subjects with *Sm* in placebo group) – (% subjects with *Sm* in vaccine group)] / (% subjects with *Sm* in placebo group). The probability of CAA detectable *Sm* infection after exposure is also called attack rate (AR). As such the formula can be rewritten as 1- ($AR_{vaccine}$ / $AR_{placebo}$) or 1-[risk ratio]. We will calculate a 95% confidence interval (CI) around the risk ratio (RR) using exact methods, and use these to derive a 95%CI around the VE estimate. Next, we will use the CI for the risk ratio to calculate a p-value to test if RR ≠ 1. These efficacy parameters will be evaluated in a per protocol analysis, which only includes participants who underwent all three immunisations, were subsequently exposed to 20 male *Sm* cercariae, and have available serum CAA data following challenge (weeks 14-24).

All participants with at least one immunisation will be included in the modified intention-to-treat analysis. The safety and reactogenicity of Sm-p80 + GLA/SE is evaluated by tabulating all adverse events for each participant in an intention to treat analysis. Adverse events will be analysed by calculating the proportion of participants in each group who reported mild, moderate or severe adverse events. Statistical testing of these proportions will be performed using chi-square tests or Fisher's exact tests. Adverse events analysis will be performed on the modified intention-to-treat population.

Immunogenicity (Sm-p80 IgG antibodies) will be assessed in the modified intention-to-treat group. We will calculate the percentage of participants achieving seroconversion (fourfold increase from baseline), approximately four weeks after each immunisation. In addition, we will calculate the geometric mean titres (with 95% CI) one and four weeks after immunisation and estimate the geometric mean fold rise.

No interim analysis will be performed except for safety data review by the DSMB. We will try to limit missing data by carefully collecting the data and if data of a certain subject is missing, extensive effort will be undertaken (i.e. documented phone calls and certified mail), to still collect the data. If this is not possible and data is still missing, we will explore the pattern and reason for missingness. If we believe the data is missing completely at random, we will proceed with a complete case analysis.

OVERSIGHT AND MONITORING

The study will be conducted in compliance with the protocol, EU Clinical Trial Regulation No 536/2014, and with the principles of good clinical practice. The study team will submit and obtain approval for substantial modifications to the original approved documents from the regulators before implementation. Data monitoring will be performed by internal monitors of the LUMC according to the monitor plan. During and after completion of the study, the data monitors will check the completeness of patient records, the accuracy of entries on the eCRFs, the adherence to the protocol and to Good Clinical Practice. A DSMB will be appointed which will be independent of the investigators and sponsor and has no conflict of interest with the sponsor of the study. This DSMB will consist of four experienced researchers/clinicians and a statistician qualified to evaluate safety data from clinical studies with schistosome infections. Their main responsibility will be assessing safety reports or serious adverse events and advising the sponsor/investigator on trial continuation. Upon completion of the study, the results of this trial will be published in an open-access, peer-reviewed journal, regardless of the study outcome. Authorship arrangements will be made based on contribution to the trial and its report.

DISCUSSION

This protocol aims to assess the safety, immunogenicity, and preliminary vaccine efficacy of the Sm-p80 + GLA-SE schistosomiasis vaccine in a CHI-S model among healthy, *Schistosoma*-naïve adults.

The development of a vaccine for schistosomiasis has been an important public health target for many years and several candidates are now in clinical testing. Controlled human infections can accelerate vaccine development through preliminary assessment of vaccine efficacy in a small group of participants (5). Previously, a controlled human infection model with schistosomes was successfully established that uses male-only infections to avoid egg-associated morbidity in study participants. A female-only CHI-S model was established with similar infectivity and tolerability, however the unexpectedly decreased susceptibility of female-only infections to praziquantel treatment limits its further use. This study compliments ongoing phase I studies of Sm-p80: in addition to preliminary efficacy data, this well-defined longitudinal sample set allows for in-depth exploration of host-pathogen interactions in vaccinated participants.

Risks for participants are related to potential side effects of the vaccine and exposure to cercariae. Based on the phase I study results, the vaccine is well-tolerated and did not result in any serious adverse events (data not published). After exposure to cercariae, nearly all develop cercarial dermatitis at the site of infection, that is expected to resolve without intervention and generally does not require symptomatic treatment. In case of severe itching, triamcinolone cream can be applied. Symptoms related to acute schistosomiasis infection are likely to occur in a subgroup of participants, starting four weeks after exposure. Previously, 6 out of 35 participants developed severe AEs suggestive of acute schistosomiasis after exposure to 20 male cercariae, which responded well to treatment with paracetamol, NSAIDs, and/or prednisolone (6). To ensure early detection and treatment, participants will be under intense follow-up. To avoid chronic infection, all participants in the study will be treated with praziquantel, which may be repeated if needed. Prior CHI-S with male schistosomes have shown that all participants can be cured using praziquantel.

It is important to note that this study will be conducted in *Schistosoma-naïve* participants from a non-endemic setting. However *Schistosoma* vaccine responses might be different in endemic settings, as seen with Ebola, BCG, and malaria, among others (9). In light of vaccine development, it is therefore especially informative to perform CHI-S studies in an endemic population, who are ultimately the target population for the vaccine. This protocol is part of a larger programme that seeks to establish a CHI-S in Uganda (10, 11) and as such study procedures are harmonised to allow comparisons between study populations in the future.

LIST OF ABBREVIATIONS

AEs	Adverse events
AR	Attack rate
CAA	Circulating anodic antigen
CHI-S	Controlled human infection with schistosomes
CI	Confidence interval
DSMB	Data safety monitoring board
eCRF	Electronic case report forms
GLA-SE	Glucopyranosyl Lipid Adjuvant in stable emulsion
LUMC	Leiden University Medical Center
RR	Risk ratio
SIS	Subject information sheet
Sm	*Schistsosoma mansoni*
Sm-p80	*S. mansoni* calpain protein [of ~80 kDa]
VE	Vaccine efficacy

DECLARATIONS

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Authors' contributions

MR, AE, AS conceived the study. JK, EH, ED, ME, JJ, EW, AS, AE, MR contributed to study design and JS, GD, PC, LvL, DC, SG, MY, AvD, CH, AA helped with implementation. JK and MR drafted the manuscript.

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Availability of data and materials

After publication, all data will undergo FAIRification and will be made available anonymised through a LUMC-based fair data point which will be made accessible through data visiting.

Ethics approval and consent to participate

The study will be conducted in accordance with the ICH guidelines for Good Clinical Practice and Declaration of Helsinki. Ethics approval is under review at the Central Committee on Research Involving Human Subjects (no. 2023-509816-27-00).

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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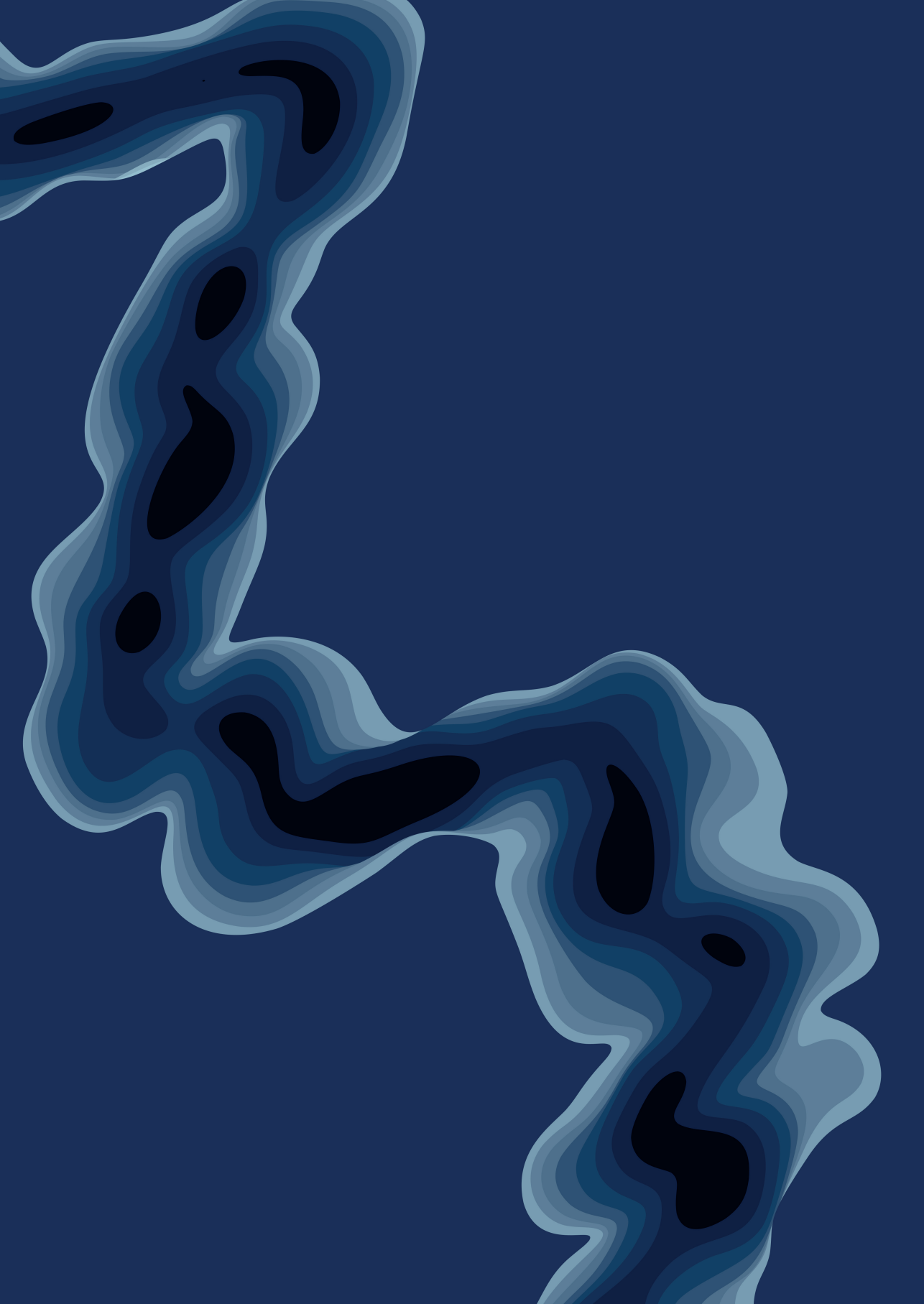

Chapter 7

Immunological considerations for *Schistosoma* vaccine development: transitioning to endemic settings

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ABSTRACT

Despite mass drug administration programmes with praziquantel , the prevalence of schistosomiasis remains high. A vaccine is urgently needed to control transmission of this debilitating disease. As some promising schistosomiasis vaccine candidates are moving through pre-clinical and clinical testing, we review the immunological challenges that these vaccine candidates may encounter in transitioning through the clinical trial phases in endemic settings.

Prior exposure of the target population to schistosomes and other infections may impact vaccine response and efficacy and therefore requires considerable attention. Schistosomes are known for their potential to induce T-reg/IL-10 mediated immune suppression in populations which are chronically infected. Moreover, endemicity of schistosomiasis is focal whereby target and trial populations may exhibit several degrees of prior exposure as well as in utero exposure which may increase heterogeneity of vaccine responses. The age dependent distribution of exposure and development of acquired immunity, and general differences in the baseline immunological profile, adds to the complexity of selecting suitable trial populations.

Similarly, prior or concurrent infections with other parasitic helminths, viral and bacterial infections, may alter immunological responses. Consequently, treatment of co-infections may benefit the immunogenicity of vaccines and may be considered despite logistical challenges. On the other hand, viral infections leave a life-long immunological imprint on the human host. Screening for serostatus may be needed to facilitate interpretation of vaccine responses.

Co-delivery of schistosome vaccines with PZQ is attractive from a perspective of implementation but may complicate the immunogenicity of schistosomiasis vaccines. Several studies have reported PZQ treatment to induce both transient and long-term immuno-modulatory effects as a result of tegument destruction, worm killing and subsequent exposure of worm antigens to the host immune system. These in turn may augment or antagonise vaccine immunogenicity.

Understanding the complex immunological interactions between vaccine, co-infections or prior exposure is essential in early stages of clinical development to facilitate phase 3 clinical trial design and implementation policies. Besides well-designed studies in different target populations using schistosome candidate vaccines or other vaccines as models, controlled human infections

could also help identify markers of immune protection in populations with different disease and immunological backgrounds.

Keywords: Schistosomiasis, vaccine-candidate, praziquantel co-administration, co-infections, endemic, vaccine response, immunological-consideration

INTRODUCTION

Schistosomiasis is a poverty associated chronic disease caused by parasitic trematodes of the *genus Schistosoma* (1). Over 190 million people globally are actively infected, of which 90% live in Africa (2) and over 200,000 deaths result from schistosomiasis disease annually in Sub-Saharan Africa (SSA) (1). The two main forms of African schistosomiasis, caused by Schistosoma haematobium and S. mansoni parasites, affect the urinary and gastro-intestinal tract, respectively. The intermediate host, fresh water snails of the genus *Bulinus* and *Biomphalaria* shed infective cercariae in water where they penetrate the skin of the human host. Water-related livelihood activities, such as fishing, thus drive transmission in resource limited settings with poor hygiene and sanitary facilities (3). Following exposure, schistosomula migrate through the blood stream and lungs to the mesenteric and/or peri-vesical vessels intestinal venules where they mature into female and male adult worms. Adult worms mate and the female produces eggs that are released from the intestinal or urinary tract to complete the cycle. Pathology results mainly from accumulation of deposited eggs that induce inflammatory responses, granuloma formation and fibrosis. This results in strictures and calcification in the urinary tract due to S. haematobium egg deposition, and liver fibrosis from S. mansoni egg deposition in the Liver. Long term consequences include hydronephrosis and kidney failure in the case of S. haematobium infection, and *hepatosplenomegaly and portal hypertension* in the case of S. mansoni infection, accounting for the morbidity and mortality in schistosomiasis disease (4, 5).

In the last decade, the World Health Organization (WHO) set an ambitious goal of controlling schistosomiasis by the year 2020 and eliminating it as a public health burden by 2025. Initially, increasing coverage of mass drug administration (MDA) of praziquantel (PZQ), the only drug currently used for schistosomiasis treatment (6), was thought to be sufficient to achieve this. Unfortunately, the increased coverage of MDA decreases the intensity of infection and thus suffices as a tool to alleviate schistosomiasis morbidity but does not prevent re-infection. Consequently, prevalence can be restored in 6-8 months after PZQ administration (7). As a result, control programs rely on the repeated administration of PZQ, whilst facing significant challenges with drug uptake, adherence and sustainability. Hence, schistosomiasis remains a paramount public health concern and economic burden in the resource limited countries (6). Also, the full dependency on a single drug poses a threat of drug resistance (7). This leaves vaccination a key approach for the control and possible elimination of schistosomiasis. An effective vaccine could contribute to

prevention and protection against re-infection. Consequently, schistosomiasis has been ranked among the top ten diseases for which a vaccine is urgently needed (8). The current overall consensus on preferred product characteristics (PPC) for an effective prophylactic vaccine is induction of 75% reduction in worm burden in immunised individuals and egg excretion in infected patients (9).

However, development of a novel schistosome vaccine faces several scientific challenges due to the immune evasive nature of adult schistosomes and schistosome-induced host immune-modulation. Illustrated by the fact that no human anti-helminth vaccines exists, the development of a schistosomiasis vaccine is likely to be more complex as compared to the recent rapid development of SARS-CoV-2 vaccines. Most importantly, the multi-stage nature of schistosomes, transforming from cercariae to Schistosomula, adult worms and ultimately eggs, involves complex antigenic switches which are life-stage specific, but can also be present across life stages. In addition, the induction of IgE responses, associated risk of allergic reactions and the potential of aggravating granulomas and fibrosis by egg-mediated responses (10) makes schistosomiasis vaccine development complex.

Moreover, poly-parasitism and co-infections, a predominant phenomenon in schistosomiasis endemic regions, mediate cross-immune-regulation whereby one infection influences susceptibility, intensity and immune responses to the other (11). In addition, there is inadequate understanding of how past and existing schistosome infections, repeated exposure, poly-parasitism and prior treatment explicitly structure the immune system in individuals, within and between the different populations. These factors are likely to increase heterogeneity in vaccine responses. Further, the need to implement vaccines in the context of the current MDA control programmes necessitates an integrated approach to praziquantel and vaccine administration (9). However, co-administration of vaccines and praziquantel presents divergent immunological dynamics that have to be considered. Here, we review the immunological challenges for schistosome vaccine development and testing in endemic settings and provide perspectives of how this scientific priority area can be accelerated.

THE PROGRESS TOWARDS A SCHISTOSOME VACCINE

The initial approach in the quest for a schistosomiasis vaccine involved vaccinating mice with live attenuated cercariae and schistosomula followed by a challenge infection to assess protectivity. These studies demonstrated that 60-70% protection was achievable with a single immunisation and could be boosted up to over 90% with subsequent immunisations (12, 13). This approach was similarly efficacious in baboons eliciting protection of over 50% to schistosome larvae challenge and 89% reduction in worm burden, upon immunisation with gamma-irradiated cercariae (14). However, despite induction of close to 100% protection (13), this approach is difficult to translate to humans (15).

Nevertheless, these studies provided a strong indication for schistosome vaccine discovery, and knowledge on induction of protective immunity. Acquired immunity was found to be mainly associated with a Th1 type IFN-γ and TNFα pulmonary inflammatory and antibody response, IL-4, IL-5, and eosinophils are of negligible importance meanwhile, IL-10 and IgG4 negates protection and are associated with re-infection (13, 14, 16). Conclusively, these studies denoted that excessive induction of either Th2 or Th1 could lead to damaging pathology and thus, induction a balanced Th1 and Th2 response is fundamental for achieving optimal protection (15).

Utilising advancements in technology, vaccine development has, in the recent past, shifted focus from irradiated parasite vaccines to specific antigen molecules. Nonetheless, vaccination with irradiated *S. mansoni* cercarie has contributed to the identification of five such molecules including the Sh28GST and the Sm14 which have proceeded to human clinical trials (12, 15) as discussed below and further reviewed by Molehin (16) and McManus et al., (15).

S. haematobium 28-kD glutathione S-transferase (r*Sh*28GST)

This glutathione S-transferase (GST) antigen is a worm detoxification enzyme which is important in preventing parasite oxidative stress (17). Ultrastructural localization of the 28GST show a dense cytosolic, as well as genital, tegumental surface and parenchyma distribution in the schistosome parasite (18). Thus, the 28GST is closely associated with the parasites' muscular organs and anti-28GST specific antibodies mediate Antibody-Dependent-Cellular-Cytoxicity (ADCC) and also inhibit transferase activity of the enzyme interfering with muscle function and ultimately in reduced worm fecundity (19, 20).

Rodent studies demonstrate that 28-kDa fraction of GST (*Sh*28GSGT) elicits an antibody response which is capable of inhibiting the native GST enzymes, conferring between 40-70% protection in rats, mice, hamsters and baboons (21). Experimental infection studies in non-human primate models revealed an anti-fecundity effect with significant reduction in tissue egg load and faecal egg excretion despite a lack of effect on adult worm burden (22).

The phase I trial reported safety and tolerability of the r28GST in Alum formulation in human subjects. The immunological read-abouts demonstrated that 28GST vaccine candidate is highly immunogenic, inducing high levels of specific IgG1, IgG2, IgG3 antibodies. A strong Th2 cytokine response was also observed, typically IL-5 and IL-13 cytokines (23). Though findings from the phase II trial are yet to be published, the phase III trial, a randomised parallel-group double-blinded trial in school children in endemic Senegal, reported a good tolerance of the vaccine with induction of long lasting vaccine specific response. High titres of IgG1, IgG2 and IgG were induced in the vaccine group. However, this vaccine candidate did not show sufficient protection and this was attributed to failure to induce the desired specific-IgG3. The investigators of this trial also attribute this to interreference by the praziquantel treatment that was administered after the first vaccine dose and before the booster dose (19). Post vaccination treatment with PZQ, which coincides with elaboration of immune response to a vaccine, has been shown to interfere with cytokine response (19, 24, 25).

S. mansoni 14-kDA fatty acid binding protein (*Sm*14)

Sm14 is one of the fatty acid binding (FABP) proteins that play a crucial role in the uptake, transport and compartmentalisation of host-derived sterols by schistosomes (26). Sm-14 protein enzymes are localised in the cytosol and in tissues adjoining the interfaces of parasite/host contact such as the basal lamella of the worm tegument and muscles layers to enable acquisition of lipids from the host (27). They are also found in the gut epithelium for lipid transportation and utilization throughout the parasite. Sm14-specific antibodies bind to tubercles on the parasite's dorsal surface and interfere with uptake of lipids essential for parasite survival as well as mediate ADCC (28).

In rodent studies, Vaccination with the rSm14 induced up to 66% and 89% protection, of Swiss mice and New Zealand white rabbits with respectively (29). Sera from immunised mice showed generally high reactivity and significant level of rSm14-specific IgM, although IgG and IgA titres were low (30).

In the phase I trial, the *Sm*14/GLA-SE formulation was safe, highly tolerable and immunogenic in adult male human subjects. It induced a strong CD4+ T cell response producing single Th1 cytokines, particularly the TNF and IL-2. Also, high titres of Sm14-specific IgG, IgG1 and IgG3 antibodies were elicited in vaccinated individuals (28, 31). The *Sm*14 vaccine has now advanced to a phase IIb trial to be conducted in school children in the endemic Senegal river basin region (26) (NCT03799510).

S. mansoni Tetraspanin-2 (*Sm*-TSP-2)

Sm-tetraspanin is a member of a four-domain-structured tetraspanin surface membrane protein linked by two extracellular loops and made of two types, TSP-1 and TSP2 (32). The main vaccine antigen in the Sm-TSP candidate vaccine is comprised of the extracellular loop and the TSP-2 type. The TSP-2 antigen was found to be more strongly recognised by IgG1 and IgG3 antibodies in the sera of naturally immune populations, unlike TSP-1 (33). The TSP-2 is a readily immune-accessible antigen on the surface of newly transformed schistosomula and a critical tegument protein for nutrient acquisition, waste excretion and immune evasion. Anti-TSP-2 antibodies interfere with these parasite survival mechanisms and elicit a protective immunity against infection in a vaccinated host (33).

Immunization with *Sm*-TSP-2 result in 57% and 62% reduction in worm and liver egg burden, respectively, and 69% reduction in faecal egg count, in rodent models (33). An increased production of Sm-TSP-2 specific antibodies and IL-4, IL-10 cytokines by spleen cells is also observed in immunised animals (34).

Phase Ia and phase Ib trials of the Sm-TSP-2/Alhydrogel (Sm-TSP-2/Al) have both been initiated to investigate safety and immunogenicity of this vaccine candidate in human subject (NCT03910972). Phase I trial of the Sm-TSP/Alhydrogel with or without glucopyranosyl lipid A (GLA-AF) formulation in non-endemic setting show the vaccine is safe and tolerable among *Sm*-naïve individuals (35). The "Sm-TSP/Al with GLA-AF" formulation elicited higher sero-response than the "Sm-TSP/Al without GLA-AF" and placebo, with a dose-response relationship exhibited by Sm-TSP/Al with GLA-AF (35).

S. mansoni Sm-p80/GLA-SE

Sm-p80 is the large subunit of calpain, a calcium activated neutral tegument protease, which is located in and on the surface epithelial syncytium and mediates tegument biogenesis for host immune evasion. *Sm*-p80 is a highly

immuno-dominant membrane antigen with no cross-reactivity with vertebrate calpains (36).

A multitude of studies performed *in vitro*, in rodents and non-human primates over the past twenty three years have demonstrated that *Sm*-p80 is a very promising vaccine candidate with prophylactic, therapeutic, anti-pathology and transmission blocking efficacies (37). Significant reduction in adult worm burden, tissue egg load and faecal egg excretion following *Sm*-p80 vaccination has been demonstrated, whereas potentially allergic IgE responses have not been registered (38). *Sm*-p80 immunisation elicits significant complement-dependent killing of schistosomula (37). Administration of PZQ preceding *Sm*-p80 vaccination is proven to profoundly reduce tissue egg retention and hatching in non-human primates (39, 40). Following the desirable responses and results from rodent and non-human primate studies, *Sm*-p80/GLA-SE has now been approved for phase I clinical trial (9).

As schistosomiasis candidate vaccines are progressing from phase I studies to testing in the target populations in several endemic settings, the distinctive disease exposure, co-infections and transmission settings that uniquely shape the immunological profiles may result in heterogeneity in vaccine responses. Hence, in addition to the existing logistical, accessibility and resource-limitation challenges, the immunological complexity presents exceptional challenges to vaccine development and testing in schistosomiasis endemic regions.

PRIOR/CURRENT INFECTION WITH SCHISTOSOMES AND ANTIGEN SENSITISATION

Generally, prophylactic vaccines are given to naïve individuals but for potential *Schistosoma* vaccines, this may be challenging as sensitization to *Schistosoma* antigens is likely to occur early in life or even *in utero* in endemic settings (41). Prior exposure to antigens included in a vaccine may enhance or suppress vaccine responses.

At birth, the offspring of *Schistosoma* infected mothers already show signs of B and T-cell sensitization to parasite antigens (42), with detectable schistosome-specific antibodies (including IgE), T cell responses in cord blood, and schistosome antigens in the urine of new-borns (41, 43). Even with zero egg counts, antibodies in infant serum recognise some worm antigens (44, 45). Following childhood exposure, infection peaks in 6-15 year old children and can be as high as 90% in <12 year olds (46, 47). The peak and dispersion in worm

burden gradually declines with age (47), which could be a reflection of reduced exposure or, in high exposure settings, the induction of naturally acquired immunity. Naturally acquired immunity is characterised by a broadening of antibody repertoire and a switch from predominantly egg-specific IgG1, IgG2 and IgM antibodies in infancy to a protective larval and adult worm-specific IgE antibodies towards adulthood and increasing duration of exposure (44, 48-50). IgE response, elicited after repeated exposure and or PZQ treatment is associated with protection (though not exclusively) in endemic populations (10). Natural or PZQ-driven death of adult worms releases parasite antigens from migrating larvae and this induces IgE response (51) which mediates antibody-dependent-cellular-cytoxicity (ADCC) killing of more parasites (32). Although not useful in vaccine development due to the potential to induce hypersensitivity (10), an IgE mediated protective immunity is subsequently developed in individuals, over many cycles of infection and or treatment (52).

This raises the question of how pre-exposure and pre-existing immunity influences vaccine responses. Depending on the vaccine antigen and the pathogen, the effect of pre-exposure may vary. For instance, reduced cytokine responses to Bacillus Calmette-Guérin (BCG) vaccination have been linked to previous exposure to other mycobacteria, but studies with hepatitis B vaccines show conflicting results with increased antibody responses after pre-exposure in one study, and lower in another study (53). For schistosome antigens, there is no evidence so far that pre-existing exposure to vaccine antigens negatively impacts schistosomiasis vaccine responses. In the r*Sh*28GST Phase 3 study, pre-existing immune responses to the 28GST antigen were observed in children aged 6-7, but there was no evidence of pre-existing anti-28GST responses negatively impacting vaccine immune responses (19). In addition, in studies with recombinant *Sm*14, high levels of the Th1 cytokines IFN-γ, and TNF-α could be observed despite a history of chronic exposure (54). This is in agreement with animal experiments which showed that successful immunization could be achieved in previously infected and cured baboons (40). As such, current data suggest that schistosome vaccines may retain immunogenicity despite prior exposure. However, the impact of prior exposure on vaccine responses may differ for each antigen and should therefore be carefully assessed in early-stage trials to avoid reduced efficacy results in phase 3 trials or implementation settings.

POPULATION DIFFERENCES IN IMMUNE PROFILE AFFECTING VACCINE RESPONSES

Differences in the baseline immune profiles between populations are known to affect both quantitative and qualitative response to vaccines (55). Since novel vaccine candidates are typically assessed for phase I safety trials in a non-endemic European population, it should be borne in mind that generally lower vaccine responses are found in African populations (56-59).

The immune profile of African cohorts, in contrast to Caucasian populations, typically contains more exhausted and activated NK cells, differentiated T and B cells and pro-inflammatory monocytes (57, 60). This immune profile was associated with a low vaccine-specific neutralizing antibody response and a poor efficacy to the yellow fever vaccine 17D (YF-17D) as compared to a Swiss cohort (57). BCG vaccination in African adults and children elicited low IFN-γ response and a mixed cytokine profile in a setting where BCG efficacy is low, but a predominant Th1 cytokine profile in the United Kingdom where BCG efficacy is 50-80% (56, 58, 61). Also, African infants did not show increase in the magnitude of T cell response to the MVA85A TB candidate vaccine unlike their UK counterparts (62). Furthermore, the HIV-adenovirus-vectored (Ad26.EnvA.01 and Ad35-EnvA) candidate vaccines induced better efficacy and greater T cell responses in an American cohort compared to South and East African subjects (59). These findings suggest that the baseline immune profile of African populations may interfere with adequate vaccine responses and this effect is especially important for the viral vectored and live vaccines such as the MVA85A and Ad26/35-EnvA, and BCG and YF-17D, respectively (56, 58, 62).

Genetic difference between populations could contribute to heterogeneity in vaccine responses. Vaccine immune responses are regulated by multiple gene complexes and networks which may be subject to genetic variations such as inherent cytokine and HLA gene polymorphisms (63, 64) and thus, difference in immune phenotype and functional response. For instance, Indonesians were shown to express the unique $CD11c^{+}$ IL-10 producing B cell subset as compared to Europeans (65), depicting a possible genetic disparity. In a malaria endemic region in Mali, both adults and children exhibited an atypical FcRL4+ expressing memory B cell population unlike their American counterparts (66). Furthermore, volunteers of Congolese origin expressed a distinctly high number of *STAT6* and *IL10RA* regulatory gene polymorphisms, a genetic evidence that explains the predominantly asymptomatic-uncomplicated malaria infection manifestation in this population (67). Differences in response

to vaccines such as the measles vaccine have also been attributed to genetic polymorphisms in the *CD46* and *SLAM* gene receptor (68).

However, there is increasing evidence suggesting that inter-population differences in immunological profiles are driven by non-inheritable environmental factors such as prior and current infection status which may typically be vastly different between rural versus urban living (60, 69, 70). For example, rural African populations express a superior immune activated state, larger memory pool and Th2 polarization as compared to urban Africans (65) and Indonesians from a non-endemic urban setting showed a similar immune profile to European subjects yet markedly differed from Indonesians from an endemic rural area (60). More so, a type 2 immune profile comprising of Th2 cells, IL-4,IL-5 and IL-13 cytokines is typical of schistosomiasis high-exposure endemic populations (71). Subjects from the endemic setting exhibited a high frequency of $CD161^{+}$Th2 cells, CCR6-$KLGR1^{+}$ ILC2, $CTLA^{+}$ T regs and $IL\text{-}10^{+}$ $CD11c^{+}$ B cells (72). Subjects from a rural setting in Uganda showed a decreased specific cytokine and antibody response to tetanus toxoid and *Mtb* purified derived protein (PPD) compared to their urban counterparts which persisted after adjusting for helminth infection (73).

Therefore, early phase clinical trials should preferably be conducted in target populations to ensure no gaps in translation at a later stage. In addition, booster regimens, potent adjuvants and higher antigen dose can be considered particularly for endemic populations such as in SSA where low vaccine response and efficacy is predominant.

CO-INFECTIONS

Besides prior and current schistosome exposure, schistosomiasis endemic populations are also highly diverse with regards to exposure to other infections. Chronic viral infections and poly-parasitism in schistosome endemic populations constantly expose the host immune system to a complex array of antigens and epitopes causing antigenic competition and immune sensitization (74). In addition, parasitic infections such as lymphatic filariasis, onchocerciasis and leishmaniasis often occur in schistosome endemic areas. This trains and pre-sets the immune phenotype and functionality, potentially distorting the host response to ,vaccine candidates.

Noteworthy, in this review we focus on the concomitant parasitic (malaria and soil-transmitted helminths) and viral (Cytomegalovirus) infections which are highly prevalent among schistosomiasis endemic populations in the low and middle income countries (LMIC) (75, 76). Parasitic co-infections are of particular importance due to the significant negative impact on immunisation especially among parasite endemic populations which are the target for schistosomiasis vaccine trials (75).

Malaria co-infection

Malaria and schistosomiasis are both parasitic diseases that share co-endemicity, and cause significant morbidity and damaging socio-economic effects (77). With schistosomiasis only ranking second to malaria, these two parasitic diseases exhibit an overlapping geographical distribution pattern and a high prevalence of co-infection across the tropics and sub tropics (77). The prevalence of malaria is highest in children (78) and is mainly caused by *Plasmodium falciparum*. In fishing communities, which are a target population for schistosomiasis vaccine trials due to the high schistosomiasis transmission and intense exposure, the risk and prevalence of malaria co-infection is very high (79). The presence of breeding sites in the lake environment such as stagnant pools of water on the shores and unused old boats, fish bait mines and finger-ponds traps which sustain a heavy vector population in these communities (80). Also, fishing activities such as nocturnal fishing promote outdoor transmission as well as the temporary makeshift housing that is porous to the malaria vector (81).

Primary malaria infection induces CD4+ T cell differentiation into CXCR+ T follicular (T*fh*) cells to provide B cell help and the highly proliferative Vγ9+Vδ2+ T cells produce high levels of IFN-γ (82). Production of pro-inflammatory cytokines and chemokines such as IL-1β, IL-6, IL-8, IL-12, IFN-γ, and TNF induce fever and other signs and symptoms in previously unexposed individuals resulting in severe and fatal malaria (83). Increased TNF levels are associated with severe and cerebral malaria infection although sustained high levels result in reduction in parasitemia and improvement of fever (84, 85). However, this Plasmodium-induced inflammatory response also controls parasite replication and resolves infection through IFN-γ and TNF mediated killing of parasite (86). Meanwhile, acute uncomplicated infection activates the less functional CXCR3+PD1+ T*fh* cells which enhances production of IL-21 and pro-inflammatory cytokines (87). *P. falciparum* malaria infection additionally prompts dysregulation of B- and CD4+T cell function subsequently, causing significant and rapid loss of

Pf-antigen-specific antibodies (87, 88). Asymptomatic malaria is an important consideration for clinical trials since it is the most common form among adults, the ideal early-phase trial subjects in endemic settings. Asymptomatic malaria is characterized by increased Treg numbers, IL-10 and TGF-β production, and dampened TNF, IFN-γ pro-inflammatory response characterises (89). *P. falciparum*-induced upregulation of IL-10 production by CD4+Foxp3- Th1 cells rather than T regs, is usually sustained in persistent asymptomatic infection (90). Malaria infection additionally affects B cell phenotype and function. In repeated malaria infections and persistent asymptomatic parasitemia, accumulation of atypical memory B cells, a hyporesponsive anergic B cell subset expressing several inhibitory receptors, is seen (91, 92). Expansion of atypical memory B cells associated with clinical immunity following acute malaria has also been reported (93). This immunological memory may have implications on vaccine response given that it may distort the balance between antibody affinity maturation and B cell clonal selection following vaccination (92, 94).

Acute malaria has been reported to lower response to tetanus and diphtheria toxoids, meningococcal polysaccharide, *Salmonella* and *Haemophilus influenza* conjugate and whole vaccines (95-97). Fever associated with malaria infection contributes to a diminishing response to *Haemophilus influenza* vaccine (96). More so, asymptomatic parasitemia in malaria infection is associated with a decreased response to the newer acellular pertussis and meningococcal vaccine (87). Similarly, malaria infection during pregnancy and in infancy decreases antibody response to infant measles vaccination (98). These findings suggest and support treatment of malaria before vaccination is necessary to alleviate the antagonising immune effects. Indeed, there is evidence that the response to some vaccines (such as the tetanus vaccine) improve following malaria chemoprophylaxis (95). However, pre-treatment does not improve response to or even impair immunogenicity of some vaccines such as the measles vaccine (95).

In co-endemic populations, it seems reasonable to test and treat malaria infections prior to vaccine administration. However, the added complexity of malaria pre-treatment in implementation settings makes it imperative to investigate the effect of asymptomatic malaria on vaccine response before phase 3 trials are performed. In addition, the optimal timing of malaria chemotherapy with regards to vaccine administration will need to be considered in order to optimize effects in different malaria transmission settings.

Soil-Transmitted Helminths (STHs) co-infection

Soil-transmitted helminths (STHs) and schistosomiasis are predominantly co-endemic (99). It is estimated that one-third of the population in SSA is infected with one or more STH (100). This prevalence is driven by the overlapping poverty-related conditions of poor environmental hygiene, improper waste disposal, in adequate water supply and pollution of water bodies (101). The common STHs are the round worm (*Ascaris lumbricoides)*, hookworms (*Necator americanus* and *Anyclostoma duodenale*), whipworm (*Trichuris tichuria)* and *Strongloides stercoralis* (99). However, hookworm and A. *lumbroicoides* infections seem the most prevalent of helminth co-infections in schistosomiasis infected individuals in many endemic areas (102, 103). Young children and males are more prone to the infections due to poor hand hygiene practices and active behaviour that exposes them to contaminated soils and water (101). This high prevalence among adult males is an indication of a greater likelihood of existing or past infection, a caution for possible distortion of host immune response to other infections and vaccines. And also a challenge to the selection of trial subjects in co-endemic settings.

STH infection impairs development of protective immunity possibly as a result of the potent chronic suppression of the Th1 response required for protection against pathogens (74). STH infections typically induce a type-2 biased immune response (104). Therefore, elevated levels of IL-4, IL-5, IL-13, IgE in addition to general modulation of both innate and adaptive immune systems can be expected in STH co-infection (104). Chronic STH infection not only induces potent local but also systemic down regulation of the immune system. For instance, human subjects challenged with *N. americanus* exhibit a strong local and systemic Th2 and T reg response with high levels of IL-10 and TGF-β production (105). In chronic STH infection, it is the increased functional activity of the $FOXP3^+$ Tregs which mediates immune suppression, rather than an increased frequency in chronically infected human hosts (106).

This potentially affects not only susceptibility and outcome of concurrent infections but response and efficacy of vaccine candidates (107). Studies in both human and mouse models report poor immunogenicity of the BCG vaccine in STH infected subjects (108). Decreased T cell proliferative response to BCG in helminth infected children was found to result from an enhanced T reg functionality and subsequent immune suppression during chronic infection (106). Besides, the immune profile to recombinant cholera toxin B subunit following live oral cholera CVD 103-HgR vaccination in helminth infected subjects is characterised by low IFN-γ, IL-2 and IL-12 (109), indicating

a suppressed immune response. Particularly, existing *Onchocerca* infection significantly decreases immune response to tetanus vaccination compared to subjects without helminth infection (110). On the other hand, STH infections do not alter IgG antibody responses to previously administered measles and tetanus vaccines (111). Hence, with the changing lifestyle, better hygiene practices and anti-helminth treatment, there is a gradual reduction in helminth infections in many LMIC and one would expect better vaccine responses. This has indeed been shown in animal studies and in humans, findings from number of studies do support that treatment of STHs alleviate immune suppression and improve vaccine responses as reviewed elsewhere (112).

Helminth treatment, usually by oral administration of benzimidazoles i.e. albendazole (400mg/kg) and mebendazole (500mg), before vaccination may be beneficial for boosting vaccine responses. Deworming of subjects with Albendazole before BCG vaccination was reported to enhance BCG vaccine-specific response (113). A similar boosting effect on oral cholera vaccine among helminth infected subjects is exhibited (114). This is possibly due to alleviation of the helminth-induced immune suppression by anti-helminth treatment. Albendazole deworming is associated with enhanced pro-inflammatory responses and down regulation of inhibitory receptor expression CTLA, by the immune suppressive Tregs (115). However, one study did not find any significant effect of albendazole treatment on influenza vaccine response, despite the increased total IgA titres in the anti-helminth treated group (116).

Nevertheless, testing and treating of helminth infections may be beneficial in phase I schistosomiasis vaccine trials. Treatment before vaccination would alleviate the immune suppression induced by helminth infection prior to vaccine administration and thus, enable better response to vaccines. Meanwhile, treatment around time of vaccine administration possibly drives antigenic unmasking to expose more antigens for host immune recognition. However, antigenic unmasking, following PZQ treatment of intense pre-existing infection which results in release of worm antigens in excess of vaccine antigen, poses a risk of antigenic competition. This may result in poor and or non-specific immune response following PZQ treatment and vaccination. In this case, intensity of pre-existing infection should be taken into consideration. It should be noted that the duration and intensity of anti-helminth treatment itself does have a differential effect on B and T cell response (117). Therefore, not only the effect of STH infections but also their treatment on vaccine response and efficacy should be investigated in late-phase trials in endemic settings.

Human Cytomegalovirus (CMV) co-infection

This double stranded DNA and enveloped virus belonging to the *Beta-herpesvirinae* infects between 40-100% of adults, varying in prevalence between populations (118). Approximately 90% of the infection incidence occurs in the LMIC of Africa, Asia and South America where up to 60% of the young adults and 90% of elderly adults are sero-positive (119-121).

Besides the high prevalence, the induced immune modulation and establishment of a periodically re-activated life-long latent infection marks CMV a very important viral infection (122, 123). CMV infection typically distorts the T cell repertoire and overall phenotype by inducing a rapid differentiation and clonal expansion of CMV-specific CD8+ T cells. This T cell subset can constitute up to 20% of the T cell repertoire, especially in older adults, but is not functional in controlling the infection (124). Meanwhile, the CMV-specific CD4+T cells increasingly produce IFN-γ, and MIP-1β that mediate re-activation of other latent infections in the host such as Tuberculosis (125). Chronic CMV infection thus causes a continuous accumulation of highly differentiated effector memory T cells. Besides, expression of co-stimulatory receptors in these T cells is inhibited, together resulting in a phenomenon known as "memory inflation" (126, 127), whilst at the same time depleting the naïve T cell pool (128).

Despite the universal host immune phenotype, the negative effect of CMV infection on vaccine responses is most pronounced in the elderly (122). CMV sero-positive elderly adults have been reported to exhibit a decreased response to *Influenza* vaccine (129, 130). A defective CD8+pSTAT1 and pSTAT3 pathway, expanding senescent CD57+KLG1+ T cell repertoire and the increasing TNF-α and IL-6 levels with infection duration, reduces cytokine responsiveness of vaccine-induced T-cells (122, 130, 131). Findings in younger CMV infected adults are conflicting. An enhanced response, characterized by high levels of circulating Th1 and Th2 cytokines to *Influenza* vaccine is seen (129), possibly due to the cross-reactive CMV-specific CD8+ T-cell epitopes (132). Meanwhile, response to Ebola candidate vaccines, ChAd3 and MVA, is impaired (122).

Given that CMV establishes a life-long infection that cannot be cleared or treated, the attention for vaccine trial design and implementation should rather be focused around different age of target populations. Phase I trials may consider testing and exclusion of CMV sero-positive subjects, especially in CMV low and moderate prevalence areas, for any vaccine targeting young children or infants. However, this may not be possible in populations where

CMV infection is prevalent and almost universal, even among children. For late-phase trials, inclusion of CMV sero-negative versus sero-positive subjects, could help decipher possible interaction between CMV and vaccine responses. Alternatively, general understanding of the possible effect of CMV could first be investigated through fundamental studies and then regimens such as booster doses for the sero-positive subjects in late phase trials can be considered and tailored accordingly.

Other Infections

Prevalent co-infections with obvious immune-modulation and even immune incompetence induction are HIV and TB.

HIV/AIDS, a life-long viral infection with no cure presently and the cause of profound immune-incompetence, is a prevalent viral infection in SSA and other developing regions of the world (133). Over 20 million people are infected in South and Eastern Africa alone (134). HIV establishes a preferential infection and depletion of CD4+ T cells, increases T cell activation, exhaustion and death, impairs antigen presentation, CD4+ and CD8+ T cell functionality (133, 135). This results in very low CD4+ T cell count (below the 500cells/mm^3 lower limit), severe immune impairment, poor vaccine response as well as rapid vaccine induced decline (135, 136).

Combination anti-retroviral therapy facilitates immune reconstitution and viral load suppression, despite persistence of the virus in latent reservoirs (135), suggesting a near-optimal immune response can be expected. Indeed improved vaccine response and reduced risk of infection following Influenza, PPV23 and PCV pneumococcal vaccinations has been reported in patients on cART (137-139). Nevertheless, immune responses in HIV infected patients may be lower, or wane off quicker, despite cART therapy as has been demonstrated in trials with hepatitis B, Influenza, experimental TB and BCG vaccination both in adults and children (140-145).

Despite the potential public health importance of vaccination in HIV infected patients given their increased susceptibility to vaccine-preventable diseases (146), they are often excluded from vaccine trials because of safety concerns and unfavourable benefit/risk profiles (147, 148). For example, the unfavourable benefit/risk profile of BCG vaccination in HIV infected has to led its constant contraindication (148-150). Therefore, testing for HIV, exclusion of sero-positives from trials and referral for cART treatment, is paramount for safety. However, given the high percentage of HIV positive individuals in some areas

and the increased use of cART and non-viable vaccines, these populations should be included at a later stage clinical testing of the vaccine to expedite roll-out in these populations after potential registration.

Tuberculosis is another prevalent co-infection among schistosomiasis endemic populations in LMICs affecting over 10 million people and causing significant immune-modulatory impact on infected hosts (134). In active TB infection, levels of IFN-γ, IL-1β, IL-18, are severely decreased and Treg numbers rise thus immune suppression ensues in the infected host. Consequently, a high FOXP3, TGF-β, and IL-4 and low IFN-γ, expression in active TB patients is associated with poor response to BCG vaccination (151, 152). However, an increased antibody response to several unrelated antigens such as measles and tetanus toxoid in active TB patients has also been reported (153).

Similar to HIV infection, active TB is a factor for vaccination deferral due to safety reasons. Nevertheless, further studies within and or outside vaccine trial studies may be important to ensure no interaction occurs between latent TB infection and schistosomiasis candidate vaccine response and efficacy.

The current COVID-19 pandemic has led to over 1.9 million deaths globally (154) inducing serious, potentially long-term immunological perturbations in the infected host that may influence vaccine responses in the same individual. The severity of the COVID-19 disease is associated with the hyper-inflammatory response, "cytokine storm", especially in the presence of co-morbidities such as diabetes and obesity which are characterised by elevated levels of pro-inflammatory IFN-γ,IL-1B,IL-12,IL-6,IL-27 and TNF cytokines (155). It has been suggested that the Th2 biased, T-reg, IL-10 mediated immune hyporesponsive and controlled inflammatory state in schistosomiasis infection (156) could counteract the damaging effects of COVID-19 disease and limit morbi-mortality (157). This theory could explain the relatively low severity and fatality rates of the COVID-19 in the helminth-endemic Africa (158, 159). However, there is currently no evidence to prove this COVID-19 and helminths interaction.

PZQ CO-ADMINISTRATION

PZQ, an acylated isoquionoline-pyrazine derivative, is the main drug used in the treatment of schistosomiasis and the basis of community-based mass drug administration (MDA) programs for the last 30 years (52, 160). PZQ, administered orally at dosages of 40-60mg.kg^{-1}, is efficacious against adult

worms with low toxicity and safe, even in pre-school age children and in third trimester pregnancy (161, 162). Although the mechanism of action of PZQ is not entirely understood, it is widely accepted that it disrupts the worm calcium homeostasis leading to worm muscle contraction and paralysis (160, 163, 164). The ensuing PZQ-induced tegument vacuolation and blebbing exposes the worm surface antigens, previously concealed in the intact live parasite to host's immune system, (**Figure 1**) (164). In vivo studies show extensive structural changes in the tegument, sub-tegumental and gastro-dermal musculature, causing leakage of tegumental cytoplasm from live worm in the first 15 minutes of PZQ treatment (165). Rodent studies have shown that PZQ-induced tegument disruption leads to exposure of a previously concealed native GST enzyme 90 minutes post PZQ treatment (166). Also in humans, drastic increases in cytokine release after PZQ treatment suggests that chemotherapy results into a pulse release of antigens (167) (**Figure 1**). This suggests that PZQ not only exposes tegument antigens but also cytosolic antigens and enzymes essential for parasite survival to the host immune system, enhancing further tegument damage and parasite killing.

As a consequence of antigenic unmasking, PZQ treatment may enhance antigen recognition of antigen-protein-isoforms that would otherwise be hidden (168). Post-treatment serum samples from *S. haematobium* patients have been shown to recognize a greater number of proteins compared to pre-treatment sera. More so, some proteins and their isoforms are recognized only in post-treatment serum samples (168). Noteworthy, the proteins that show enhanced recognition post treatment are generally those that are associated with parasite musculature and glycolytic metabolism (169), consistent with the disruptive mechanism of action of PZQ. This enhanced antigen recognition permits better neutralization by antibodies raised by vaccination (166, 170). Increased antigen recognition consequently enhances affinity maturation of immunoglobulin responses such as worm specific IgE. For instance, high IgE titres following PZQ chemotherapy ensues from directed IgG affinity maturation induced by PZQ (169), an effect which may also be conferred to co-administered vaccines and moreover lead to a more sustained antibody response following vaccination (166). Besides, antibody titres IgG1, IgG2, IgG4, IgM against worm antigens and IgG2, IgE to egg antigens are boosted following PZQ administration (171).

On the other hand, antigenic competition as a result of PZQ-induced parasite killing could suppress response to the single vaccine antigens. This is because PZQ-induced parasite killing avails large amounts and or a variety of crude worm antigens into the blood stream, in excess of vaccine antigen load. This can

potentially overshadow vaccine response or elicit non-specific or undesirable immune response (172). In the phase 3 human trials of the r*Sh*28GST vaccine candidate, PZQ was administered before and after the initial vaccination as well as just before and after the booster vaccination. No significant delay in schistosomiasis recurrence was seen in the vaccine group compared to the control group despite vaccine induced immunity (19). Although this may be attributed mainly to the failure to induce the desired IgG3 and IgA protective antibody response but rather IgG4 responses, interference by the repeated PZQ treatment was also suggested to have, partly, contributed to the observed results (19). Previous studies have demonstrated PZQ-induced modulation of cellular cytokine response to GST (24, 25). Likewise, more subjects exhibit an increased Sh28GST specific cytokine with a shift towards a more pro-inflammatory phenotype following PZQ treatment than before treatment (24, 25). However, a definite conclusion on the role of the PZQ treatment in the outcome of this trial cannot be made. Preferably, next studies should include a control arm whereby the timing of PZQ treatment is shifted several weeks before or after the vaccine administration.

Indeed, PZQ treatment markedly alters both the polarization and magnitude of schistosome specific cytokine responses with longer-term immunological impact than just transient clearance of infection. In the human host, Th2 biased response in form of increased parasite-specific IgE, eosinophil numbers, soluble-high and low affinity IgE receptors on eosinophils and CD23+B cells following PZQ administration is observed (24, 173, 174), which has been related to resistance to reinfection (174-176). In addition, PZQ chemotherapy alleviates the immune suppression induced by schistosomes by downregulating the immune dampening T-reg, Th17 cell numbers and IL-10 cytokine levels (24, 25, 177, 178). Clearance of infection following PZQ administration results in increased effector T cell frequency, increased Schistosome-specific cytokine response and decreased levels of T reg. This contrasts with the immune suppressive profile during infection such as antigen specific hypo-responsiveness, T cell memory pool distortion and increased CD4+CD25+FOXP3+T cell numbers (179). Consequently, in a randomised trial in Entebbe, Uganda, PZQ treatment significantly, although transiently, improved vaccine response to measles immunisation in *S. mansoni* infected pre-school children (171). Noteworthy, this effect seems greatest when PZQ is administered a few weeks before immunisation (171).

In conclusion, although PZQ has been deemed to be non-immunogenic in itself and thus have no impact on vaccine efficacy, PZQ-induced antigenic release

elicits both transient and long term immune modulatory effects (24, 167, 177, 180) (**Figure 1**) which in turn, potentially augment or mask vaccine responses. This is particularly important given that future schistosome vaccine trials in endemic settings target adult subjects, whose immunological and exposure profile has been shaped by repeated exposures and or PZQ treatment. As such PZQ-vaccine co-delivery could either be a very effective, more wholistic strategy to enhance vaccine response and efficacy or be detrimental to detecting vaccine efficacy in late-stage clinical trials. Therefore, different vaccine schedules with PZQ chemotherapy needs to be assessed in order to select the most appropriate vaccine-PZQ combination and immunisation-chemotherapy treatment schedule before embarking on such strategies in phase 3 studies.

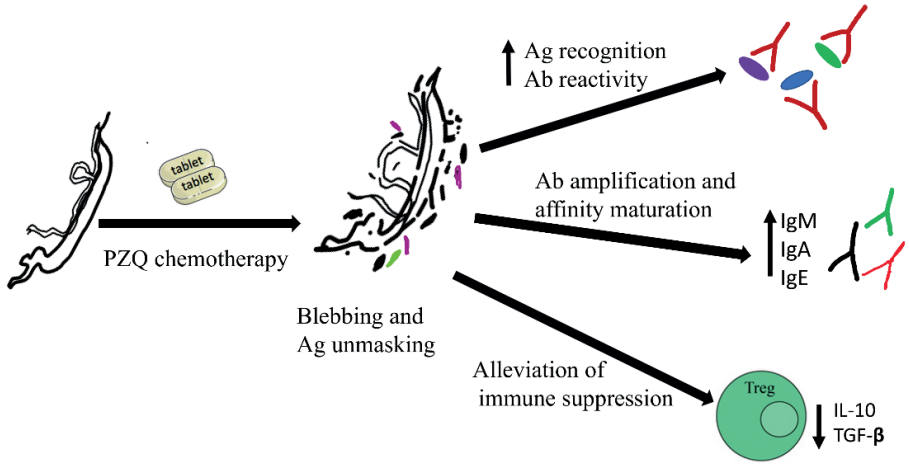

Figure 1. Schematic summary of PZQ-induced immune-modulatory responses that potentially impact vaccine response upon co-administration/delivery. PZQ acts on parasite tegument causing vacuolation and blebbing. This unmasks worm antigens and elicits an increased antigen recognition, antibody reactivity, amplification and affinity maturation, and alleviates the immune suppression induced by live parasites.

OUTLOOK

Given the potentially complex interaction of the above mentioned factors within the target population (44), the appropriate selection of trial subjects in which confounders have been taken into account appropriately, has become a true challenge to vaccine development and testing. Recognising the fact that

large phase 3 efficacy trials will be performed in a randomised fashion, most likely correcting for potential unknown or unidentified confounders within the trial, understanding or recognising the potential of these factors will ultimately be important for extrapolation of trial results and implementation of any schistosome vaccine across sub-Sahara Africa. Moreover, the phase 3 design will have to take into account the treatment of co-infections in the context of already existing MDA programmes.

Therefore, we argue that considerable investment should be made during early phase clinical testing in an attempt to understand and disentangle these confounders. As a start, thorough screening of subjects for co-infections, chronic diseases and physiological disorders should be done in order to identify confounders in early-stage clinical trials, and enable appropriate selection of trial populations with sufficient heterogeneity at a later stage. The inclusion of several trial arms with different population subsets (e.g. high vs low pre-exposure) is helpful to unravel the interaction between co-infections and vaccine responses. For treatable co-infections, the effects of pre-treatment such as PZQ, artemisinin and or albendazole administration at the same time or prior to vaccination should be carefully incorporated into trial designs especially from phase II trial stage. Additionally, timing of treatment and vaccine administration maybe a crucial determining factor for PZQ masking of or synergy with vaccine response and efficacy. Understanding the interplay between drug administration and vaccine responses will prove to be essential for implementation of novel vaccines in MDA settings. In the case of co-infections such as HIV/AIDS (which can be treated but not eliminated), testing and exclusion of sero-positive volunteers is the current practice in phase 1 trials. However, including seropositive individuals in phase II and III trials should be promoted to ensure population-wide implementation of the vaccine after registration.

In larger, late-stage stage clinical trials, the predominantly age dependent distribution of infection intensity and development of protective immunity among endemic populations (47) supports the need for careful selection of analysis methods and tools for handling trial data. Implementing approaches such as pre-defined sub-group analysis, hierarchical clustering analysis and stratified randomization with regards to age, and minimal and intense prior exposure in trials is likely to be useful. This would help to explicitly identify the immunologically distinct heterogenous groups and give useful insights into differential vaccine efficacy.

Controlled Human infection (CHI) models present an opportunity to not only disentangle the different parameters at play in endemic settings, but also accelerate the vaccine development pipeline in general. In the implementation of the CHI model, healthy volunteers are intentionally infected with a pathogen with the aim of generating knowledge on natural history of a disease, testing vaccines or therapeutics, and developing reliable and defined models of infection for future studies (181). These studies are controlled in terms of dose and route, production and selection of the pathogen strain administered. Additionally, a "controlled" condition/environment is key to prevent natural infection during the study period (181). Therefore, signs and symptoms of disease, and evolution of responses following a defined timing of the exposure can be observed in a well described and managed manner. This makes it possible to study the natural history of infection, host-pathogen interactions, evolution of immune responses and provide a preliminary assessment of vaccine efficacy (182, 183). The CHI models are also proof of concept studies and furthermore enable identification of correlates of protection. This approach allows for testing and early selection of promising vaccine candidates in a smaller group of healthy volunteers (less than 100), within a shorter period of time under controlled conditions compared to the classical vaccine testing methods (184). Hence, CHI studies reduce the burdening cost and time requirements, and the high downstream vaccine efficacy failure, associated with classical clinical trials approach. Noteworthy, for safety and ethical concerns, the CHI approach is applicable only for infections/diseases that are self-resolving and or are treatable such as schistosomiasis (181, 182).

The recent development of a controlled Human Infection model for Schistosomiasis (CHI-S), utilised single-sex cercariae for infection of healthy Dutch volunteers (183). This approach prevents the risk of egg-related pathologies as seen in the "natural" mixed-sex schistosome infections, while preserving the ability to cause infection and mature into adult schistosomes in human subjects (185). The highly sensitive diagnostic test, Circulating Anodic Antigen (CAA) test is used to detect infection that usually occurs between 6-12 weeks after the CHI procedure (183). Subjects are then treated with PZQ at 8 or 12 weeks post exposure to ensure clearance of infection (183). The first CHI-S trial demonstrates that the male-only CHI-S is safe and tolerable among naïve Dutch volunteers with about 82% infection rate, and absence of schistosome egg production (183). Despite obvious limitations of the single-sex nature of the CHI-S model, it can nevertheless be used as a screening tool for future schistosomiasis vaccine candidates and new drugs. More importantly, the implementation of the CHI-S model in endemic settings provides a unique

opportunity to disentangle the aforementioned distinctive immune responses and potential natural resistance, to *Schistosoma* infection and vaccines among endemic populations (186). This would not only accelerate vaccine development and testing but also help unravel the heterogeneity in vaccine immunogenicity, safety and efficacy.

CONCLUSION

The urgent need for a vaccine to eliminate the burden of the schistosomiasis in LMICs calls for acceleration in the testing and subsequent approval of the current vaccine candidates. However, endemic populations exhibit a multiplicity of distinctive disease transmission, exposure, pathogenesis, genetic and environmental factors that result in immunological challenges. These potentially impact vaccine responses and efficacy and amplify the chances of vaccine failures. Therefore, significant consideration has to be conceded to these immunological challenges in order to accelerate vaccine development and testing. Studies to investigate and accurately establish the impact and extent to which these immunological challenges affect host immune and vaccine response should become a fundamental facet of baseline and or preparatory studies as well the actual trial protocols for vaccine testing in endemic settings.

Author Contributions:

Conceptualization: MR, AME, JPK, ED. Writing (original draft preparation): ED, JPK. Writing (review and editing): MR, AME, JPK, AAS, MY, SC. Supervision: MR, AME. All authors contributed to the article and approved the submitted version.

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Table 1. Summary of the immunological considerations for schistosome vaccine development.

	Immune-Modulation	Key References	Vaccine Response	Key References
1. Prior/Current Schistosome Exposure				
a. In *Utero* exposure	B and T cell sensitization	Malhotra et al, 1997	↓ BCG, *Pf* Sporozoite Vaccine	Zimmerman et al, 2019
b. Early childhood exposure	Specific cord blood IgG, IgE	Dauby et al, 2012	No effect on r28GST response	Sissoko et al, 2017
				Jongo et al, 2018
c. Age-linked cumulative exposure	IgG2 and IgM	Attallah et al, 2003	↑ r*Sm*14 vaccine	
	↓ IgE			Riveau et al, 2018
	↑ Adult worm specific-IgE, IgM	Gryseels et al, 2006		
	↑ Antigen recognition			Brito et al, 2000
	↓ Egg-specific IgG1, IgG2	Mutapi et al, 2008		
	Age-dependent acquired immunity	Rujeni et al, 2013		

	Immune-Modulation	Key References	Vaccine Response	Key References
2. Difference in baseline immune-profile/micro-environment	Activated immune micro-environment in African cohort ↑ Exhausted/activated NK cells ↑ Differentiated B and T cells ↑ Pro-inflammatory monocytes Genetic differences; cytokine and HLA gene polymorphisms • $CD11c^{+}$ IL-10+ B cell expression in Indonesians • Atypical FcRL4+ memory B cell in Malian subjects • High *STAT6* and *IL10RA* gene polymorphism among Congolese subjects ↑ Activated, memory pool Th2 polarisation in rural populations	Muyanja et al, 2014 De Ruiter et al, 2020 Weiss et al, 2009 Koukouikila-Koussounda et al, 2013 Mbow et al, 2014 De Ruiter et al, 2020	↓ Yellow fever, HIV, BCG, MVA85A TB vaccines in African cohorts ↓ HIV Ad26.EnvA.01 and Ad35-EnvA candidate vaccines in African cohort Measles vaccine heterogeneity due to *CD46* and *SLAM* gene polymorphism ↓ Tetanus toxoid, Mtb PPD in rural subjects	Muyanja et al, 2014 Black et al, 2002 Lalor et al, 2011 Gilmour et al, 2013 Pathan et al, 2012 Gilmour et al, 2013 Haralambieva et al, 2013 Kabagenyi et al, 2020

	Immune-Modulation	Key References	Vaccine Response	Key References
3. Co-infections				
a. Malaria	Primary infection malaria ↑ CXCR+ T follicular (T*fh*) ↑ IFN-γ producing Vγ9+Vδ2+ T cells Previously unexposed persons ↑ IL-1β, IL-6, IL-8, IL-12, IFN-γ, TNF Asymptomatic malaria ↑ IL-10, TGF-β, CD4+Foxp3- Th1 cells, Tregs ↓ TNF, IFN-γ Repeated and persistent infection ↑ Atypical Memory B cells	Moormann et al, 2019 Collins et al, 1999 Tran et al, 2020 Perez et al, 202	↓ Tetanus-diphtheria, meningococcal, salmonella, *Haemophilus influenza,* pertussis and meningococcal vaccines ↑ Measles vaccine upon malaria treatment ↑ Malaria RTS,S and r*Pf* circumsporozoite candidate Vaccine	Rosen et al, 2005 Usen et al, 2000 Williamson et al, 1978 Banga et al, 2015
b. Cyto-megalovirus (CMV)	↑ CMV-specific CD8+ T cells ↑ IFN-γ and MIP-1β production Memory "Inflation" ↓ T cell co-stimulatory receptor expression ↑ Senescent CD57+KLG1+ T cellS	Miles et al, 2008 Portevin et al, 2015 Karrer et al, 2003	↓ *Influenza* vaccine in older adults ↑ *Influenza* vaccine in young adults ↓ Ebola candidate vaccines, ChAd3 and MVA	Furman et al, 2015 Trzonkowski et al, 2003 Bowyer et al, 2020

	Immune-Modulation	Key References	Vaccine Response	Key References
c. Soil trans-mitted Helminth (STHs)	↓ Th1 response	Padmini and Gause, 2012	↓ BCG, Cholera, Tetanus vaccines	Wammes et al, 2010
	↑ IL-4, IL-5, IL-13, IgE	Colombo and Grencis, 2020	No effect on previous measles and tetanus vaccinations	Elias et al, 2008 Cooper et al, 2001 and 1999
	↑ Treg, IL-10 and TGF- β	Gaze et al, 2012		Storey et al, 2017
d. Human Immuno-deficiency Virus (HIV)	↑ T cell activation, exhaustion and death	Elfaki, 2014	↓ Hepatitis B, Influenza, measles vaccines	Vigano et al, 2008,mNair et al, 2009,
	↓ Antigen presentation	Boaso et al, 2019	↑ Influenza, PPV23 and PCV pneumococcal vaccine following cART	Kim et al, 2009, Haban et al, 2017, Evison et al,2009, Teshale et al, 2008, Rodriguez-Barradas et al, 2008
	↓ T cell number and function	Kemeis et al, 2014		
	↑ Antibody decay			
e. Tuberculosis (TB)	↓ IFN-y, IL-1β, IL-18 in Active TB	Teri et al, 2017)	↑ Tetanus toxoid, other antigens	Kimuda et al, 2020
	↑ T reg in Active TB	de Martino et al, 2019		
	↑ Th1 in prior BCG vaccination	Biraro et al, 2014		

	Immune-Modulation	Key References	Vaccine Response	Key References
PZQ co-administration	↑ Antigen unmasking, recognition and Antibody neutralization ↑ Antibody maturation, isotype switching and Affinity maturation ↑ Specific IgE, IgA, IgG4, IgG2 and eosinophils ↑ Effector T cell frequency ↑ Th2 IFN-y, IL-5, IL-4 and IL-13 ↓ Tregs, Th17, IL-10	Mutapi et al, 2005 Fitzsimmons et al, 2004 Oliveira et al, 2012 Joseph et al, 2004 Scot et al, 2000 Lucja et al, 2020	↑ Sm28GST and measles vaccine, ↓ rSh28GST in phase 3 trials	Bourke et al, 2014 Tweyongyere et al, 2010 Riveau et al, 2018

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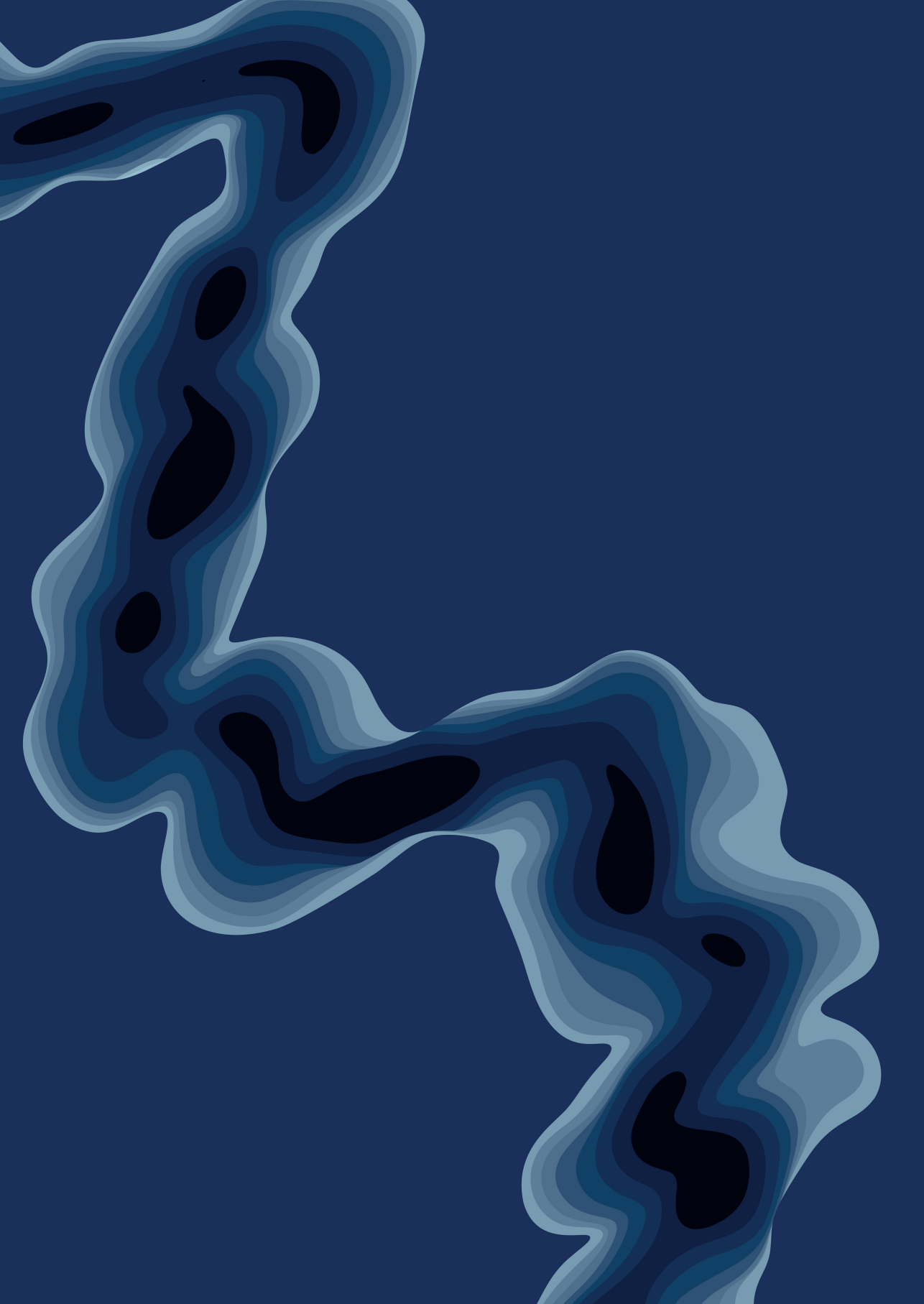

Chapter 8

Methodological considerations for future *Schistosoma* vaccine studies

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BACKGROUND

Schistosomiasis, a parasitic infection with blood flukes of the genus *Schistosoma*, remains highly prevalent with over 150 million infections worldwide (1), despite extensive praziquantel treatment in mass drug administration (MDA) programs of at-risk populations. Praziquantel treatment has significantly contributed to schistosomiasis morbidity reduction, but has its limitations: it is ineffective against juvenile worms and does not prevent reinfection. Moreover, reliance on a single drug raises concerns of drug resistance. A schistosomiasis vaccine is expected to help meet the WHO disease control targets more rapidly, according to modelling studies (2). However, a serious challenge to schistosomiasis vaccine development is the limited available research funding, as for all neglected tropical diseases. Four vaccine candidates are currently in clinical testing, which have been extensively reviewed elsewhere (3), and some of these will soon be evaluated for efficacy. These pivotal studies are costly and require careful planning, not least because conventional vaccine studies incompletely address issues such as praziquantel pre-treatment or do not capture effects on transmission. There is no immunological correlate for protection in schistosomiasis and studies therefore will rely on clinical or infection endpoints. However, whereas the efficacy of vaccines is often measured in relation to the incidence of disease (i.e. clinical endpoint) (4), this is not possible for schistosomiasis, because of the long lag time spanning multiple years between exposure to schistosomes and (chronic) disease manifestations. Consequently, trial endpoints will be focussed primarily on infection incidence. There are several ways to diagnose infection, including egg-based microscopy, antibody-based tests, antigen-based tests, and molecular techniques (5), each with advantages and limitations. For example, egg-based microscopy, currently the gold standard, lacks sensitivity in low infection intensity or prevalence settings. Vaccine efficacy estimates are determined by choices in diagnostic methods and effect measures, and therefore warrant careful consideration. Prior exposure to schistosomes, coinfections, and praziquantel treatment are each likely to affect vaccine immunogenicity and efficacy(6), however the direction and magnitude of these effects are still unclear. As such, heterogeneity in vaccine responses is expected to result from these complex immunological interactions within individuals, and should be considered in schistosomiasis vaccine efficacy studies. Here, we elaborate on the aspects raised above and provide suggestions for design of future studies.

CHOOSING THE MOST SUITABLE SCHISTOSOMIASIS DIAGNOSTIC FOR VACCINE STUDIES

As briefly introduced, microscopic egg detection is widely used for schistosomiasis diagnosis in endemic settings. However, sensitivity of microscopic egg detection in low-intensity infections is suboptimal, risking overestimation of vaccine effects when vaccinated are wrongly classified as protected. PCR detection of eggs has higher sensitivity than microscopy and PCR Ct values have shown good correlation with egg output, making it possible to more reliably assess vaccine effects in low-intensity infections (7). Alternatively, infection can be diagnosed with detection of worm-excreted circulating anodic (CAA) antigen levels in serum. This test has high sensitivity, even in low infection intensity settings, and is a surrogate for worm burden (8).

A schistosomiasis vaccine can have different (biological) mechanisms of action. A schistosomiasis vaccine may kill already present or incoming worms (reduce worm burden) and/or interfere with worm fertility (reduce number of eggs produced or reduce viability of excreted eggs). These effects are also referred to as anti-worm and anti-fecundity effects, respectively. Because egg production is responsible for most severe disease and facilitates onward transmission, egg-based diagnostics are an important potential measure of vaccine effects on transmission. However, it should be noted it only indirectly provides information about the anti-worm effect, which might be the primary mechanism of action of a vaccine. For those vaccines where direct anti-worm effects are expected, we therefore recommend antigen detection as to directly measure the mechanism of action. Ideally for vaccines that are expected to have more than one effect, a combination of diagnostics as co-primary outcomes should be employed to enhance our understanding of the potential impact of vaccines on schistosomiasis infection incidence, transmission and potential burden of disease.

CHOOSING RELEVANT EFFECT MEASURES TO DERIVE VACCINE EFFICACY

Another key component of the vaccine efficacy estimate is the effect measure used (9). Effect measures can be used to 1) compare the incidence of infection after immunisation in the vaccinated and unvaccinated group [risk ratio or rate ratio], 2) compare the average time to recurrent infection between groups [hazard ratio], or 3) compare the (geometric) mean egg output or CAA levels between groups. A phase III rSh28GST schistosomiasis vaccine study in a

highly endemic setting (prevalence 60%) in Senegal used time-to-recurrence as primary outcome, defined as having haematuria in combination with egg detection in urine (10). Notably, infection was assessed routinely only from 82 weeks after administration of the first vaccination (30 weeks after one year booster), by which time 41% of individuals (104 of 249) had recurrent infection. The main finding was that there was no statistically significant difference in time-to-recurrence based on log-rank test, however no hazards ratio and consequently vaccine efficacy was calculated. In some cases, it is possible to look at different pre-defined endpoints and effect measures within one trial, as with the RTS,S malaria vaccine, which used risk ratios for severe malaria, rate ratios for all episodes of clinical malaria, and hazard ratios for first episodes of clinical malaria (11). This is also an attractive approach for schistosomiasis. Through regular active case detection and treatment of cases, for instance every three months, the effect on total number of schistosomiasis episodes can be evaluated (Fig 1A).

Prior-exposure, age, and coinfections are all thought to affect vaccine responses. We will only be able to gain understanding into this interplay if future studies incorporate these within their designs: data on coinfections in individuals can be collected and be explored in pre-defined subgroup analyses, while stratified randomisation may be considered with regards to prior exposure level and age group. These however require a significant increase in study participants to provide sufficient statistical power, incurring further study costs.

USE OF LESS CONVENTIONAL STUDY DESIGNS

As an alternative or addition to more classical phase II clinical trials, novel tools allow for more disruptive trial designs with the potential to inform on vaccine effects. An important development in this space is the recent establishment of a controlled human infection model for schistosomes (CHI-S). In these CHI-S studies, healthy participants are exposed to a low dose of only male cercariae, to avoid egg-associated risks, and followed-up intensively to measure infection by CAA detection. CHI-S studies only require a few participants because everyone is exposed at the same time, and they can be completed relatively quickly (~ one year). Controlled human infection studies are therefore increasingly used as a tool to obtain an early estimate of vaccine efficacy for many different pathogens. Although there are limitations to the model, for instance that it can only assess effects on worm burden, it can help us better understand key issues in schistosomiasis vaccines, particularly when the model

has successfully been set-up in Uganda, where schistosomiasis is endemic (12). It will allow for the exploration of vaccine effects in populations with different levels of prior exposure and coinfections . Moreover, using for instance 2x2 factorial design in which participants are randomised to 1) PZQ treatment and 2) vaccination (**Figure 1B**), the influence of PZQ-codelivery can be investigated before moving to larger field studies.

Up until now, we only considered measuring vaccine effects in studies that individually randomise participants to either vaccine or control arm, which will give an estimate of the direct effect: immunity conferred to vaccinated individuals by vaccination. However, if a vaccine reduces egg output, transmission may also decrease. Hence, future vaccine studies should ideally also evaluate this indirect effect (i.e. reduction in incidence resulting from lower force of infection in community), which can be evaluated in cluster-randomised studies (13). In these studies, a cluster usually consisting of all eligible study participants on school, village, or district level, are randomised to either the vaccine or control arm. It requires a larger sample size that is determined by the number and size of clusters. Previously, Ross et al. performed a cluster-randomised study to investigate the effect of bovine vaccination in combination with other interventions on human schistosomiasis (14). They included 18 pairs of villages and used a 2x3 factorial design consisting of a first randomisation step determining vaccination (or not) of bovines and a second randomisation step for additional intervention (human PZQ prophylaxis, molluscicides, or no additional control measure). In this specific example, decreases in infection incidence in groups that did not receive human PZQ can be ascribed to decreased transmission. As reduction in transmission is an important vaccine goal, we recommend exploring cluster-randomised design studies. Moreover, these studies can incorporate OneHealth elements, such as monitoring of snails, the intermediate hosts, and environmental DNA, a tool for detecting cercariae in freshwater sample (15), allowing us to better understand how *Schistosoma* vaccines impact the epidemiology of schistosomiasis (**Figure 1C**).

CONCLUSION

With several promising schistosomiasis vaccine candidates in early stages of clinical testing, the discussion around the optimal design for a vaccine efficacy study becomes consequential. This includes how infection is measured and how the trial endpoint is defined. Conventional study designs do not appropriately address questions of disease transmission, the influence of PZQ

treatment, coinfections and prior-exposure, and we have therefore suggested alternative study designs, such as a factorial CHI-S study to study effects of PZQ co-delivery and a cluster-randomised study to study transmission. These will hopefully increase the chance of successful licensure and roll-out of a schistosomiasis vaccine in the future.

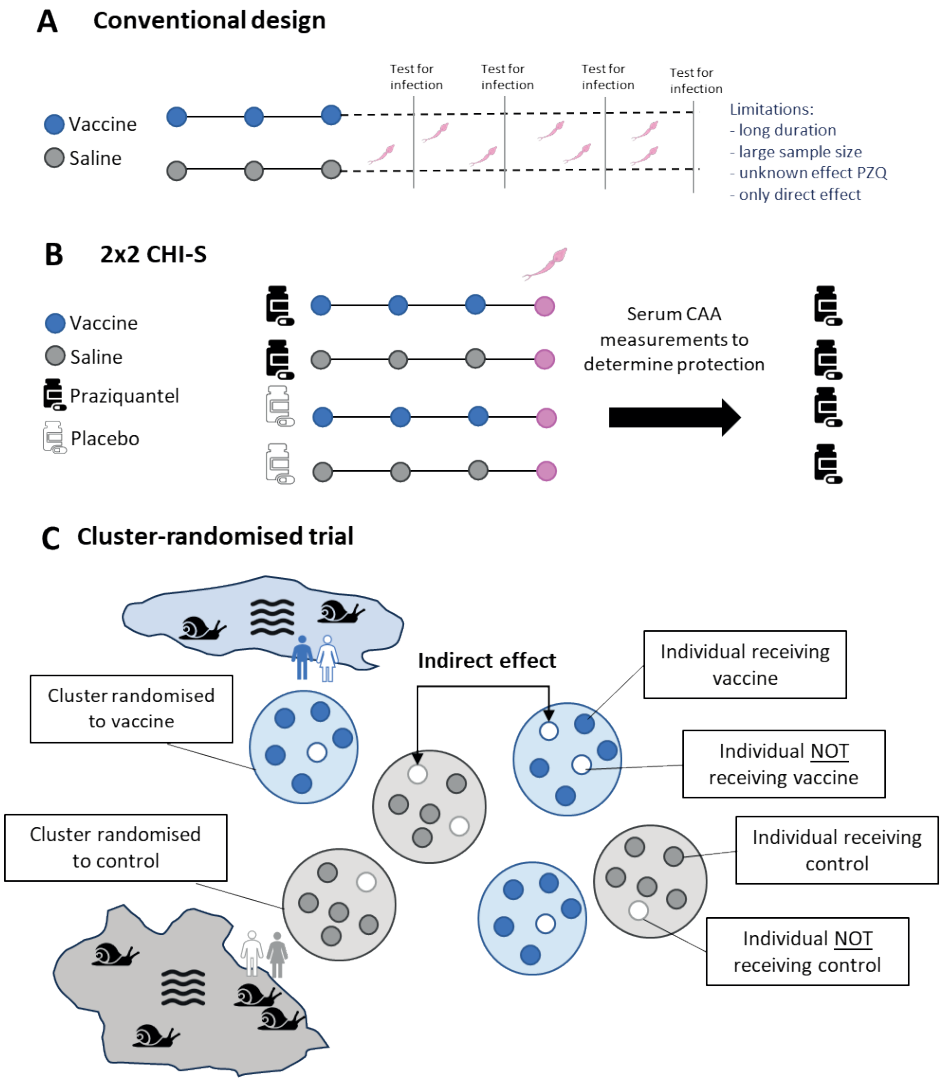

Figure 1. Different study designs for future schistosomiasis vaccine studies.

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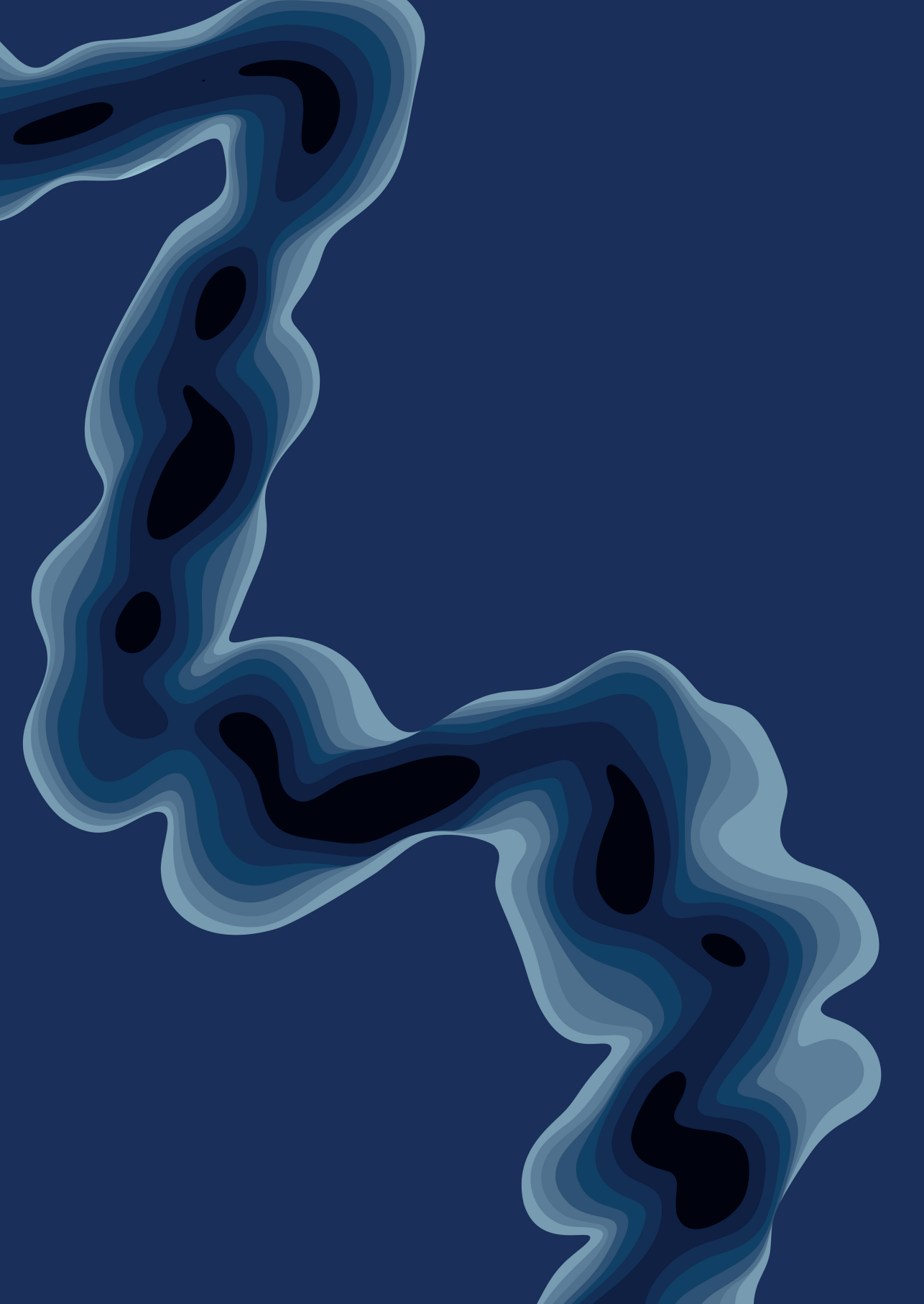

Chapter 9

Summarising discussion and conclusion

To this day, schistosomiasis remains a major cause of morbidity in large parts of the world, particularly in Sub-Sahara Africa (1), despite frequent mass drug administration with praziquantel; water, sanitation and hygiene interventions(2); and health education(3). The development of an efficacious vaccine should be a top research priority to limit further suffering, but the road to eventual vaccine licensure is long and time-consuming, as vaccines against helminths are challenging. In its co-evolution with humans, *Schistosoma* species, much like other parasites, have developed ingenious mechanisms to evade human immune responses. Within the human host, schistosomes undergo critical developmental stages while transiting through skin, blood, and lungs. Yet despite all these developmental changes, the parasite manages to escape immune attack at each life cycle stage (4, 5). Eventually, immunity against schistosomes does develop in chronically-exposed individuals, albeit slow: the prevalence and/or infection intensity decreases with age (6), independent of water-contact (7). In addition to these epidemiological findings, other research has focussed on understanding how some animals, for instance rhesus macaques, are able to self-cure, hoping to find new vaccine targets (8). Perhaps the most promising data in support of vaccination, are immunisation studies with irradiated cercariae in several animal models showing up to 80% reduction in worm burden upon cercarial challenge (9). Together, these epidemiological and animal-model studies have led to the identification of several promising vaccine targets, for which vaccine candidates have been developed. A handful of these schistosomiasis vaccine candidates have now advanced to clinical testing (10), but progress has been slow in part due to a lack of commercial interest.

To speed up schistosomiasis vaccine development, we have established a controlled human infection model (CHIM) for *Schistosoma mansoni*. Controlled human infections with schistosomes (CHI-S) can be used to evaluate vaccine or drug candidates, understand pathogenesis or immune responses, and study infection-derived immunity (11), jointly contributing to vaccine development. Particularly, our understanding of early stages of infection in humans is limited, because timing of exposure is often unknown and infection is only detected at a later, egg-producing stage. In this thesis, we report on the three CHI-S using *Schistosoma mansoni (Sm)* performed to date (**chapter 2, 3, and 5**). In the next paragraphs, we will integrate the findings of the these CHI-S studies and discuss what we have learned so far.

SYMPTOMS AFTER CHI-S

In all controlled human infection studies, safety of participants is paramount and for that reason measures are taken to limit harm (11-13). Given that many of the pathogens for which CHIMs have been developed, have affected human populations for many years, we generally have good knowledge of the symptoms they may cause. For schistosomes, these are: 1) skin reactions immediately after exposure; 2) acute schistosomiasis, a flu-like syndrome; and 3) chronic (egg-producing) schistosomiasis (6, 14). Individual host and/or pathogen factors are believed to influence the risk of severe complications in chronic (egg-producing) schistosomiasis, but the exact mechanisms are not entirely clear. Our single-sex (egg-free) approach, exposing participants to only male or only female cercariae, of the CHI-S model mitigates this risk and is a novelty in the CHIM field. It should be noted, however, that this adaptation does make the CHI-S less comparable to natural infection, and the harm to individuals exposed to a very low number of eggs, resulting from a low cercarial dose and prompt treatment, is likely minimal.

Perhaps the least studied clinical presentation is acute schistosomiasis (AS), previously also called Katayama syndrome, as this is often only recognised in returning travellers (14). AS is a self-limiting illness, that has been attributed to systemic inflammatory responses to the migrating schistosomula and egg deposition. The presentation of AS can be varied, but often includes fever, headache, cough, and urticaria, and a combination thereof. It may also manifest as abdominal pain, diarrhoea, myalgias and neck pain. Estimates on the risk of AS differ substantially between studies (15) and may depend on infectious dose, *Schistosoma* species, duration of water contact and number of exposures. These factors are all effectively controlled for in CHI-S, which provides the opportunity to better understand the development of AS.

Previously, many have attributed AS to onset of egg production, but our findings in single-sex studies clearly show that AS is not limited to egg-producing infection. Moreover, In the small group of participants in the reinfection study who could have potentially developed a mixed-sex, egg-producing infection, we did not see an increase or exacerbation of AS symptoms. Increases in symptoms suggestive of AS were observed three weeks after exposure to male or female cercariae. AS is characterised by diverse symptoms that each by themselves are non-specific and have a high incidence in the general population, making diagnosis challenging. In our initial dose-finding, around half of participants exposed to 20 male or 20 female cercariae developed moderate to severe

systemic symptoms suggestive of AS. This number may have been an overestimation as there was no control group in these studies, and there is considerable overlap between AS symptoms and other intermittent infectious illnesses. Based on the reinfection study that did include a control group, the risk of moderate-severe AS approaches 25-35% after primary exposure to 20 male cercariae. To alleviate symptoms, we have started treating severe AS with prednisolone for at least five days, before tapering off the dose. This strategy has been successful: participants experience improvement immediately after ingestion of the first dose.

Another important finding from the reinfection study is that clinical tolerance to AS symptoms already develops after the initial exposure, with fewer symptoms being reported with subsequent exposures. These results agree with what is commonly described in the field: people in endemic settings appear not to develop acute schistosomiasis except for *S. japonicum* infections, possibly because of early-life exposure to *Schistosoma* antigens (14).

In our studies, we also observe a maculopapular rash at the exposure site in 80% (44/54) of participants, after male or female cercarial exposure. This incidence seems higher than what previous studies have reported in travellers (risk 11-36%) (16), which may be because of recall bias in observational studies, or that the skin reaction has been overlooked as it is only present for a short period of time and is not always pruritic. Similar skin reactions are also reported in Europe after exposure to avian schistosomes in freshwater bodies. These *Schistosoma* species, such as *Trichobilharzia* spp, are not well adapted to the human host and are therefore believed to die in the skin only causing a local, allergic reaction instead of infection. Interestingly, sensitisation to avian schistosomes may lead to worsening of skin reactions upon re-exposure (17). This has also been seen in a controlled human hookworm study where albendazole treatment was given two weeks after exposure as an immunisation method (18) and is associated with protection to hookworm infection. However, repeated exposure did not lead to an increase or decrease in skin reactions in our schistosome reinfection study.

As discussed in **chapter 3,** four participants exposed to 20 female cercariae had a more long-term single sex infection, because of PZQ failure. These participants did not report any symptoms during follow-up, suggesting that more chronic, single-sex infection causes limited harm, in comparison to chronic mixed-sex infections.

INFECTION PARAMETERS AND VALIDITY OF THE CHI-S MODEL

Different diagnostic tools are available for schistosomiasis detection, with each its strengths and weaknesses. In CHI-S, participants have a known time and dose of exposure and are followed up and sampled frequently allowing us to study the kinetics of various diagnostic tests.

In the absence of eggs, we rely on the very sensitive, lab-based UCP-LF circulating anodic antigen (CAA) test to determine infection status for our primary outcome (19). This CAA is excreted by juvenile and adult worms indicating active infection. Although excretion patterns of CAA was thought to differ between single-sex male and single-sex female infections based on animal work and *in vitro* worm cultures (20), the probability of CAA detectable infection, defined as having at least one CAA value ≥1.0 pg/mL, did not differ substantially. Moreover, there is consistency in attack rates of 20 male cercariae between the initial dose-finding study and primary exposures in reinfection and infection controls in the reinfection study, supporting the validity of the male-only model in this *Schistosoma*-naïve population. This is important, because attack rates are used to calculate sample size for future studies and therefore directly impact statistical power. Moreover, unlike for other challenge agents, it is currently not possible to set up a master bank of cercariae (21); for each production we harvest fresh miracidia from hamsters that are used to sustain the parasite lifecycle which, some have argued, might introduce heterogeneity in infectivity.

The studies in this thesis have demonstrated the usefulness of CAA over antibody testing. This has even led to the incorporation of the CAA test in the clinical microbiology lab at LUMC, available to clinicians nationwide. We have convincingly shown that CAA can detect infection and treatment effect, without the downside of antibodies. This is particularly useful in returning travellers to discriminate between active worm infection vs. merely exposure to worms, evidenced by antibody seroconversion in CAA negative participants.

CHI-S have improved our understanding of antibody-based diagnostics. In response to CAA, highly sensitive and specific anti-CAA IgM and IgG antibodies can be detected three to four weeks post-exposure, which can be explored as a novel antibody based diagnostics (22). Antibodies against soluble egg antigen are also present in a subgroup of participants, even in male-only infections, suggesting cross-reactivity of cercarial and egg antigens. However, titres are lower than in (potential) mixed-sex infections as seen in our repeat infection study. Nonetheless, shared cercarial and egg antigens warrant further

examination as these could be ideal vaccine targets that could affect two crucial parasite life stages.

In our studies, we have used PCR on stool to exclude egg production after single-sex exposure as additional quality and safety check. The positive PCR test result led us to discover the accidental exposure to female cercariae in the reinfection study. This triggered a thorough investigation, in which all production-related data were checked and stored cercarial samples retested. Having incorporated strict procedures in line with good manufacturing principles (GMP) and back-up diagnostics allowed us to uncover the error, and demonstrates the value of having such measures in place.

DIFFERENCES IN PRAZIQUANTEL SUSCEPTIBILITY BY CERCARIAL SEX

As mentioned before, treatment of schistosomiasis relies on a single drug, praziquantel of which more than 250 million tablets are distributed yearly (23). Dependency on a single drug raises concerns of drug resistance, which is why recent studies have investigated alternative treatment options, such as artemisinin-based therapies and mefloquine (24-26). Cure rates after praziquantel are ~80% based on Kato-Katz, but are lower when more sensitive antigen tests are used (27). Earlier *in vitro* and animal studies have suggested that unpaired worms may be less susceptible to praziquantel (28), however in humans this has been difficult to corroborate. In our studies, we noticed the following: firstly, a single-dose of 40 kg/mg was not sufficient to clear male-only infections based on CAA values in a substantial number of participants. In order to achieve cure, a higher second dose of 60 mg/kg needed to be administered. This dose has now become the standard dose after CHI-S.

Next, female-only infections are less susceptible to praziquantel treatment, even at 60 mg/kg dose, given 8 and 12 weeks post-exposure or when given three days consecutively. Four (out six) participants had recurring CAA levels, after initial decreases, indicating persistence of the female worms. The underlying reason is not entirely clear, however some explanations have been suggested as discussed in **chapter 3** and include pharmacogenetic variations, immaturity of unpaired worms, location of worms, and intrinsic biological differences in worm biology. We speculate that if the decreased susceptibility of unpaired female worms holds true in the field, it may provide an explanation as to why after repeated treatment with praziquantel the infection intensity is only

temporarily reduced (27), but does not lead to clearance. Moreover, surviving female worms are able to pair with new incoming male worms, and lead to egg-producing infection, as seen in our reinfection study. Decreased treatment susceptibility unfortunately limits the future use of the female CHI-S model, but highlights an important, but overlooked limitation of praziquantel treatment. It further underscore the need for research into new treatment strategies, e.g. combination therapy, and novel therapeutics.

IMMUNE RESPONSES TO CHI-S

Immunology of schistosomiasis is complex and characterised by two distinct phases: the early infection stage and the chronic egg-producing stage (29). Most human data available is limited to the chronic egg-producing stage, as this is when diagnosis is made (30). However, with CHI-S we have now been able to investigate the early infection stage, which are an important target for vaccines. Some animal studies have previously suggested that immune responses to unpaired male or female cercariae differ (31), but we observe similar mixed immune responses consisting of Th1, Th2 and regulatory profiles four weeks post-exposure using flow cytometry. These findings were later confirmed using mass cytometry for high-dimensional profiling (32). Both studies also demonstrated increases in worm-specific IgG1, which has previously been associated with protection in animal studies with irradiated cercariae (9, 33), as well as in endemic populations (8). This raises the question if these responses can be protective. Although we observed boosting in the reinfection study, this did not lead to protection. Apart from the dose that is much higher in animal studies, the difference may be explained by the quality and specificity of the IgG response that may be shaped by the number of cumulative exposures, which is higher in endemic settings. In the reinfection study, we observed that participants accidentally exposed to male-female-male cercariae showed higher eosinophil CCL23, CCL4, and TNFα levels, as well as higher IgG antibody titres against soluble egg antigen, compared to single-sex exposed participants. These findings are consistent with initial responses to egg production in mice (34-36).

Table 1. Summary of CHI-S key findings

Key findings of CHI-S	Supporting data
Symptoms • AS occurs in the absence of eggs • Clinical tolerance to acute schistosomiasis rapidly develops after exposure	• AS is observed after male-only infection • AS symptoms decrease after first exposure in repeated infection study
Treatment • Female-only infections are less susceptible to praziquantel treatment	• Treatment failure after female-only infection
Immune responses • Immune responses to male-only and female-only cercariae are similar • Increases in worm-specific IgG1 responses are not protective • Egg-production is characterised by distinct immune responses	• Both show mixed Th1, Th2, and regulatory profiles • Absence of protection in repeated infections, despite boosting of IgG1 • Increase in eosinophils, CCL23, CCL4, TNFα, and SEA IgG in those exposed to male-female-male cercariae

AS = acute schistosomiasis, SEA= soluble egg antigen

ADAPTATIONS TO THE CHI-S MODEL

As outlined in the previous sections, we have gained many new insights into *Schistosoma* infections by performing these studies in quick succession (summarised in **Table 1**). Along the way, we have been making adjustments to our study procedures based on earlier findings to improve the model. Examples include the adaptive dose design in the female-only study or the higher PZQ dose for treatment in the female-only and reinfection study. Apart from these, there may be a few more modifications to consider for future studies.

With regard to the production of single-sex cercariae, there is need for an additional check to ensure cercariae are of the desired sex, as per protocol. This is achieved in two steps: 1) after the first shed, snails producing the “wrong” cercarial sex are immediately discarded; and 2) at a second shed, PCR on the tentatively selected snail is repeated to confirm the sex of the cercariae. The production of cercariae is a challenging, time-sensitive process with several variables that are difficult to control, such as the take rate of mono-miricidial infection i.e. probability of a snail become infected after exposure to a single miracidium, snail death, and the ratio of female to male secreting snails. Moreover, if this all goes well, the shelf-life of cercariae is only four hours after shedding. Mitigation of some of these risks would be a major step forward for CHI-S and may be brought about in the following ways: further optimisation of cercarial production processes and cryopreservation of cercariae. We collect

all production-related data in a dedicated database, that can be reviewed to examine particular steps in the production process and make the necessary alterations. The ability to freeze and store cercariae, as also done for other challenge inocula, would move the production process closer to GMP principles, where it is common practice to develop a well-characterised master bank (21). Additionally, it would remove the logistical challenge of producing cercariae for fixed-date study visits, as with the reinfection study. It also improves the scalability of CHI-S which, for logistical reasons, is in our setting capped at 24 people per cohort, as well as the transferability of the model.

On the clinical side, we would reduce the frequency of follow-up visits after PZQ treatment at the end of the study to once every four weeks, instead of every two weeks. We observed that CAA that can show a rebound after initial decreases in the first weeks, making the in-between visits of little clinical importance to decide whether to give additional treatment.

TRANSFER OF THE CHI-S MODEL TO ENDEMIC SETTINGS

The benefits of CHIM have been extensively discussed, but it should be noted that CHI studies are complex and require considerable expertise to navigate ethical and logistical challenges. This also applies to CHI-S. The studies described in **chapter 2, 3, and 5** were all conducted in *Schistosoma*-naïve participants in The Netherlands. It is important to note that people living in endemic settings may respond differently to CHI-S, because of prior exposure to schistosomes and PZQ treatment, coinfections and other environmental exposures. The same is to be expected for *Schistosoma* vaccine responses, as seen with Ebola, BCG, and malaria, among others (37). In light of vaccine development, it is therefore especially informative to perform CHI studies in endemic population, who are ultimately the target population for the vaccine. Experiences with controlled human malaria infections in for instance Tanzania and Kenya prove that CHI studies can be successfully performed in endemic settings and high-quality evidence can be obtained on vaccine hypo responsiveness and the influence of prior immunity on controlled human infection (38, 39). For schistosomiasis, we have been working closely together with the MRC/UVRI and LSHTM Uganda Research Unit to facilitate the transfer of the CHI-S model to Uganda, where schistosomiasis is highly endemic and causes considerable morbidity in affected communities. The team in Uganda has extensively engaged with stakeholders to discuss the ethical and scientific considerations surrounding a Ugandan CHI-S and identified key next steps to

move forward, which included the risk assessment in **chapter 4**. In parallel, the Ugandan team completed studies to engage with the target communities from which CHI-S participants might be recruited (40). Using in-depth key informant interviews and group discussions, they observed that communities were willing to take part in future CHI-S studies, but that enough time should be taken for the informed consent process to ensure study procedures, risk, and benefits are well understood. This study convincingly illustrates the value of qualitative research into CHI participation with findings that can be directly integrated in study processes and procedures. Together with the MRC/UVRI and LSHTM Uganda Research Unit, we plan on evaluating the Sm-p80 + GLA-SE vaccine in adults in a non-endemic (Dutch) and endemic (Ugandan) setting using CHI-S. The study protocol for the Leiden study can be found in **chapter 6,** which will also be used as a blue print for the Entebbe (Ugandan) study. Harmonisation of study procedures will enable us to make valid and meaningful comparisons between the study populations.

MAXIMISING SCIENTIFIC BENEFIT OF CHI-S

We focussed here on the main clinical findings of the CHI-S studies, which have already led to many new insights even before the model has been used to test a vaccine (**table 1**) through comparisons between 1) single-sex male vs single-sex female infections, 2) single vs. repeat infection, 3) and, unexpectedly, single-sex vs. mixed-sex potentially egg producing infection. Yet, there are many more insights to be gained from these trials. Because of intensive longitudinal sampling, the dynamics of particular (bio)markers or other outcomes can be assessed over time, as well as the effects of PZQ treatment. Moreover, because of the controlled nature of the studies, these trials are well-suited to explore host-pathogen interaction in the early stages of infection using for instance glycan and/or protein arrays and transcriptomics. While in the first study we performed sputum induction in three participants, that showed a mixed type-1/type-2 inflammatory cytokine profile compared to baseline (41), in the next studies we used nasosorption as a minimally invasive sampling procedure (42), that can be used to investigate pulmonary immune responses. Future exploratory analyses on the sample set is likely to increase the scientific contribution of these studies, but will depend on the following: extensive collaboration, data management and integration, statistical expertise, and data sharing.

To comprehensively understand *Schistosoma* infection, we need input from different research disciplines and involve clinicians, immunologists, biologists, and data specialists to approach it from multiple angles. Also collaboration across research institutes and across borders i.e. internationally, is crucial, especially for neglected tropical diseases, and must aim to build equitable partnerships. The integration of different datasets, for example clinical and immunological or immunological non-endemic and immunological endemic, is necessary to make informative comparisons and is therefore very important, however requires a thorough data management strategy for multi-omics data. The large number of potential outcome variables and repeated measurements make the statistical analyses challenging and requires specific statistical expertise. Lastly, to further increase scientific and societal value, the datasets should be made available to other researchers, in line with Open Science principles. All these fundamental new insights can feed back into vaccine development pipeline hopefully help identify new promising vaccine targets and/or strategies against schistosomiasis.

NEXT STEPS IN *SCHISTOSOMA* VACCINE DEVELOPMENT

Now that some vaccine candidates have shown good tolerability and immunogenicity, further studies in endemic populations are to be initiated. In **chapter 7**, we have discussed key immunological challenges that these vaccine candidates will likely encounter transitioning to phase II and III in endemic settings. In brief, prior exposure to schistosomes, prior or concurrent infections with other pathogens, and praziquantel co-delivery, can all potentially impact vaccine responses. However, whether these augment or antagonise vaccine efficacy is still unclear. Recognising these complexities is essential to designing future *Schistosoma* vaccine trials (**chapter 8**). To help understand these challenging immunological interactions, future studies should at least carefully screen participants for co-infections and previous exposure, and incorporate PZQ, artemisinin and/or albendazole administration at the same time or prior to vaccination. The study outcomes need to also be carefully defined, as there are multiple effect measures, e.g. risk ratio, hazards ratio, mean egg reduction, that can be used to calculate vaccine efficacy (43), but that each refer to different effects. Reducing egg excretion is an important goal of schistosomiasis vaccines, as this will result in reduced onward transmission. Traditional RCTs in which individuals are randomised to either vaccine or control arm, give only an estimate of the direct effect, immunity conferred to vaccinated individuals by vaccination, but do not evaluate indirect effect (i.e.

reduction in incidence resulting from lower force of infection in community). These indirect effects, however, can be evaluated in cluster-randomised studies, that allocate clusters consisting of all eligible study participants on school, village, or district level, are randomised to either the vaccine or control arm (44), and should be considered for future schistosomiasis vaccine studies. To understand how *Schistosoma* vaccines impact the epidemiology of schistosomiasis, it is worth considering adopting a OneHealth approach that includes monitoring of snails, the intermediate hosts, and environmental DNA, a tool for detecting cercariae in freshwater samples.

Also, optimisation of vaccine delivery should be continued. During the COVID-19 pandemic, we witnessed the expedited use of novel vaccine platforms with great success. The use of these platforms, such as mRNA vaccines, should be explored for *Schistosoma* vaccines as well, as currently the vaccine candidates are all subunit vaccines adjuvanted with either GLA-SE or Alhydrogel®.

Lastly, in light of increased reporting on vaccine hesitancy across many parts of the world (45), it is vital to start engaging with communities that either participate in vaccine studies or that will ultimately be provided vaccines upon successful licensure, well ahead of time. The successful roll-out of a vaccine depends strongly on context-specific factors, such as accessibility, timing, and perceived risks, requiring specialist knowledge. The importance of community acceptance and also political will, should not be underestimated. After all, an efficacious vaccine is only of limited value if the uptake is low.

CONCLUDING REMARKS

In this discussion, we have elaborated on the key findings from the CHI-S studies that in short time have contributed a great deal to our understanding of early-stage *Schistosoma* infection. Moreover the male-only CHI-S can now be used to evaluate vaccine candidates. To maximise the use of CHI-S, we have identified steps to optimise the model and enable transfer to endemic settings. Through extensive collaboration and data integration we will be able to explore *Schistosoma* infections in an unprecedented way, that will hopefully lead to fundamental new insights that can feed back into the vaccine development pipeline. We have summarised the challenges vaccine candidates may encounter transitioning to phase II and III studies and have suggested strategies to address these. Altogether, this work (**graphically summarised in figure 1**) hopefully enables us to expedite *Schistosoma* vaccine development,

culminating in successful licensure of an efficacious vaccine to help control this debilitating parasitic disease.

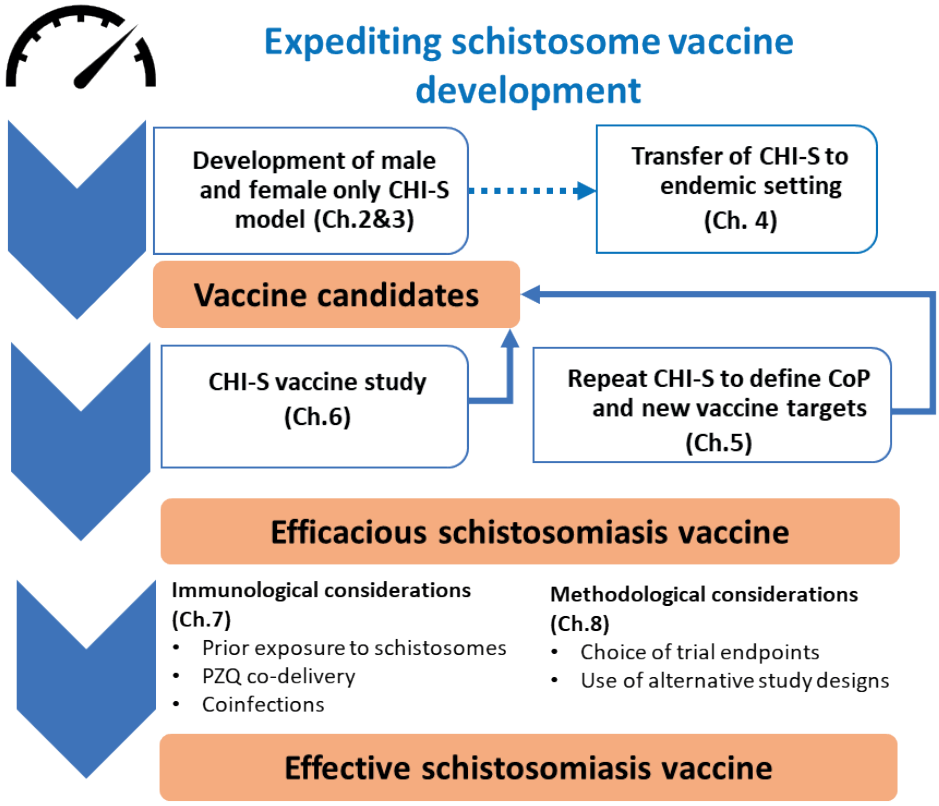

Figure 1. Contributions of this thesis to expediting schistosome vaccine development. CHI-S = controlled human infection with schistosomes; CoP = correlates of protection; PZQ = praziquantel; Ch = chapter.

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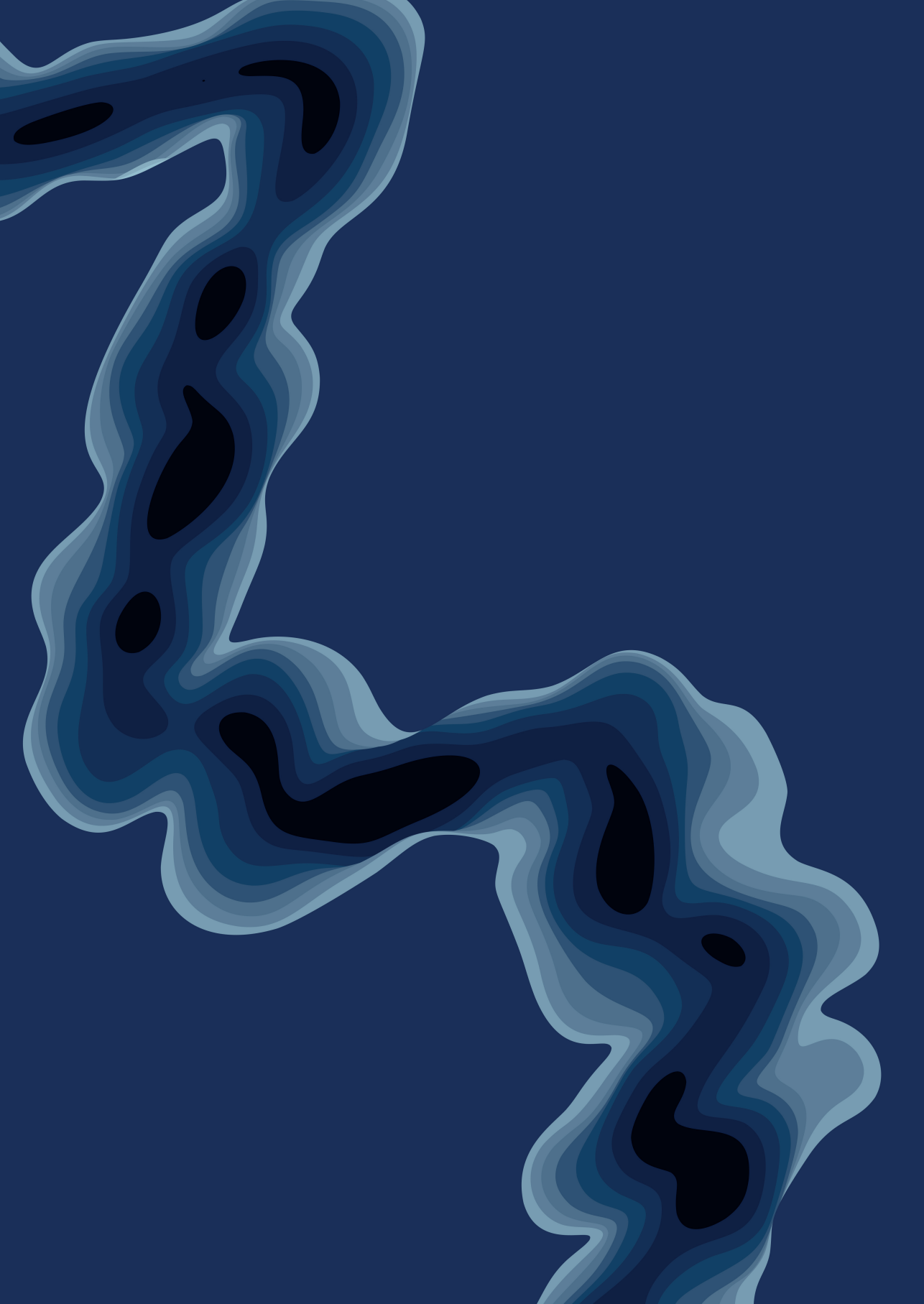

Appendices

Nederlandse samenvatting

Dankwoord (acknowledgements)

Curriculum Vitae

PhD Portfolio

List of publications

NEDERLANDSE SAMENVATTING

Schistosomiasis is een parasitaire infectie met *Schistosoma* wormen. Wereldwijd zijn ruim 150 miljoen mensen geïnfecteerd en schistosomiasis is dan ook één van de belangrijkste verwaarloosde tropische ziekten. Besmetting vindt plaats door contact met zoet water waarin de infectieuze larven, ofwel cercariën, leven. De cercariën kunnen via de huid het lichaam binnendringen en komen vervolgens via de longen terecht in de bloedvaten rond de darmen (voor *Schistosoma mansoni*) of in de urinewegen/genitaliën (*Schistosoma haematobium*). Hier worden wormparen gevormd door mannelijke en vrouwelijke wormen, welke eieren produceren die via ontlasting of urine worden uitgescheiden. De acute fase van de infectie presenteert zich als een lokale huiduitslag (cerariële dermatitis) en het acute schistosomiasis syndroom. Dit is een systemische afweerreactie met griepachtige symptomen. De chronische fase van de infectie levert echter de meeste gezondheidsproblemen op, doordat eieren vast komen te zitten in weefsel en daar ontstekingen veroorzaken. Schistosomiasis wordt op grote schaal behandeld met praziquantel, wat heeft geleid tot een afname in infecties. Een verdere afname lukt echter niet, omdat praziquantel niet werkt tegen onvolwassen wormen en de kans op herinfectie groot is. De Wereldgezondheidsorganisatie heeft als doel gesteld om vóór 2030 schistosomiasis uit te roeien als volksgezondheidsprobleem. Een vaccin kan daarbij helpen, maar op dit moment is er nog geen vaccin geregistreerd dat werkzaam is tegen schistosomiasis. Dit komt doordat vaccins tegen parasieten ingewikkeld zijn. Parasieten hebben allerlei slimme methodes ontwikkeld om te ontsnappen aan ons immuunsysteem. Ook is er slechts weinig onderzoeksgeld beschikbaar is voor de ontwikkeling van een vaccin tegen schistosomiasis, net als voor meer verwaarloosde tropische ziekten. Desondanks worden er op dit moment vier vaccinkandidaten getest in klinische studies. Om vaccinontwikkeling te versnellen, kunnen gecontroleerde humane infecties ingezet worden. Dit zijn experimentele infecties van gezonde deelnemers met een ziekteverwekker onder gecontroleerde omstandigheden. Doordat iedereen op een vast moment wordt blootgesteld, kan een vaccin in relatief korte tijd met weinig proefpersonen worden getest op werkzaamheid (effectiviteit). Voor een groot aantal infectieziekten worden deze gecontroleerde humane infectiemodellen reeds gebruikt en hebben zo een belangrijke bijdrage geleverd aan de ontwikkeling van vaccins. Ook hebben deze modellen geleid tot nieuwe inzichten in de pathofysiologie van infectieziekten. Een gecontroleerd infectiemodel voor schistosomiasis kan daarom van toegevoegde waarde zijn. In dit proefschrift beschrijven we de ontwikkeling van een gecontroleerd infectiemodel met schistosomen. Omdat eiproductie een gezondheidsrisico

met zich meebrengt voor studiedeelnemers, gebruikten we alleen mannelijke of vrouwelijke cercariën voor infectie. Hierbij werd de ultragevoelige circulerende anodische antigeen (CAA) test gebruikt om de infectie in bloed te detecteren. Na infectie werden deelnemers behandeld met praziquantel.

In **hoofdstuk 2** beschrijven we de allereerste gecontroleerde infectie met mannelijke *Schistosoma mansoni (Sm)* cercariën. We zagen dat blootstelling aan 20 cercariën leidt tot detecteerbare infectie in 82% (9/11) van de deelnemers en dat acute schistosomiasis frequent voorkomt, ook zonder eiproductie.

Nadat dit infectiemodel met mannelijke cercariën succesvol was opgezet, wilden we een soortgelijk model ontwikkelen voor vrouwelijke cercariën. Dit geeft de mogelijkheid om de gastheer-pathogeen interacties te vergelijken. Daarnaast kunnen vaccins dan ook in beide modellen getest worden. Dit kan relevant zijn, omdat in diermodellen één van de vaccinkandidaten (Sm-p80) beter lijkt te werken tegen vrouwelijke schistosomen.

In **hoofdstuk 3** laten we zien dat gecontroleerde infecties met vrouwelijke *Sm* cercariën leidde tot vergelijkbare klachten en immuunreacties als bij mannelijke infecties, maar dat deze infecties minder gevoelig waren voor praziquantel. Ondanks herhaalde behandeling met praziquantel en alternatieve behandeling met artemether-lumefantrine bleef infectie detecteerbaar in vier (uit 13) deelnemers. Drie daarvan hebben de infectie spontaan geklaard. Door deze verminderde praziquantelgevoeligheid is het gebruik van dit infectiemodel met vrouwelijke cercariën helaas beperkt.

Tot dusver zijn deze studies uitgevoerd in deelnemers die nooit eerder met schistosomen in aanraking zijn gekomen. Zoals al eerder is aangetoond voor andere infectieziekten, kan de effectiviteit van vaccins verschillen tussen endemische en niet-endemische populaties. Dit kan te maken hebben met eerdere blootstelling aan schistosomen, eerdere behandelingen tegen schistosomen, andere infecties, omgevingsfactoren en andere factoren. Daarom worden gecontroleerde infectiestudies steeds vaker in endemische gebieden uitgevoerd. Samen met het MRC/UVRI & LSHTM Uganda Research Unit werken we aan het opzetten van gecontroleerde infectiemodel in Oeganda, waar schistosomiasis endemisch is. In aanloop hiernaartoe voerden we in **hoofdstuk 4** een risicoanalyse uit. We brachten hier de verschillende risico's in kaart, zoals de import van slakken en schistosomen vanuit het LUMC of het risico op natuurlijke infecties tijdens de studie. Tevens opperden we maatregelen die de risico's zoveel mogelijk kunnen beperken.

In **hoofdstuk 5 en 6** laten we zien hoe we het infectiemodel kunnen inzetten om vaccinontwikkeling te versnellen. Allereerst wilden we uitzoeken hoe bescherming tegen herinfectie werkt. In endemische gebieden wordt namelijk gezien dat de prevalentie van schistosomiasis afneemt met leeftijd, wat wijst op natuurlijke bescherming. Daarnaast is het mogelijk om in diermodellen hoge bescherming op te wekken na immunisatie met bestraalde, verzwakte *Schistosoma* parasieten. In beide situaties lijkt IgG mogelijk een belangrijke rol te spelen, wat ook wordt opgewekt bij gecontroleerde infectie met schistosomen (CHI-S). In **hoofdstuk 5** zagen we dat twee keer blootstellen en behandelen niet leidde tot bescherming, ondanks toenames in IgG, maar dat klinische tolerantie snel optrad: in de deelnemers die meerdere keren werden blootgesteld, nam het aantal symptomen direct na de eerste infectie af. Daarnaast werden in deze studie vijf deelnemers per ongeluk blootgesteld aan vrouwelijke cercariën bij de tweede blootstelling, waardoor bij de derde infectie met mannelijke cercariën mogelijk wormparen zijn gevormd met eiproductie tot gevolg. Deze deelnemers hadden meer eosinofielen, CCL23, CCL4 en TNF, en hogere IgG SEA antistoffen. **Hoofdstuk 6** beschrijft het studieprotocol voor de CHI-S studie waarin het Sm-p80 + GLA-SE schistosomiasis vaccin getest wordt.

Wanneer vaccinkandidaten potentie laten zien in fase II studies, zullen ze vervolgens voor effectiviteit getest worden in grotere fase III studies. In **hoofdstuk 7** bespreken we de immunologische factoren waarmee deze vaccinkandidaten te maken kunnen krijgen, zoals eerdere blootstelling aan schistosomen, eerdere praziquantel behandeling en andere infecties. Wat de invloed hiervan gaat zijn op vaccinreacties is vooralsnog onduidelijk, maar het erkennen van deze factoren helpt om vaccinstudies zo goed mogelijk te ontwerpen (**hoofdstuk 8**). Zo moet er een relevante uitkomstmaat gekozen worden en worden nagedacht over het integreren van behandeling met praziquantel. Mogelijk is het ook van belang om verder te kijken dan het directe effect van vaccinatie op het individu en moet het indirecte effect van vaccinatie ten gevolge van minder transmissie ook onderzocht worden.

Ten slotte vatten we in **hoofdstuk 9** de belangrijkste bevindingen van de CHI-S-studies samen die in korte tijd veel hebben bijgedragen aan onze kennis over de vroege fase van *Schistosoma* infecties. Daarnaast kan CHI-S, met alleen mannelijke cercariën, nu worden gebruikt om vaccinkandidaten te evalueren. Om de wetenschappelijke waarde van CHI-S te maximaliseren, hebben we stappen geïdentificeerd die het infectiemodel optimaliseren en het uitvoeren ervan in endemische gebieden mogelijk te maken. Door uitgebreide samenwerking en data-integratie zullen we *Schistosoma* infecties op een nieuwe

manier kunnen benaderen, wat hopelijk zal leiden tot fundamentele nieuwe inzichten die bijdragen aan vaccinontwikkeling. Dit werk stelt ons hopelijk in staat om de ontwikkeling van het vaccin te versnellen met als eindresultaat een werkzaam en geregistreerd vaccin tegen schistosomiasis.

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Other schisto-aficionados, Eric, Lisette, Govert, Paul, Linda, Anna, Pytsje, Eva thank you for being so supportive of the CHI-S work and all your input interpreting the study findings.

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Als laatste, wil ik mijn ouders bedanken die me altijd hebben gestimuleerd om verder te kijken dan wat je kent of weet.

CURRICULUM VITAE

Jan Pieter Koopman was born in Bergen op Zoom, The Netherlands, on the 3rd of February 1992 and grew up in Zeeland. He attended a bilingual Dutch-English secondary school in Bergen op Zoom, before studying medicine at Leiden University in 2010. During his second year in medical school, he developed an interest in infectious diseases and started a research internship in the department of Infectious Diseases at Leiden University Medical Center (LUMC) examining genetic risk factors for urinary tract infections. In parallel, he took a half minor in Spanish language and culture and spent several months learning Mandarin in Shanghai and Taipei. For his extracurricular activities, he was awarded a Leiden University Honours College certificate in Medicine. During his clinical rotations, he travelled to Cape Town, South Africa where he was a medical elective in infectious diseases and emergency medicine at Tygerberg hospital. His scientific research internship was spent in Makassar, Indonesia where he investigated intestinal permeability and helminth infections in children from high vs low socioeconomic backgrounds, under supervision of dr. Firdaus Hamid (Hasanuddin University), dr. Erliyani Sartono (LUMC), and prof. Maria Yazdanbakhsh (LUMC).

Soon after receiving his medical degree in February 2018, he started working as a trial physician at the LUMC on several controlled human infection studies led by prof. Meta Roestenberg. He left the LUMC in September 2018 for an MSc degree in Epidemiology at the London School of Hygiene and Tropical Medicine, which he passed with merit. He returned to the LUMC in October 2019 to start his PhD project that focussed on controlled human *Schistosoma* infections to expedite vaccine development with prof. Meta Roestenberg. During his PhD, he was involved in several other studies, such as on rabies vaccination and controlled human hookworm infections, and regularly taught medical and biomedical students. In addition, he followed the Epidemiologist B training program at the department of Clinical Epidemiology, supervised by prof. Rolf Groenwold, which included a one-year secondment and advanced courses on epidemiological methods.

In 2024, he started working as clinician (ANIOS) in Internal Medicine at Haaglanden Medical Center in The Hague. Late 2025, he returned to the Leiden University Centre for Infectious diseases (LUCID) at LUMC as ANIOS for the post-COVID expertise centre.

PHD PORTFOLIO

PhD Training	Year
Courses	
Teach the teachers	2021
Analysis of repeated measurements	2021
Survival analysis	2022
Capita Selecta (epidemiology)	2022-2023
Prediction modelling and intervention research	2022
Skills for the practising epidemiologist	2023
Meta analysis	*2023*
Causal inference	2023
Laboratory Diagnosis of Human Parasitic Infections	2024
Congress attendance and poster or oral presentation	
Wellcome Trust human infection networking meeting	2020
IABS 3rd Human Challenge Trials in Vaccine Development	2020
Oral presentation at BSP	2021
Oral and poster presentation at AIGHD Masterclass: "Future of Vaccination"	2021
Poster at ASTMH	2021
Invited speaker at BSP	2022
Wellcome Trust meeting: use and utility of human infection study data	2022
Invited speaker at ICOPA	2022
Invited speaker at HIC-VAC annual meeting	2022
Invited speaker at Uganda Schistosomiasis Symposium I	2023
Oral presentation at BSP	2023
Invited speaker at UK Clinical Vaccine Network	2023
Oral presentation at ASTMH	2023
Oral presentation at Leiden Vaccine Symposium	2023
Invited speaker at "Havensymposium"	2023
Invited speaker at "MMM+M Reizigersgeneeskunde"	2024
Invited speaker at LUMC Top Research Seminar Theme Infection	2024
Teaching activities	
Half-minor fieldwork in Indonesia	2019
Docent-coach (BSc medicine)	2020
Infection in health and disease (BSc medicine)	2020-2023
Pathogen-host interactions (MSc biomedical Sciences)	2020-2024
Co-supervising student research project (MSc medicine)	2021
Global health and immunity (BSc liberal arts and sciences)	2021-2024
Global health (BSc medicine)	2021-2022
Mechanism of disease 1 (BSc medicine)	2021, 2023
Infectious agents and immunity (BSc biomedical sciences)	2022-2023
Design and analysis of biomedical studies (BSc biomedical sciences)	2022
Practical research skills (MSc medicine)	2022
Academic and scientific training year 1 (BSc medicine)	2023
Methods and techniques for scientific research (BSc biomedical sciences)	2023
Critical Appraisal of a Topic (BSc medicine)	2023-2024
Honours college lecture series (BSc medicine)	2023
Parasitology (MSc infection biology and control, CERMEL)	2024
Awards and prizes	
NVP travel award (ICOPA, Copenhagen)	2022
BSP travel award (BSP Spring meeting, Edinburgh)	2023
Best oral presentation Leiden Vaccine Symposium	2023
Other	
Peer review for Vaccine, Parasites & Vector, and PLoS Neglected Tropical Diseases	

LIST OF PUBLICATIONS

*Contributed equally

1. Driciru E, **Koopman JPR**, Steenbergen S, Sonnet F, Stam KA, Bes-Roeleveld L, Iliopoulou E, Janse JJ, Sijtsma J, Nambuya I, Hilt ST, König M, Kruize Y, Casacuberta-Partal M, Egesa M, van Dam GJ, Corstjens PLAM, van Lieshout L, Mpairwe H, MacDonald AS, Yazdanbakhsh M, Elliott AM, Roestenberg M, Houlder EL. T cell responses in repeated controlled human schistosome infection compared to natural exposure. Nat Commun. 2025 Jul 24;16(1):6827. doi: 10.1038/s41467-025-62144-8.
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