Vaccine 92 (2026) 129129

Contents lists available at ScienceDirect

Vaccine

journal homepage: www.elsevier.com/locate/vaccine

Original article

Antibody-mediated functional responses induced by the SchistoShield® schistosomiasis vaccine in disease-naïve and endemic human populations

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ARTICLE INFO

Keywords:
SchistoShield®
Vaccine
Schistosoma mansoni
Functional antibodies
Immunogenicity
Schistosomiasis
Calpain
Sm-p80

ABSTRACT

Schistosomiasis (bilharzia) is a neglected tropical disease caused by *Schistosoma spp*. Clinical manifestation of chronic schistosomiasis include but not restricted to anemia, growth stunting, hepatosplenomegaly, cognitive impairment in children and male/female genital schistosomiasis. Praziquantel (PZQ) remains the principal standard treatment for schistosomiasis, but concerns about its reduced effectiveness against larval stages, reinfections, and emerging drug resistance reinforces the urgent need for a vaccine. SchistoShield®, composed of Sm-p80 antigen with GLA-SE adjuvant, is a promising vaccine candidate that has successfully completed phase 1 and 1b clinical trials in the USA and two countries in Africa (Madagascar and Burkina Faso). In this study, SchistoShield® vaccine specific total IgG antibody titers were measured from serum samples collected at multiple time points from both the USA and Africa trials. Results demonstrate that total IgG titers increased at week 5 after the first booster and peaked at weeks 9 and 12. Furthermore, *in vitro* schistosomula killing assays and heterologous passive transfer of purified total IgG in mice were performed to evaluate the role of vaccine-induced antibodies against schistosomes. Sera collected from individuals enrolled in the USA, Burkina Faso, and Madagascar trials, exhibited 69.2%, 55.1%, and 34.3% *in vitro* schistosomula killing, respectively, indicative of potent anti-worm antibody responses. Passive transfer of human total IgG from vaccinated individuals in mice revealed notable reductions in worm burden, egg counts, and egg-hatching ability compared to groups given pre-vaccination sera across all trials. Overall, these findings support that SchistoShield® induces generation of functional antibodies that may play a crucial role in antibody-mediated protection against schistosomiasis.

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https://doi.org/10.1016/j.vaccine.2026.129129
Received 1 July 2026; Received in revised form 31 August 2026; Accepted 1 September 2026
Available online 7 September 2026
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1. Introduction

Schistosomiasis (bilharzia) is a neglected tropical disease (NTD) and remains one of the most debilitating chronic parasitic infections. Schistosomiasis is caused by a digenetic trematode of the genus *Schistosoma.* Two distinct types of diseases are caused by different species of blood flukes: hepatic/intestinal schistosomiasis, which is caused by *S. mansoni*, *S. japonicum*, *S. mekongi*, and *S. intercalatum*; and urogenital schistosomiasis, which is caused by *S. haematobium*. The clinical symptoms can vary depending on the infecting schistosome species both in children and adults. In school-aged children, chronic schistosomiasis infection frequently leads to severe sequelae, anemia, growth stunting, malnutrition, and cognitive deficits [1–4]. In adults, chronic infections cause periportal fibrosis, portal hypertension, variceal bleeding; while urogenital schistosomiasis causes urinary tract obstruction, renal failure, and squamous cell bladder carcinoma [5,6]. Urogenital schistosomiasis also causes male genital schistosomiasis through egg deposition in the seminal vesicles and prostate, leading to hematospermia and infertility [7]. Similarly, female genital schistosomiasis causes pelvic pain, contact bleeding, and increased susceptibility to HIV transmission, though this relationship with HIV remains underdiagnosed [8–10]. The World Health Organization (WHO) estimates that 251 million people are currently affected by schistosomiasis and 800 million people are at risk of acquiring new infections [11]. Over 90% of infected individuals with schistosomiasis live in Africa. The effectiveness of mass drug administration (MDA) with the lead drug praziquantel is questionable due to its inability to clear the larval stages, recurrence of infection, and the risk of emergence of drug resistance [12–15].

SchistoShield® vaccine is a leading candidate that targets the large subunit of *S. mansoni* calcium-activated neutral protease, Sm-p80 [16], which plays an important role in surface membrane turnover thereby playing a pivotal role in the immune evasion by the worm [17,18]. Potent vaccine efficacy of Sm-p80-based formulations have been observed in three experimental animal models of infection and disease (mouse, hamster, and baboon). Sm-p80 with GLA-SE showed a significant reduction in adult worm count, and in egg counts in the liver and intestine, as well as reduced egg-hatching ability, compared with control groups [19–22]. The cross-species protective efficacy of SchistoShield® has been demonstrated in rodents (mice and hamsters) and non-human primate (baboon) models of both hepatic/intestinal and urogenital schistosomiasis [23,24]. Pre-clinical baboon trials have consistently shown that vaccination with SchistoShield® elicits strong immune responses and confers significant protection against cercarial challenge, as evidenced by a marked reduction in worm burden, egg counts in the liver and intestine, egg-hatching, and egg excretion in feces relative to adjuvant controls [21,25–28]. In summary, SchistoShield® preclinical data demonstrated prophylactic, therapeutic, anti-pathological, and transmission-blocking efficacy supporting its progression to human clinical trials [22,23,25].

In a phase 1 first-in-human dose-escalation clinical trial conducted in a disease-naïve US population, SchistoShield® was evaluated for safety, reactogenicity, and immunogenicity in 45 healthy volunteers. This trial demonstrated the safety of the vaccine with no serious adverse events including in high dose groups mirroring the results of our pre-clinical trials [29]. Serum antibodies from this trial and other preclinical studies have recently shown to inhibit the Sm-p80 catalytic activity *in vitro*, an indication that these functional responses could interfere with the parasite's biology [30]. We have also demonstrated robust cell-mediated effector and memory responses induced by SchistoShield® in subjects who participated in the phase 1 and 1b clinical trials [31].

Assessing the antibody response induced by the SchistoShield® vaccine in various populations is crucial for validating its performance in different geographical settings. Furthermore, demonstrating a functional antibody response provides early confidence that the vaccine may have protective efficacy in the field. Therefore, we tested sera from both disease-naïve and endemic-population subjects after SchistoShield® vaccination using key functional assays, including *in vitro* schistosomula killing, and heterologous passive transfer of total IgG in a mouse model of schistosomiasis.

2. Methods

2.1. Ethics statement, mice, and parasites

All experimental procedures involving mice were performed in accordance with the guidelines established by the Institutional Animal Care and Use Committee (IACUC) of Texas Tech University Health Sciences Center under approved protocol number 20010202. Female C57BL/6 mice (6–8 weeks of age) were sourced from Charles River Laboratories (Wilmington, MA, USA). For the *S. mansoni* cercarial challenge, *Biomphalaria glabrata* snails infected with the Puerto Rico PR-1 strain of *S. mansoni* were obtained from the Schistosome Resource Center (Biomedical Research Institute, Rockville, MD, USA).

2.2. Study design and ethics statement for the phase 1 and 1b SchistoShield® human clinical trials

A phase 1, open-label, randomized clinical trial aimed to assess the tolerability, safety and immunogenicity of the Sm-p80 + GLA-SE (SchistoShield®) vaccine in healthy adult volunteers was carried out at the Kaiser Permanente Washington Health Research Institute in Seattle, WA, USA (Registration date: 23/03/2022; ClinicalTrials.gov ID, NCT05292391) using an IRB approved protocol (DMID 18–0018; IRB-approval through Advarra Inc., Columbia, MD, USA). Informed consent was secured from each participant prior to their enrollment in the trial. The study was conducted in accordance with the International Council for Harmonization (ICH) and Good Clinical Practice (GCP) guidelines. A total of 45 healthy adults aged 18 to 55 years were recruited. Participants were assigned to five groups (Groups A, B, C, D and E, with 9 participants per group) and were vaccinated three times intramuscularly. For volunteers in group A, the vaccine contained 100 μg of the recombinant Sm-p80 protein antigen and no GLA-SE adjuvant. In the remaining groups, the vaccine doses contained 5 μg of GLA-SE but with varying doses of Sm-p80 (groups B, D, and E) and a different second booster vaccination day (group C). Volunteers in groups B, D, and E received low-dose (10 μg Sm-p80 + 5 μg GLA-SE), mid-dose (30 μg Sm-p80 + 5 μg GLA-SE), and high-dose (100 μg Sm-p80 + 5 μg GLA-SE) vaccines on day 0, 28, and 56, respectively. Participants in group C received mid-dose (30 μg Sm-p80 + 5 μg GLA-SE) vaccines on day 0 and 28 similar to other groups, but with a delayed booster was given at day 179.

A phase 1b trial (ClinicalTrials.gov ID: NCT05762393) was conducted as a multicenter, randomized, placebo-controlled, observer-blinded, dose-escalation trial to evaluate the safety, tolerability, and immunogenicity of the SchistoShield® vaccine in healthy adults in Burkina Faso (Registration date: 2023-03-17) and Madagascar (Registration date: 2023-02-28) using the approved IVI VASA 001 protocol from the International Vaccine Institute (IVI) Institutional Review Board (IRB number 2022–011). In the phase 1b trial, a total of 120 healthy volunteers aged between 20 and 59 years were recruited across Madagascar and Burkina Faso. Participants were assigned to three cohorts (Cohort A, B, and C), each contains 20 participants. Within a cohort, 5 out of 20 volunteers were randomly selected as placebo controls. Three doses were administered to the low-, mid-, and high-dose cohorts using the same amount of Sm-p80 and GLA-SE, as in the phase 1 trial. To study the antibody response to the SchistoShield® vaccine in both endemic and disease-naïve populations, pre-vaccination (Day 0) serum samples were used as a control for each experimental cohort/group. All the volunteers received the SchistoShield® vaccine intramuscularly on Day 0, Day 28, and Day 56. Serum samples were collected on Day 0, 7, 28, 35, 56, 63, 84, and the last time point was collected on Day 180 for the phase 1 USA trial and on Day 224 for the phase 1b African trial. After receipt from clinical sites, serum samples were immediately stored in a dedicated −80 °C freezer and aliquoted into 50 μL in 1.5 mL centrifuge tubes to avoid frequent freeze-thawing cycles. Additional groups in the phase 1 USA trial received high dose (100 μg

Sm-p80) with unadjuvanted, mid-dose with a delayed booster (30 μg Sm-p80 + 5 μg GLA-SE) followed different dosing regimens and were not included in this study. Similarly, placebo groups from each cohort in the phase 1b trials (Madagascar and Burkina Faso) were not included.

2.3. Human serum ELISA

Serum from all participants and time points across all three trials (USA, Madagascar, and Burkina Faso) was used to determine total IgG titers per participants in this study (Suppl. Table 1 and Suppl. Fig. 1). Endpoint titers were determined as follows: 384-well microtiter plates (Corning, Kennebunk, ME, USA) were coated with recombinant Sm-p80 (30 ng/well) (Quratis Inc., Seoul, South Korea). Plates were blocked with blocking buffer (10% BSA) for 2 h at room temperature. Serially diluted samples (4-fold) were added to the plates and incubated for 1 h at room temperature. Goat anti-human IgG-HRP secondary antibody (1:25000) (Cat. No: 2040–05, Southern Biotech, AL, USA) was added and incubated for 1 h at room temperature. TMB substrate solution (SeraCare, MA, USA) was added and incubated for 10 min at room temperature. Finally, the reaction was stopped using TMB stop solution (1% HCl) (SeraCare, MA, USA). Plates were read at 450 nm in triplicate using SoftMax Pro® and a SpectraMax iD3 microplate reader (Molecular Devices, CA, USA). Raw optical density (OD) values for all samples from the three human clinical trials were uploaded using the Lab Upload Tool provided by the Vaccine Immunology Statistical Center (VISC) at the Fred Hutchinson Cancer Center in Seattle, WA, USA.

Statistical analysis of the total IgG response across all three trials was performed by VISC using R software packages (CRAN R 4.4.0). Assay repeatability and variability were evaluated, and plate-level replicate values were summarized using the arithmetic mean, geometric mean, and median. The geometric mean was calculated by taking the mean of the natural log-transformed optical density (OD) values and back-transforming to the original scale. Distributions of OD values on both the raw and log-transformed scales were examined to determine the most appropriate plate-level summary measurement (Suppl. Fig. 2). Total IgG responses were summarized as endpoint titers derived from OD *versus* serum dilution titration curves. For each participant and time point, the corresponding pre-vaccination (baseline) sample was used to define a cutoff value, calculated as the mean OD plus two standard deviations [32]. The endpoint titer was defined as the highest dilution at which the OD value remained above the cutoff.

2.4. Heterologous IgG passive transfer

A total of 108 C57BL/6 female mice were included in the passive transfer experiment using serum samples collected from the three clinical trial sites. Total IgG antibody was purified from the sera of study participants used in our recent study [31] at selected time points using a standard protein A/G spin kit (Cat No: 89950, Thermo Scientific, MA, USA). Purified total IgG was analyzed by SDS-PAGE to assess purity. After measuring the concentration of purified total IgG on NanoDrop ND-1000 Spectrophotometer (Thermo Fisher Scientific, DE, USA), samples were stored at -80 °C until use. Three doses of purified total IgG were transferred to the mice intravenously on days 0, 3, and 9. Each mouse from the control group received an intravenous injection of purified total IgG (75 μg) isolated from Day 0 (pre-immunization) control human sera, while those in the experimental group received purified total IgG (75 μg) from Sm-p80- immunized human sera collected on Days 56, 84, and 180 of the US trial and Days 56, 84 and 224 of the Africa trials. Three mice were used for each purified total IgG individual sample for each time point across the three trials. Each mouse was challenged with 150 *S. mansoni* cercariae on day 6 *via* tail immersion in water to allow for percutaneous infection. Mice were euthanized six weeks post-cercarial challenge, and adult worms were recovered from the mesenteric vasculature and hepatic portal system by perfusion. The percent reduction in adult worm burden was determined by comparing the number of worms recovered from the experimental groups (I) to the control group (C). Protection (P) level was calculated using the formula: % P = [(C – I)/C × 100]. To assess the schistosome egg burden in the livers and intestines, euthanized mouse samples were digested with 4% potassium hydroxide at 37 °C overnight, washed, and resuspended in a 1.2% (w/v) sodium chloride solution. Light microscopy was used to determine the number of eggs per gram of liver and intestinal tissue. Tissue egg-hatching rates were also determined: Briefly, a 0.5–1 g section of liver was collected from each mouse following euthanasia. Tissue samples were minced using razor blades before being placed into 50 mL tubes containing PBS. Collagenase B (0.1 U/mL), penicillin (33 U/mL), and streptomycin (40 μg/ml) were added to each sample, which was then incubated at 37 °C overnight with agitation at 220 rpm. The samples were then centrifuged at 4 °C and 1000 rpm for 5 min. The supernatant was discarded, and the pellet resuspended in 20 mL of cold 1.2% NaCl. The homogenized samples were then passed through a series of sieves (425, 180, 106, and 45 μm mesh opening). *S. mansoni* eggs were collected from the 45 μm sieve and centrifuged at 1000 rpm for 10 min. The egg pellet was resuspended in fresh water after discarding the supernatant and seeded into 24-well plates. The plates were placed under a light source for 2 h to induce egg-hatching. Mature eggs, dead eggs, and hatched eggs were counted using a light microscope, and the egg-hatching rates were expressed as the percentage of eggs hatched per gram of liver. An unpaired Student's *t*-test (GraphPad Prism 9) was applied to compare the statistical difference in averages ± SEM in total worm counts, liver and intestine egg per gram, and egg-hatching capacity in each group compared to Day 0 (pre-immunization) *versus* post-immunization in a heterologous total IgG passive transfer experiment. *P*-values less than 0.05 were considered statistically significant.

2.5. In vitro schistosomula killing assay

Schistosomula killing assays were performed using sera collected from subjects enrolled in clinical trials of SchistoShield® vaccine. Serum from randomly selected nine human volunteers was used in this study: three each from the USA, Madagascar, and Burkina Faso clinical trials. Sera were heat inactivated by incubating samples at 56 °C for 30 min. *S. mansoni* cercariae were shed from snails by exposure to light at 28 °C for 1 h and 30 min. Cercariae were mechanically transformed into schistosomula by repeated passes through a 22-gauge needle-syringe. Schistosomula were incubated in the presence of heat-inactivated Sm-p80-immunized and control human sera (1:50 dilution) with exogenous guinea pig complement at a 1:50 dilution (Sigma-Aldrich, MO, USA). The treated schistosomula were cultured overnight at 37 °C with 5% CO_2 in complete RPMI-1640 media (Cytiva-HyClone, UT, USA) supplemented with 100 μg/mL penicillin G (Sigma Aldrich, MO, USA), 100 μg/mL streptomycin (Sigma Aldrich, MO, USA), 10 μg/mL gentamycin (Sigma Aldrich, MO, USA), and 10% fetal bovine serum (FBS) (ThermoFisher Scientific, NC, USA) at a density of approximately 50 schistosomula per well in 2 mL of media using 24-well plates (Corning Costar) [33]. Schistosomula were observed under bright field at 10× magnification with an inverted microscope (Nikon, Model TMS) to determine whether they were alive or dead based on morphological changes (blebbing, granularity, and/or loss of membrane integrity) and motility. The number of dead and viable schistosomula was counted in each well. Percentage in schistosomula killing was calculated using the following formula: (% protection) = (% killing of schistosomula in experimental groups – % killing of schistosomula in control groups) / % killing of schistosomula in experimental groups × 100. Each assay was carried out with three technical replicates. Data collected from *in vitro* schistosomula killing assays were analyzed by unpaired Student's *t*-test using GraphPad Prism 9. Data were presented with error bars denoting the standard error of the mean (mean ± SEM). In addition, paired Student's *t*-test was used to determine confidence intervals (CI) for three volunteers of each trial site using GraphPad Prism 9.

3. Results

3.1. SchistoShield® vaccine induces a robust IgG antibody response

Antibody responses were determined for non-endemic and endemic subjects, and titers were assessed for differences due to endemicity. Among phase 1 and 1b trial participants, total IgG titers began to increase at week 5 (7 days after the first booster) across all trials (Fig. 1). Titer continued to rise thereafter, with peak total IgG titer was observed in week 9 (7 days after the second booster), and week 12 (28 days after the second booster) across all groups in the phase 1 and 1b trials. When total IgG titer induced by SchistoShield® were evaluated across trials, participants in the Burkina Faso trial exhibited lower total IgG titers across all time points than those in the Madagascar trial (Fig. 1). A slightly higher antibody response was observed particularly early in the endemic sites compared with the non-endemic USA trial.

3.2. Heterologous passive transfer of SchistoShield® vaccine elicited-IgG antibodies in mice

To assess the protective efficacy of Sm-p80-specific total IgG against *S. mansoni* infections, C57BL/6 mice were infused with purified antibodies from sera obtained from clinical trial participants and subsequently challenged with cercaria (Fig. 2a). Results demonstrated that the passively transferred Sm-p80-specific total IgG antibodies protected the mice from *S. mansoni* challenge. Significant reductions were observed in adult worm numbers from each of the trials (USA, Madagascar, and Burkina Faso, respectively) as follows: total worms: 39.5% ($P = 0.02$), 44.5% ($P = 0.006$), and 62.6% ($P = 0.002$); female worms: 49.3% ($P = 0.01$), 40.1% ($P = 0.01$, and 73% ($P = 0.001$); (Fig. 2b and c). Liver and intestine tissue samples from control and experimental groups were also assessed for tissue egg burden. A significant reduction in egg burden was observed in the livers of experimental groups: 55.9% ($P = 0.0001$), 35.9% ($P = 0.001$), 70.5% ($P = 0.00001$); and intestine: 54.2% ($P = 0.0001$), 40.8% ($P = 0.0003$), and 76.9% ($P = 0.00002$) compared to the control groups (Fig. 2d and e). To assess egg viability, we compared hatching rates of eggs isolated from mouse liver tissue samples in the control and experimental groups. Hatching rate analysis of eggs isolated from the tissues of mice which had received Sm-p80-specific total IgG showed a significant reduction of 50.4% ($P = 0.005$), and 53.9% ($P = 0.02$) in the liver (USA and Burkina Faso clinical trials, respectively) when compared to that of the control mice (Fig. 2f). We also observed a 33.4% reduction in egg-hatching ability in the livers of mice that had received IgG from the Madagascar trial, but the reduction was not statistically significant ($P = 0.06$) (Fig. 2f).

3.3. In vitro killing of schistosomula by SchistoShield® vaccinated human sera

There was a significant increase in *in vitro* schistosomula killing induced by serum samples collected from the USA and Burkina Faso clinical trial sites at different time points post-SchistoShield® immunization, compared to pre-vaccination controls (Day 0) when the sera were supplemented with exogenous complement. A 69.2% of the schistosomula were killed by serum from volunteers in the phase 1 (USA) trial at different time points, as depicted in Fig. 3b. Serum samples collected from vaccinated volunteers in the phase 1b (Madagascar and Burkina Faso) trials exhibited 34.3% and 55.1% killing, respectively (Fig. 3b). Across all post-vaccinated groups, a notable increase in schistosomula killing compared to pre-immunization was observed.

4. Discussion

First-in-human phase 1 and follow-on phase 1b SchistoShield® clinical trials conducted in endemic and non-endemic populations, respectively, have demonstrated that this vaccine formulation robustly elicited antigen-specific anti-Sm-p80 antibodies in vaccinated individuals. In this report, we have established that these vaccine-induced immune responses result in potent parasite-neutralizing efficacy in a mouse model of schistosomiasis using passive transfer and *in vitro* killing of schistosomula, respectively, using purified total IgG or serum samples. Notably, vaccine-induced antibodies mediated multifaceted protection against *S. mansoni*, as evidenced by a significant reduction in adult worm burdens, tissue egg deposition, and egg viability, alongside a marked level of *in vitro* schistosomula killing. These results from human clinical trial samples provide a promising link to the potent efficacy demonstrated in pre-clinical trials [21,22,25].

We evaluated antibody responses between the disease-naïve US and endemic African (Madagascar and Burkina Faso) trials to assess the effect of geography and disease exposure across equivalent cumulative low-, mid-, and high-dose groups. We had previously reported phase 1 immunogenicity and safety data [29]. Now that the phase 1b samples were available, we evaluated variations in response magnitude across populations using an endpoint titer ELISA. Sm-p80-specific total IgG antibodies were detected five weeks after the primary immunization and showed a significant increase at subsequent time points, with peak responses at weeks 9 and 12 in vaccinated individuals across all three clinical trial populations. The total IgG antibody titer analysis of disease-naïve and endemic populations indicates that the Sm-p80 antigen elicits consistent humoral antibody kinetics irrespective of baseline geographical or endemic variations. Furthermore, these clinical immunogenicity

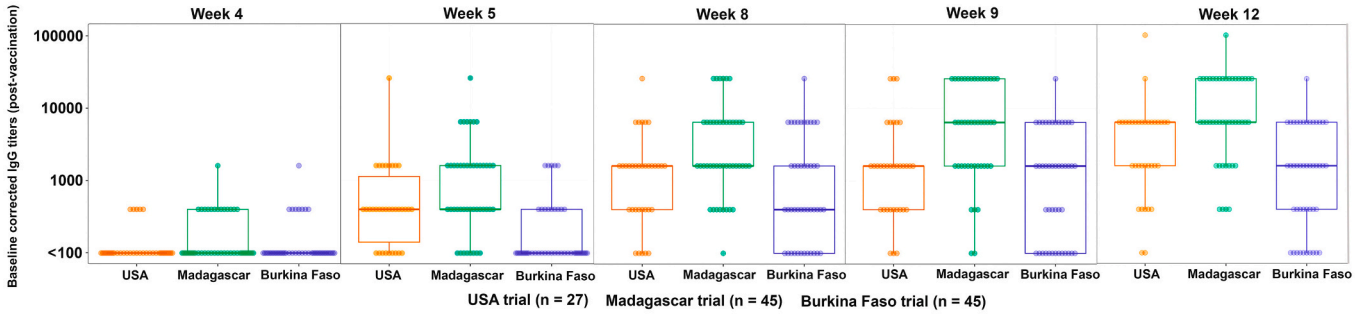


Fig. 1. Immunogenicity analysis of anti-Sm-p80 antibody by end-point ELISA titer determined the highest total IgG response in post-vaccination SchistoShield® treatment group of phase 1 (USA) and 1b trials (Madagascar and Burkina Faso) on the modified intent to treat (mITT) population. At each time point and for each participant, the pre-vaccination sample was used to calculate a cut-off value of 2 standard deviations above the mean optical density. Baseline-corrected serum IgG titers following vaccination across three clinical trials (USA, $n = 27$; Madagascar, $n = 45$; Burkina Faso, $n = 45$), shown at weeks 4, 5, 8, 9, and 12 post-vaccinations. Titers were plotted in a box plot on a $\log_{10}$ scale, with values below the assay's lower limit of detection shown as <100. Titers from individual participants at each timepoint were shown as dots. In each box plot, the horizontal line within the box denotes the median, whereas the lower and upper box boundaries mark the first and third quartiles (25th and 75th percentiles). The whiskers extend to the most extreme values, lying within 1.5 × IQR of the nearest quartile, and dots beyond the whiskers were shown as outliers.

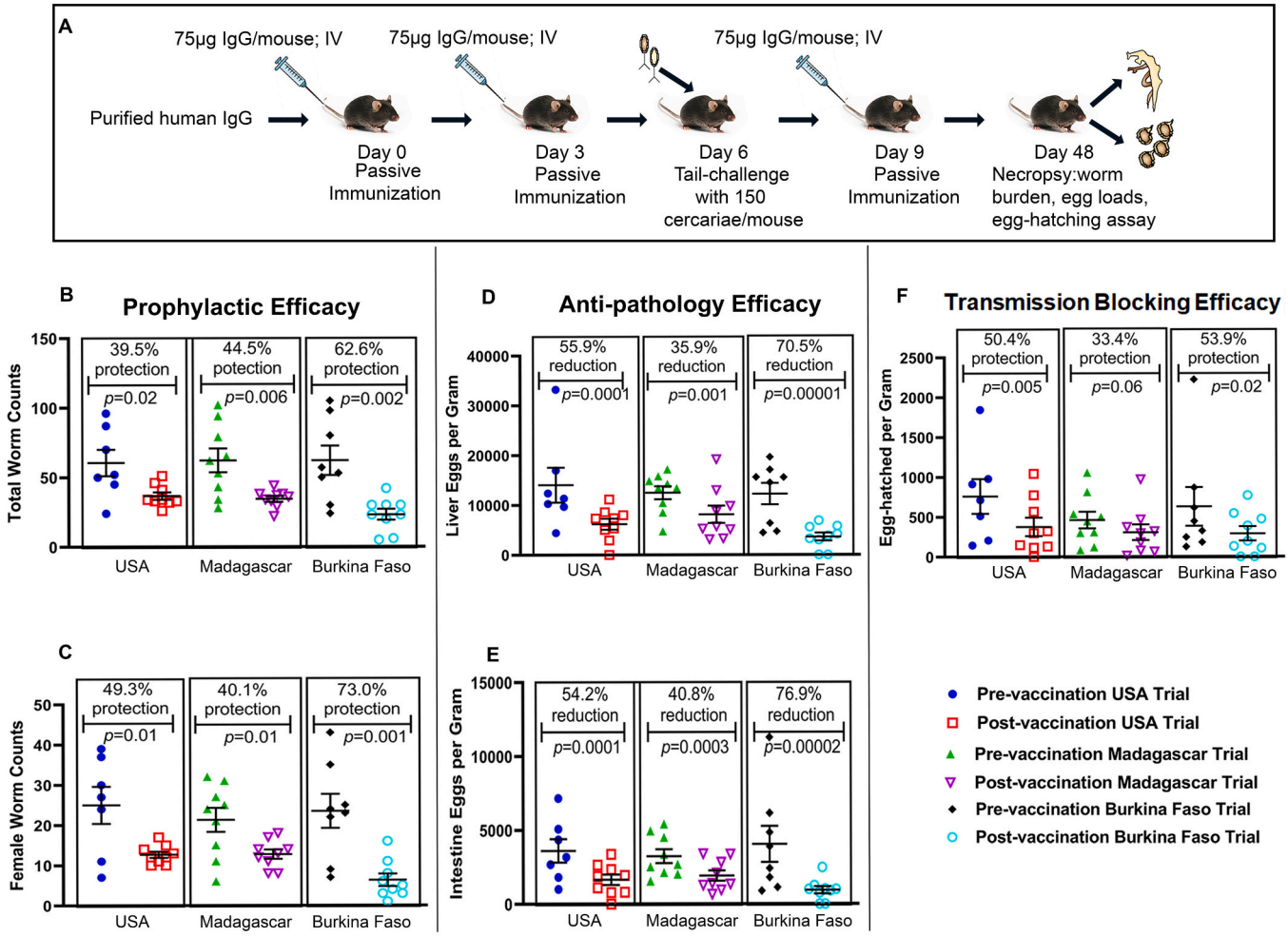


Fig. 2. Anti-Sm-p80 purified antibodies from serum samples collected from volunteers in the US, Madagascar, and Burkina Faso SchistoShield® clinical trials conferred protection in a mouse model of schistosomiasis. Either pre-vaccination or post-vaccination purified IgG (75 µg/mouse) was transferred to the mice intravenously on day 0, 3, and 9; and each mouse was challenged with 150 *S. mansoni* cercariae at day 6 *via* tail immersion technique. All mice were sacrificed at 6 weeks post *S. mansoni* cercariae challenge. Experimental timeline (A) recovered adult total worms (B), and female worms (C) following passive transfer of purified IgG from human volunteers immunized with SchistoShield® vaccine and control (pre-immunization). Liver egg burden (D) and intestine egg burden (E) at necropsy. Hatching rates of eggs recovered from livers (F) of mice passively transferred with post-immunization purified IgG antibodies compared to mice received IgG purified from pre-immunization sera. Each point represents one mouse. $p < 0.05$ considered to be statistically significant.

outcomes mirror the robust humoral responses previously established in the pre-clinical trials using baboons immunized with SchistoShield® [25,29]. The antibody titer analysis also showed variations in the total levels of Sm-p80-specific total IgG induced across populations. Vaccinated volunteers from Madagascar had higher titers at all time points after the primary immunization than the disease-naïve population or the endemic Burkina Faso population. We also observed detectable baseline total IgG in pre-vaccination samples from a few participants in the Madagascar trial, unlike with the disease-naïve and endemic Burkina Faso populations. However, due to the low titers in these individuals, it is plausible that total IgG induced by natural infection is negligible compared with the robust immune response induced by SchistoShield® vaccination in populations.

Here, we have also demonstrated antibody-mediated protection in a heterologous passive-transfer mouse model of schistosomiasis using purified total IgG from serum samples collected pre- and post-vaccination in our phase 1 and 1b SchistoShield® vaccine trials. There were significant reductions in the worm burden observed across all the mice that received purified total IgG from post-vaccinated sera compared to those that received total IgG from the pre-vaccinated sera. Mice that received total IgG from the Burkina trial showed the highest reduction in total worm numbers compared to mice that received purified total IgG from either the USA or Madagascar trials. Similarly, the highest reduction in female worms was observed in mice that received total IgG from the Burkina Faso volunteers, compared with mice in the other groups.

Another important outcome of the passive transfer study was a reduction in egg numbers in tissues such as the liver and intestine. Significant reductions in egg numbers were observed across all groups that received total IgG from post-vaccination sera compared to those that received total IgG from pre-vaccination sera. Again, serum from Burkina Faso volunteers showed the greatest reduction in eggs, followed by the USA and Madagascar. As expected, this correlates with the percentage reduction in female worms observed in this study. The reduced number of tissue eggs in the current study may reflect either an immediate anti-fecundity effect induced by the vaccine or a delayed initiation of egg laying. These results suggest that the SchistoShield® vaccine will directly affect the parasites themselves and will reduce egg loads in tissues, thereby counteracting pathology and impacting transmission.

The egg-hatching assay demonstrated pronounced protection in the groups that received total IgG from vaccinated individuals from the Burkina Faso trials, followed by the USA trial. The group of mice that received purified total IgG from vaccinated individuals in Madagascar

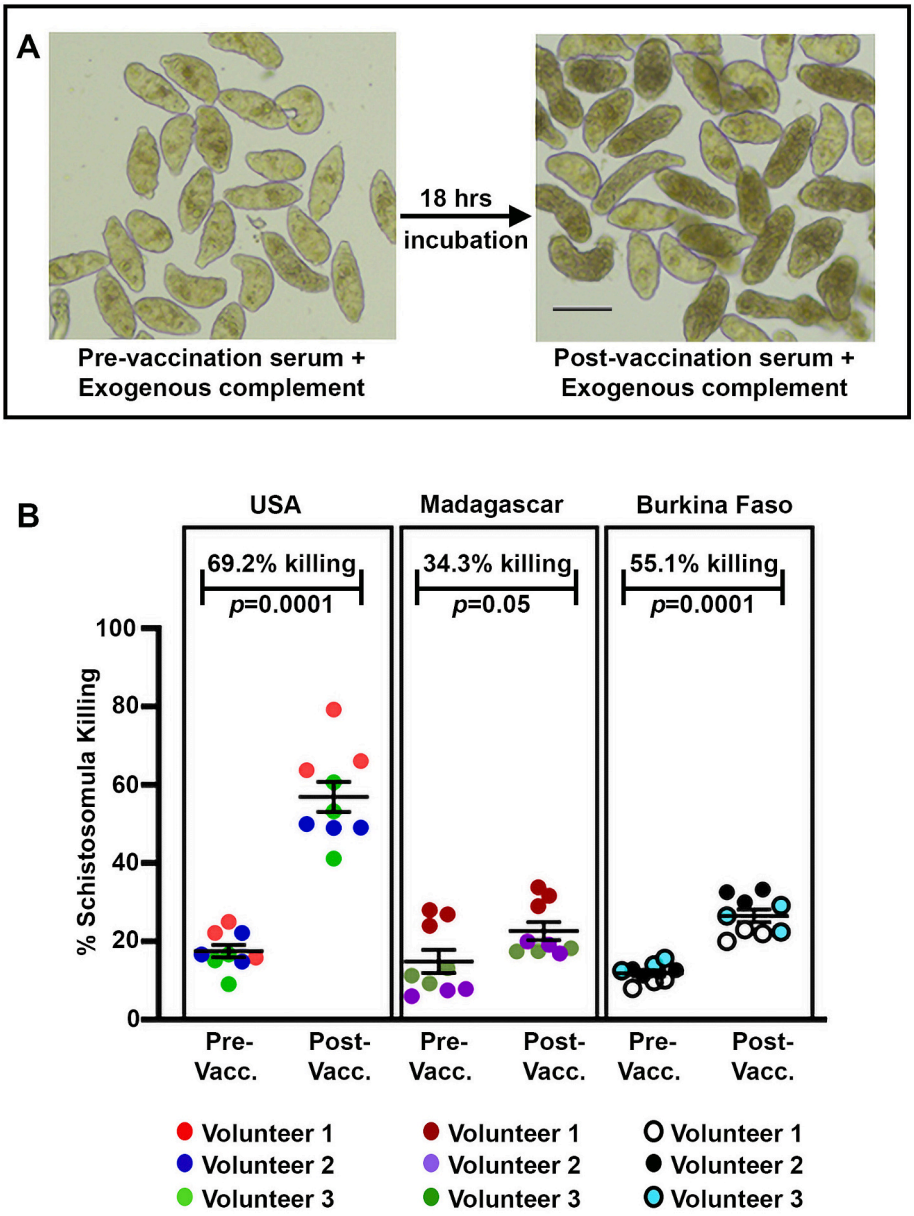


Fig. 3. *In vitro S. mansoni* schistosomula killing by human serum samples supplemented with exogenous complement in SchistoShield® vaccine trials in the USA, Madagascar, and Burkina Faso. Mechanically transformed schistosomula were incubated in complete media supplemented with sera and exogenous complement. Panel (A) illustrates schistosomula in RPMI-1640 media with serum. In contrast, morphological changes indicative of schistosomula killing were evident when incubated with pooled sera with exogenous component from vaccinated volunteers. Percentage of schistosomula killing for experimental samples were shown for post-vaccination sera for USA trial, Madagascar trial, and Burkina Faso trial (Panel B) compared to sera samples collected at pre-vaccination. Percentage increase in schistosomula killing was determined by comparing the experimental to the control groups. Plotted graphs represent error bars showing means and standard error of the mean (mean ± SEM). V = Day of vaccination. Unpaired student's *t*-test between pre- and post-vaccination groups with $p < 0.05$ was considered to be statistically significant. The 95% confidence intervals for the three volunteers in each trial were as follows: USA (95% CI: 17.83–60.97), Madagascar (95% CI: −0.6367 - 16.17), and Burkina Faso (95% CI: 3.821–25.51). Schistosomula were observed under bright-field at 10× magnification with an inverted microscope (Nikon, Model TMS). The microscopic fields presented in Fig. 3A were randomly selected from the micrographs taken in the study.

showed slightly less protection than the other groups. A substantial proportion of the eggs were not viable and could not transmit disease in the environment, indicating the potential for transmission-reducing activity.

We further explored the role of antibodies induced by the SchistoShield® vaccine using an *in vitro* schistosomula killing assay. Here, significant parasite killing was induced by post-vaccination sera compared to pre-vaccination sera. This is indicative that Sm-p80 antigen-specific antibodies could be sufficient for protection against schistosomiasis. The outcome of the *in vitro* schistosomula killing studies agrees with our previously published data [33]. Functional assays are increasingly being developed and evaluated as potential biomarkers and composite efficacy readouts for schistosomiasis vaccine clinical trials [33]. Although the *in vitro* assay does not establish the precise *in vivo* mechanism of protection, it provides supporting evidence that vaccination elicits antibodies capable of recognizing and killing schistosomula and hence antibodies are functionally active. There are numerous previous studies demonstrating antibodies play an important role in schistosomula killing [34–38]. However, future studies with additional experimental controls, including purified total IgG from placebo recipients, unrelated

human total IgG, infected mice receiving no human total IgG, or post-vaccination total IgG depleted of Sm-p80-specific antibodies by antigen adsorption are warranted.

In addition, our planned optimization of SchistoShield® in a controlled schistosome human challenge infection [39], will provide further insights into, for example by comparing infection establishment, circulating anodic antigen kinetics, surrogate measures of worm burden, and relationships between Sm-p80-specific antibody function and infection intensity. Finally, the present work provides preliminary evidence of functional vaccine-induced antibodies and supports further field efficacy clinical trials. Lastly, some of the conclusions maybe constrained by the smaller sample size, therefore, further studies are required to confirm the data presented in the present exploratory study with additional human volunteer samples from both the USA and Africa trials.

5. Conclusion

In summary, the SchistoShield® vaccine induced a robust humoral immune response in human volunteers. Furthermore, sera from vaccinated individuals were able to kill schistosomula *in vitro*. Reduced worm, egg deposition, and decreased egg viability were observed following heterologous total IgG passive transfer. These results imply that the vaccine in humans could have protective efficacy and interrupt disease transmission in endemic settings.

CRediT authorship contribution statement

Aravindan Kalyanasundaram: Writing – review & editing, Writing – original draft, Methodology, Formal analysis, Data curation. **Desalegn W. Kifle:** Writing – review & editing, Validation, Methodology, Formal analysis. **Jennifer Davis:** Writing – review & editing, Methodology, Formal analysis. **Mumtaz Y. Balkhi:** Writing – review & editing, Methodology. **Aryandra Arya:** Writing – review & editing, Methodology. **Bryan T. Mayer:** Writing – review & editing, Visualization, Validation, Software, Formal analysis, Data curation. **Gabrielle Lemire:** Writing – review & editing, Visualization, Validation, Formal analysis, Data curation. **Dinesh Krishnappa:** Writing – review & editing, Project administration, Methodology. **Mohamadou Siribie:** Writing – review & editing, Supervision, Data curation. **Hyonjin Jeon:** Writing – review & editing, Resources, Data curation. **Birkneh T. Tadesse:** Writing – review & editing, Resources, Investigation, Data curation. **Njariharinjakamampionona Rakotozandrindrainy:** Writing – review & editing, Methodology, Investigation. **Ravomialisoa Razafimanantsoa:** Writing – review & editing, Investigation. **Henry Bere Noellie:** Writing – review & editing, Methodology, Investigation. **Nebié Issa Ouedraogo:** Writing – review & editing, Methodology, Investigation. **Amidou Diarra:** Writing – review & editing, Methodology, Investigation. **Thirumalaisamy P. Velavan:** Writing – review & editing, Resources. **Sean A. Gray:** Writing – review & editing. **Jinhee Lee:** Writing – review & editing, Resources. **Raphaël Rakotozandrindrainy:** Writing – review & editing, Resources, Investigation, Funding acquisition. **Sodiomon B. Sirima:** Writing – review & editing, Resources, Investigation, Funding acquisition. **Lisa A. Jackson:** Writing – review & editing, Supervision, Resources, Investigation, Funding acquisition. **Florian Marks:** Writing – review & editing, Supervision, Resources, Investigation, Funding acquisition, Conceptualization. **Darrick Carter:** Writing – review & editing, Supervision, Methodology, Funding acquisition. **Afzal A. Siddiqui:** Writing – review & editing, Writing – original draft, Supervision, Resources, Project administration, Investigation, Funding acquisition, Formal analysis, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

We thank the many contributors who provided advice and assistance with this study, specifically: NIAID (Ranjodh Gill, Lee Hall and Annie Mo), IDCRC (Chris Posavad), the Gates Foundation (Jonathan Arm and Gary Means). Above all, we are grateful to the participants themselves for their altruism and commitment to these trials. Specimens for the phase 1 trial in the USA were provided by the Infectious Diseases Clinical Research Consortium (IDCRC) through the NIAID, part of the NIH, under the award number UM1AI148684. This study was conducted using samples collected for secondary research under protocol DMID 18-0018. The Africa-phase 1b trials were funded by European Union Horizon 2020 (DLV-815643). Funding support from NIAID/NIH (R44AI103983); Gates Foundation (INV-004809, INV-027499), RIGHT Foundation (Investment ID, RF-2021-V02), Wellcome Trust (Grant # 218454/Z/19/Z) and TTUHSC School of Medicine (H251374) are acknowledged for their support of the Sm-p80-based vaccine development effort.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.vaccine.2026.129129.

Data availability

Data will be made available on request.

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