JCI The Journal of Clinical Investigation

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

Jan Pieter R. Koopman, … , Cornelis H. Hokke, Meta Roestenberg

J Clin Invest. 2024. https://doi.org/10.1172/JCI185422.

Clinical Medicine In-Press Preview Infectious disease

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 […]

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

Authors: Jan Pieter R. Koopman[1], Emma L. Houlder[1], Jacqueline J. Janse[1], Olivia. A.C. Lamers[1], Geert V.T. Roozen[1], Jeroen C. Sijtsma[1], Miriam Casacuberta-Partal[1], Stan T. Hilt[1,2], M.Y. Eileen C. van der Stoep[3,4], Inge M. van Amerongen-Westra[3,4], Eric A. T. Brienen[1], Linda J. Wammes[1], Lisette van Lieshout[1], Govert J. van Dam[1], Paul L.A.M. Corstjens[2], Angela van Diepen[1], Maria Yazdanbakhsh[1], Cornelis H. Hokke[1], Meta Roestenberg[1]

Affiliations:

[1] Leiden University Center for Infectious Diseases, Leiden University Medical Center, Leiden, The Netherlands

[2] Department of Cell and Chemical Biology, Leiden University Medical Center, Leiden, The Netherlands

[3] Department of Clinical Pharmacy and Toxicology, Leiden University Medical Center, Leiden, The Netherlands

[4] Center for Cell and Gene therapy, Leiden University Medical Center, Leiden, The Netherlands

Corresponding author:

Prof. Meta Roestenberg

Leiden University Centre for Infectious Diseases,

Leiden University Medical Centre

Building 1, Albinusdreef 2, 2333 ZA Leiden

Tel: +31 71 5265071

Email: M.Roestenberg@lumc.nl

Conflict-of interest statement:

The authors have declared that no conflict of interest exists.

Role of funding source:

The funder of the study (European Union) had no role in study design, data collection, data analysis, data interpretation, or writing of the report.

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.

Funding: ERC Starting grant (no. 101075876).

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 (**Fig. 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**).

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 Fig. 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.

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 Fig. 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 Fig. S3).**

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) $\alpha = 0{\cdot}05$ 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.

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.

ACKNOWLEDGMENTS

The authors wish to thank Perry van Genderen, Amaya Bustinduy, and Anne Wajja for their valuable advice as members of the safety monitoring committee.

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.

REFERENCES

1. McManus DP, Dunne DW, Sacko M, Utzinger J, Vennervald BJ, and Zhou XN. Schistosomiasis. *Nat Rev Dis Primers.* 2018;4(1):13.
2. Colley DG, Bustinduy AL, Secor WE, and King CH. Human schistosomiasis. *Lancet.* 2014;383(9936):2253-64.
3. Colley DG, and Secor WE. Immunology of human schistosomiasis. *Parasite Immunol.* 2014;36(8):347-57.
4. Gaze S, Driguez P, Pearson MS, Mendes T, Doolan DL, Trieu A, et al. An immunomics approach to schistosome antigen discovery: antibody signatures of naturally resistant and chronically infected individuals from endemic areas. *PLoS Pathog.* 2014;10(3):e1004033.
5. Mbanefo EC, Huy NT, Wadagni AA, Eneanya CI, Nwaorgu O, and Hirayama K. Host determinants of reinfection with schistosomes in humans: a systematic review and meta-analysis. *PLoS Negl Trop Dis.* 2014;8(9):e3164.
6. Wilson RA. Models of Protective Immunity against Schistosomes: Implications for Vaccine Development. *Pathogens.* 2023;12(10).
7. Bickle QD. Radiation-attenuated schistosome vaccination--a brief historical perspective. *Parasitology.* 2009;136(12):1621-32.
8. Koopman JPR, Houlder EL, Janse JJ, Casacuberta-Partal M, Lamers OAC, Sijtsma JC, et al. Safety and infectivity of female cercariae in Schistosoma-naive, healthy participants: a controlled human Schistosoma mansoni infection study. *EBioMedicine.* 2023;97:104832.
9. Langenberg MCC, Hoogerwerf MA, Koopman JPR, Janse JJ, Kos-van Oosterhoud J, Feijt C, et al. A controlled human Schistosoma mansoni infection model to advance novel drugs, vaccines and diagnostics. *Nat Med.* 2020.

10. Ross AG, Vickers D, Olds GR, Shah SM, and McManus DP. Katayama syndrome. *Lancet Infect Dis.* 2007;7(3):218-24.
11. Malhotra I, Ouma J, Wamachi A, Kioko J, Mungai P, Omollo A, et al. In utero exposure to helminth and mycobacterial antigens generates cytokine responses similar to that observed in adults. *J Clin Invest.* 1997;99(7):1759-66.
12. Houlder EL, Stam KA, Koopman JPR, Konig MH, Langenberg MCC, Hoogerwerf MA, et al. Early symptom-associated inflammatory responses shift to type 2 responses in controlled human schistosome infection. *Sci Immunol.* 2024;9(97):eadl1965.
13. Karanja DM, Hightower AW, Colley DG, Mwinzi PN, Galil K, Andove J, et al. Resistance to reinfection with Schistosoma mansoni in occupationally exposed adults and effect of HIV-1 co-infection on susceptibility to schistosomiasis: a longitudinal study. *Lancet.* 2002;360(9333):592-6.
14. Mangold BL, and Dean DA. Passive transfer with serum and IgG antibodies of irradiated cercaria-induced resistance against Schistosoma mansoni in mice. *J Immunol.* 1986;136(7):2644-8.
15. Pearson MS, Becker L, Driguez P, Young ND, Gaze S, Mendes T, et al. Of monkeys and men: immunomic profiling of sera from humans and non-human primates resistant to schistosomiasis reveals novel potential vaccine candidates. *Front Immunol.* 2015;6:213.
16. Vignali DA, Devey ME, Bickle QD, and Taylor MG. The role of antibody affinity and titre in immunity to Schistosoma mansoni following vaccination with highly irradiated cercariae. *Immunology.* 1990;69(2):195-201.
17. Pearson MS, Tedla BA, Becker L, Nakajima R, Jasinskas A, Mduluza T, et al. Immunomics-Guided Antigen Discovery for Praziquantel-Induced Vaccination in Urogenital Human Schistosomiasis. *Front Immunol.* 2021;12:663041.
18. Grzych JM, Pearce E, Cheever A, Caulada ZA, Caspar P, Heiny S, et al. Egg deposition is the major stimulus for the production of Th2 cytokines in murine schistosomiasis mansoni. *J Immunol.* 1991;146(4):1322-7.
19. Pearce EJ, and MacDonald AS. The immunobiology of schistosomiasis. *Nat Rev Immunol.* 2002;2(7):499-511.
20. Du X, Li F, Zhang C, Li N, Huang H, Shao Z, et al. Eosinophil-derived chemokine (hCCL15/23, mCCL6) interacts with CCR1 to promote eosinophilic airway inflammation. *Signal Transduct Target Ther.* 2021;6(1):91.
21. Matsumoto K, Fukuda S, Hashimoto N, and Saito H. Human eosinophils produce and release a novel chemokine, CCL23, in vitro. *Int Arch Allergy Immunol.* 2011;155 Suppl 1:34-9.
22. Burke ML, McManus DP, Ramm GA, Duke M, Li Y, Jones MK, et al. Temporal expression of chemokines dictates the hepatic inflammatory infiltrate in a murine model of schistosomiasis. *PLoS Negl Trop Dis.* 2010;4(2):e598.
23. Costain AH, Phythian-Adams AT, Colombo SAP, Marley AK, Owusu C, Cook PC, et al. Dynamics of Host Immune Response Development During Schistosoma mansoni Infection. *Front Immunol.* 2022;13:906338.
24. Amiri P, Locksley RM, Parslow TG, Sadick M, Rector E, Ritter D, et al. Tumour necrosis factor alpha restores granulomas and induces parasite egg-laying in schistosome-infected SCID mice. *Nature.* 1992;356(6370):604-7.
25. Janse JJ, Langenberg MCC, Kos-Van Oosterhoud J, Ozir-Fazalalikhan A, Brienen EAT, Winkel BMF, et al. Establishing the Production of Male Schistosoma mansoni Cercariae for a Controlled Human Infection Model. *J Infect Dis.* 2018;218(7):1142-6.
26. Corstjens PL, De Dood CJ, Kornelis D, Fat EM, Wilson RA, Kariuki TM, et al. Tools for diagnosis, monitoring and screening of Schistosoma infections utilizing lateral-flow based assays and upconverting phosphor labels. *Parasitology.* 2014;141(14):1841-55.
27. Meurs L, Brienen E, Mbow M, Ochola EA, Mboup S, Karanja DM, et al. Is PCR the Next Reference Standard for the Diagnosis of Schistosoma in Stool? A Comparison with Microscopy in Senegal and Kenya. *PLoS Negl Trop Dis.* 2015;9(7):e0003959.
28. Deelder AM, van Zeyl RJ, Fillie YE, Rotmans JP, and Duchenne W. Recognition of gut-associated antigens by immunoglobulin M in the indirect fluorescent antibody test for schistosomiasis mansoni. *Trans R Soc Trop Med Hyg.* 1989;83(3):364-7.
29. van Dam GJ, Stelma FF, Gryseels B, Falcao Ferreira ST, Talla I, Niang M, et al. Antibody response patterns against Schistosoma mansoni in a recently exposed community in Senegal. *J Infect Dis.* 1996;173(5):1232-41.

30. Stek MF, Minard P, Dean DA, and Hall JE. Immunization of Baboons with Schistosoma mansoni Cercariae attenuated by gamma irradiation. *Science.* 1981;212(4502):1518-20.
31. Soisson LA, Reid GD, Farah IO, Nyindo M, and Strand M. Protective immunity in baboons vaccinated with a recombinant antigen or radiation-attenuated cercariae of Schistosoma mansoni is antibody-dependent. *J Immunol.* 1993;151(9):4782-9.

474 **FIGURES**

475

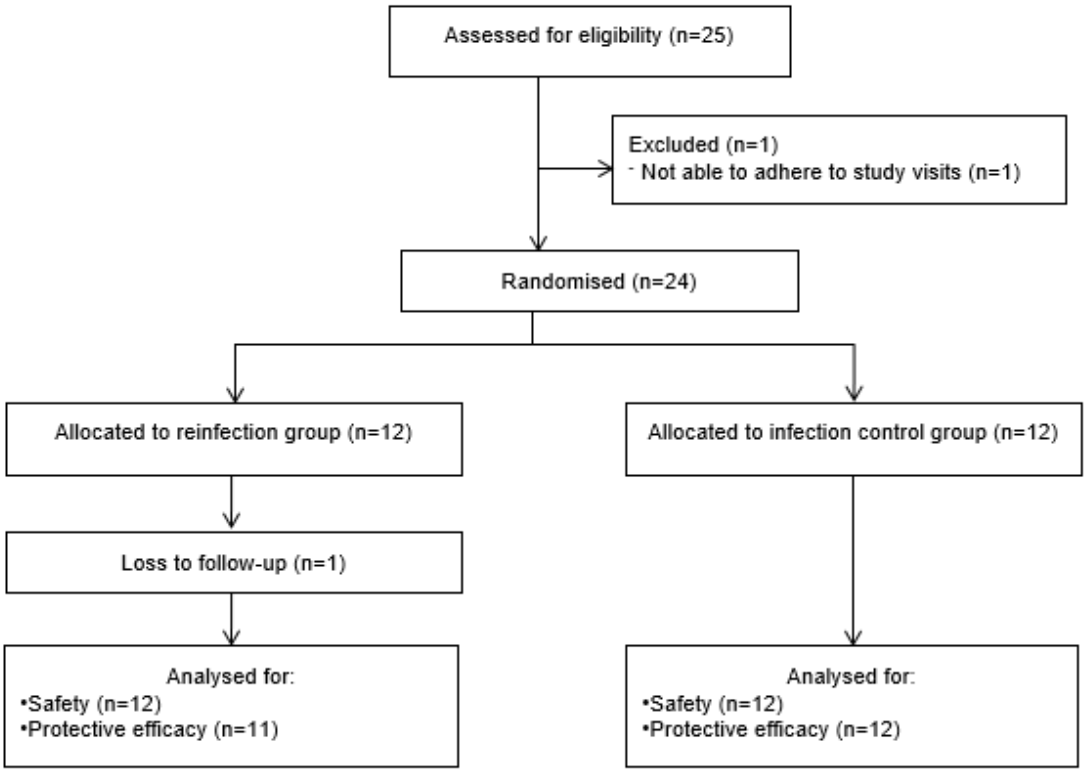

Figure 1: Consort flow for study participants.

476

477

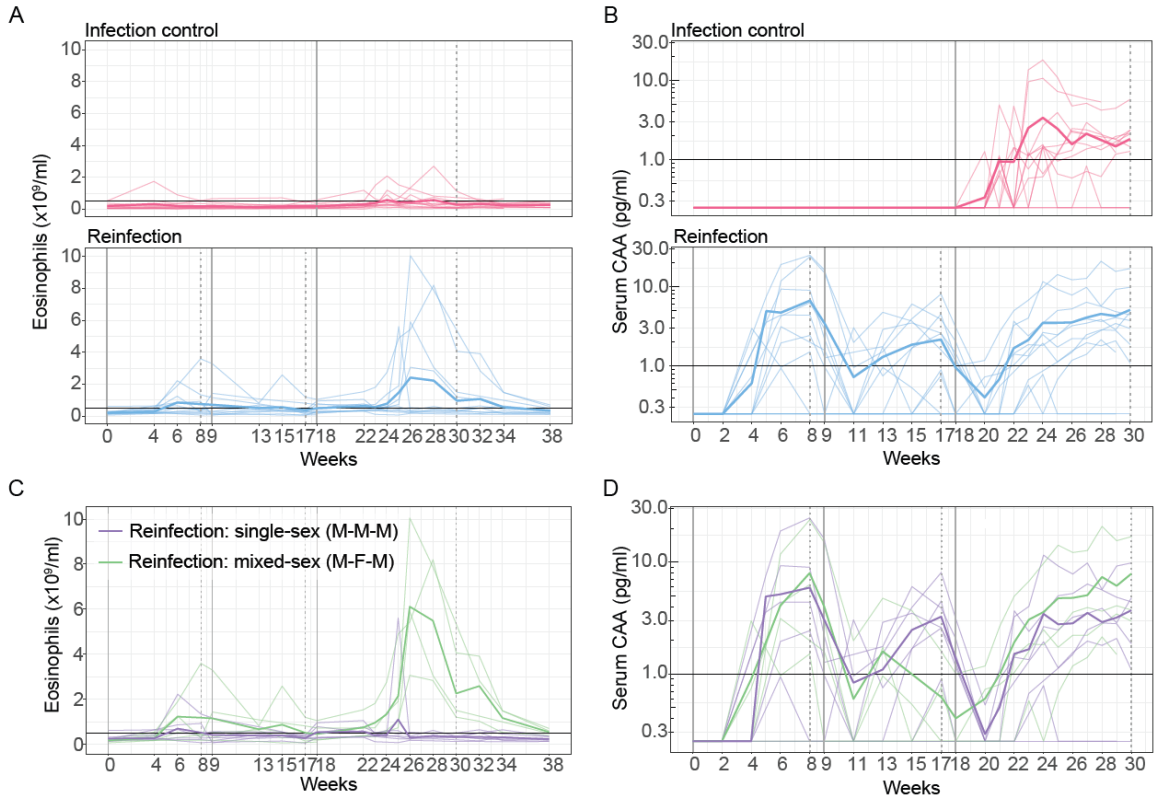

Figure 2: Eosinophil counts and CAA levels after (re)exposure to *Sm* cercariae. Plots show the changes over time in eosinophils (A) and CAA (B) in infection control (pink, n=12) and reinfection (blue, n=12) participants. Eosinophils (C) and CAA (D) in the reinfection group is then plotted stratified 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.

478

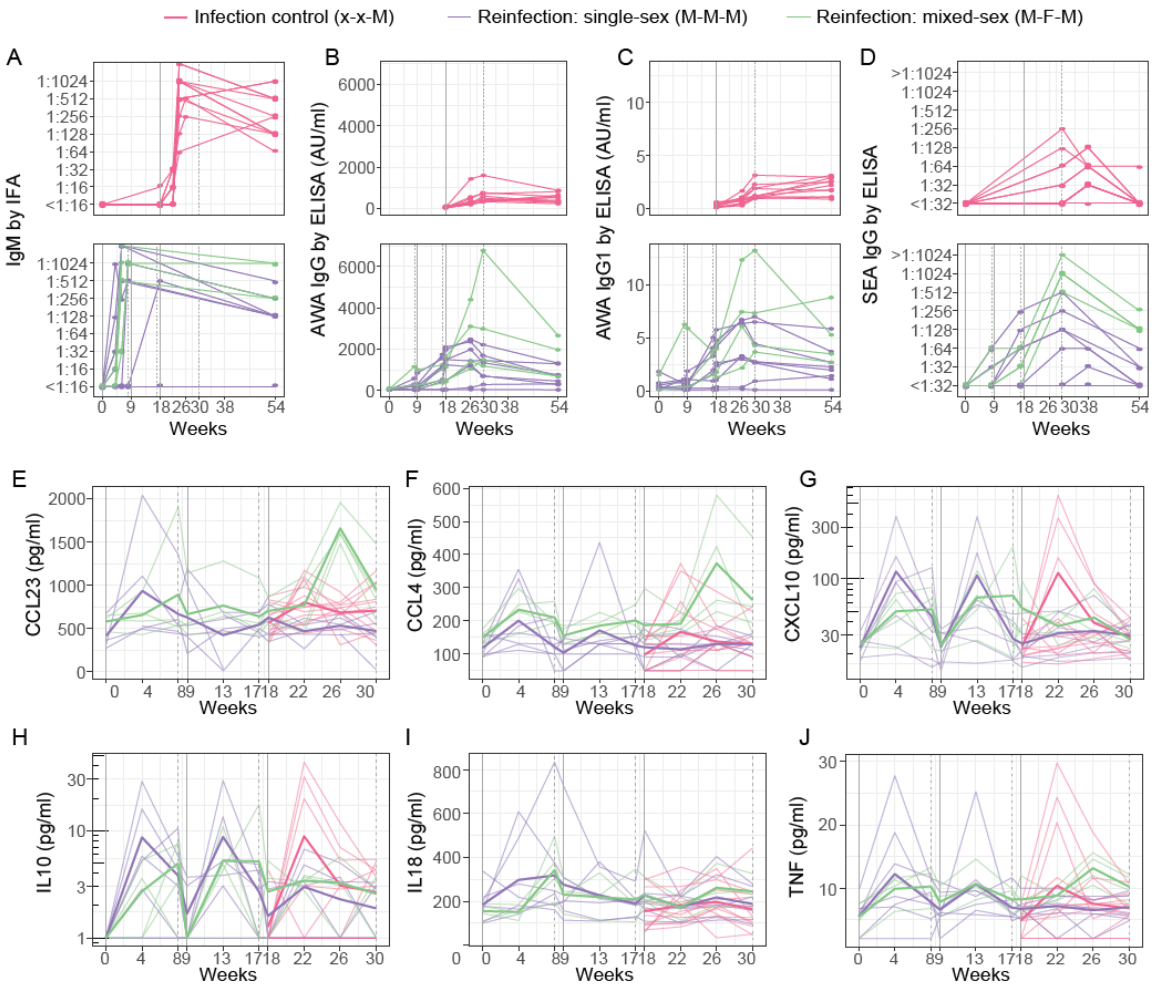

Figure 3: Antibody, chemokine, and cytokine responses after (re)exposure to *Sm* cercariae. Plots show the individual changes in antibody levels in worm-specific IgM (A), AWA-specific IgG (B), AWA-specific IgG1 (C), and SEA IgG (D). For CCL23 (E), CCL4 (F), CXCL10 (G), IL-10 (H), IL18 (I), and TNF (J) 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

TABLES

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]

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