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Summary

Background A human hookworm vaccine is being developed to protect children against iron deficiency and anaemia associated with chronic infection with hookworms. *Necator americanus* aspartic protease-1 (*Na*-APR-1) and *N americanus* glutathione S-transferase-1 (*Na*-GST-1) are components of the blood digestion pathway critical to hookworm survival in the host. Recombinant *Na*-GST-1 and catalytically inactive *Na*-APR-1 (*Na*-APR-1[M74]) adsorbed to Alhydrogel were safe and immunogenic when delivered separately or co-administered to adults in phase 1 trials in non-endemic and endemic areas. We aimed to investigate the safety and immunogenicity of these antigens in healthy children in a hookworm-endemic area.

Methods This was a randomised, controlled, observer-blind, phase 1, dose-escalation trial, conducted in a clinical research centre, in 60 children aged six to ten years in Lambaréné, a hookworm-endemic region of Gabon. Healthy children (determined by clinical examination and safety laboratory testing) were randomised 4:1 to receive co-administered *Na*-GST-1 on Alhydrogel plus *Na*-APR-1(M74) on Alhydrogel and glucopyranosyl lipid A in aqueous formulation (GLA-AF), or co-administered ENGERIX-B hepatitis B vaccine (HBV) and saline placebo, injected into the deltoid of each arm. Allocation to vaccine groups was observer-masked. In each vaccine group, children were randomised 1:1 to receive intramuscular injections into each deltoid on two vaccine schedules, one at months 0, 2, and 4 or at months 0, 2, and 6. 10 µg, 30 µg, and 100 µg of each antigen were administered in the first, second, and third cohorts, respectively. The intention-to-treat population was used for safety analyses; while for immunogenicity analyses, the per-protocol population was used (children who received all scheduled vaccinations). The primary outcome was to evaluate the vaccines' safety and reactogenicity in healthy children aged between six and ten years. The secondary outcome was to measure antigen-specific serum IgG antibody levels at pre-vaccination and post-vaccination timepoints by qualified ELISAs. The trial is registered with ClinicalTrials.gov, NCT02839161, and is completed.

Findings Between Jan 23 and Oct 3, 2017, 137 children were screened, of whom 76 were eligible for this trial. 60 children were recruited, and allocated to either 10 µg of the co-administered antigens (n=8 for each injection schedule), 30 µg (n=8 for each schedule), 100 µg (n=8 for each schedule), or HBV and placebo (n=6 for each schedule) in three sequential cohorts. Co-administration of the vaccines was well tolerated; the most frequent solicited adverse events were mild-to-moderate injection-site pain, observed in up to 12 (75%) of 16 participants per vaccine group, and mild headache (12 [25%] of 48) and fever (11 [23%] of 48). No vaccine-related serious adverse events were observed. Significant anti-*Na*-APR-1(M74) and anti-*Na*-GST-1 IgG levels were induced in a dose-dependent manner, with peaks seen 14 days after the third vaccinations, regardless of dose (for *Na*-APR-1[M74], geometric mean levels [GML]=2295·97 arbitrary units [AU] and 726·89 AU, while for *Na*-GST-1, GMLs=331·2 AU and 21·4 AU for the month 0, 2, and 6 and month 0, 2, and 4 schedules, respectively). The month 0, 2, and 6 schedule induced significantly higher IgG responses to both antigens (p=0·01 and p=0·04 for *Na*-APR-1[M74] and *Na*-GST-1, respectively).

Interpretation Co-administration of recombinant *Na*-APR-1(M74) and *Na*-GST-1 to school-aged Gabonese children was well tolerated and induced significant IgG responses. These results justify further evaluation of this antigen combination in proof-of-concept controlled-infection and efficacy studies in hookworm-endemic areas.

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Research in context

Evidence before this study

Children in lower-income regions in tropical and subtropical countries bear the majority of the burden of disease due to hookworm. There are no licensed vaccines to prevent this parasitic infection. Recombinant *Necator americanus* glutathione S-transferase-1 (*Na*-GST-1) and modified enzymatically inactive *N americanus* aspartic protease-1 (*Na*-APR-1[M74]) are the two most advanced candidate vaccine antigens. Vaccination with these recombinant proteins induces antibodies that are hypothesised to neutralise the native enzymes, both of which perform essential functions in the adult hookworm's blood digestion pathway. The end goal is prevention of worm development or parasite death, resulting in reduced disease manifestations and interrupted transmission. In clinical trials these vaccines have been well tolerated and safe both when administered separately or when co-administered to healthy adults. Co-administration of *Na*-GST-1 and *Na*-APR-1(M74) to school-age children is meant to evaluate the safety and immunogenicity of the combination in this age group, and to guide the choice of dosing schedule. We searched PubMed and the Cochrane Library for research articles published between Jan 1, 1980, and Dec 5, 2023, using the terms "hookworm", "vaccine", "clinical trial", "phase", and "children". No language restrictions were applied. The six manuscripts identified included four that reported the results of phase 1 trials with long-term safety or immunogenicity follow-up; three reported on trials that tested either *Na*-GST-1, *Na*-APR-1(M74), or both in adults. The trials of *Na*-GST-1 and *Na*-APR-1(M74) showed that these investigational vaccines were safe, well tolerated, and induced significant antigen-specific IgG responses.

Added value of this study

Our study describes the first phase 1 clinical trial of any novel vaccine candidates (and in this case, two) against human hookworm infection (*Na*-APR-1[M74] and *Na*-GST-1) conducted in children. To our knowledge it is the first report on the safety and immunogenicity of two leading hookworm vaccine candidates administered to healthy school-age children living in an *N americanus*-endemic area of Central Africa.

Implications of all the available evidence

A paediatric vaccine against hookworm would be a useful tool in preventing disease, particularly iron deficiency and anaemia, in high-risk populations in endemic areas. Co-administration of *Na*-GST-1 and *Na*-APR-1(M74) to school-age children resulted in rapid production of IgG antibody responses against both vaccine antigens, with the highest levels observed after the third dose of each antigen. Antibody responses were strongest when vaccinations were administered according to a month 0, 2, 6 schedule. Further clinical studies, including controlled infection and field efficacy trials of these vaccines, are warranted.

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Introduction

Hookworm is a neglected tropical disease with an immense yet under-appreciated effect on global health. Up to 400 million individuals worldwide are infected with one of three hookworm species (*Necator americanus*, *Ancylostoma duodenale*, or *Ancylostoma ceylanicum*), primarily in rural areas of low-income and middle-income countries with inadequate access to safe water and sanitation.[1] Chronic infection results in iron deficiency and anaemia, complications that particularly affect children due to their increased iron requirements and low stores. Children in hookworm-endemic regions are often infected while young, and can remain chronically infected into adulthood due to prolonged parasite survival in the host intestinal tract, in addition to an immune response to infection that fails to protect upon re-exposure.[2,3] Chronic infection in childhood can result in permanent disability due to negative impacts on physical and cognitive development.[4]

Children of pre-school and school age are the principal focus of hookworm control efforts, which currently primarily consist of periodic distribution of anthelminthic medications in mass drug administration campaigns.[5,6] However, rapid post-treatment re-infection and potential emergence of anthelminthic drug resistance have prevented adequate disease control in children in endemic areas, thereby justifying investment in alternative or supplemental prevention tools such as an effective paediatric vaccine.

N americanus glutathione S-transferase-1 (*Na*-GST-1) and *N americanus* aspartic protease-1 (*Na*-APR-1), modified to make it enzymatically inactive (*Na*-APR-1[M74]), are the lead candidates being developed as components of the human hookworm vaccine. Both antigens target enzymes that are components of the blood digestion pathway of adult hookworms parasitising the human host intestinal tract.[7] Antibodies to *Na*-GST-1 and *Na*-APR-1 induced by vaccination are hypothesised to interfere with this pathway, thereby impairing the parasite's ability to feed, protecting the host against intestinal blood loss.[8]

Na-GST-1 and *Na*-APR-1(M74) have been expressed as recombinant proteins and formulated on Alhydrogel (Biosector, Frederikssund, Denmark).[9,10] Phase 1 trials of each product conducted in healthy, hookworm-naive adults in the USA[11,12] and in hookworm-exposed adults in Brazil[11] have demonstrated both the safety and immunogenicity of each antigen when delivered singly. The addition of a synthetic monophosphoryl lipid A-containing immunostimulant (glucopyranosyl lipid A) in aqueous formulation (GLA-AF) resulted in enhanced IgG antibody responses to *Na*-APR-1(M74) on Alhydrogel,[12] but not to *Na*-GST-1 on Alhydrogel,[11] leading to discontinuation of the latter formulation in subsequent clinical trials.

To increase the likelihood of efficacy, the human hookworm vaccine is being developed as a bivalent product containing both *Na*-APR-1(M74) and *Na*-GST-1, targeting the hookworm blood digestion pathway at two critical points. Given the results of the phase 1 trials of each antigen administered alone, a phase 1 trial of co-administered *Na*-APR-1(M74) on Alhydrogel and *Na*-GST-1 on Alhydrogel was conducted in adults in the hookworm-endemic region of Lambaréné, Gabon.[13] In this study, co-administration of the vaccines was well tolerated and neither negatively nor positively affected either the strength or the duration of IgG responses to either antigen.

Clinical development has therefore advanced to testing the co-administered antigens in school-aged children aged between 6 and 10 years in sub-Saharan Africa (Gabon, in this report), where there is a high burden of hookworm disease in this age group.[14] To our knowledge, this is the first hookworm vaccine trial to be conducted in children and was designed to provide crucial safety and immunogenicity information on co-administration of *Na*-APR-1(M74) and *Na*-GST-1 in this population.

Methods

Study design and participants

In this observer-masked, randomised, controlled dose-escalation phase 1 clinical trial of co-administered *Na*-APR-1(M74) and *Na*-GST-1 vaccines, conducted at the Centre de Récherches Médicales de Lambaréné, a clinical research centre located in Lambaréné, Gabon, we recruited children aged between six and ten years who were long-term residents of the study area. Lambaréné is a region where infection with *N americanus* in school-aged children is common.[14] Complete eligibility criteria are listed in appendix; children with concurrent medical conditions or clinical laboratory abnormalities were excluded (pp 38–39).

See Online for appendix

Examination for ova and parasites was performed by microscopy on faecal and urine samples during screening, and children were treated with an appropriate medication at least two weeks before initiation of vaccinations if infection with an intestinal helminth or *Schistosoma haematobium* was detected.

The study was approved by the National Ethics Committee of Gabon (#009/2016/SG/CNE) and conducted under an investigational new drug application (IND#016184) to the US Food and Drug Administration. Parents or legal guardians of potential participants provided written informed consent. If the parent or guardian subsequently passed an informed consent comprehension questionnaire, the child signed an informed assent form before screening procedures were initiated. The trial is registered with ClinicalTrials.gov, NCT02839161.

Randomisation and masking

Eligible children were enrolled consecutively into three cohorts. Assignment to cohorts was done via an open sequential method, whereas within each cohort, vaccine allocation was done in a randomised observer-masked way. In the first, second, and third cohorts, children received 10 µg, 30 µg, and 100 µg of each antigen (co-administered *Na*-APR-1[M74] on Alhydrogel and *Na*-GST-1 on Alhydrogel; henceforth referred to as the co-administered antigens), respectively (n=16); or ENGERIX-B hepatitis B vaccine ([HBV], GlaxoSmithKline Biologicals, Rixensart, Belgium) co-administered with saline placebo (n=6). Within each cohort, children were randomly allocated to receive vaccinations on study days 0, 56, and 112, or on days 0, 56, and 180 per-protocol (with months 0, 2, and 4, and months 0, 2, and 6 acting as practical equivalent periods), such that eight children received the co-administered antigens, and two children received HBV with saline according to each schedule.

Standard block randomisation was used, with each block of four containing one control participant (ENGERIX-B HBV and normal saline); the randomisation list was provided to the study pharmacist in a sealed envelope. Only those who prepared the vaccines were aware of vaccine allocations.

Saline was selected as a control in children randomly allocated to receive ENGERIX-B so all participants would receive injections in both arms. Syringes were masked using opaque tape, and vaccinators were not involved in evaluation of reactogenicity or adverse events. Study personnel conducting immunological assays were masked.

Procedures

Recombinant *Na*-GST-1 and *Na*-APR-1(M74) were manufactured as 0·1 mg/mL suspensions and adsorbed to 0·8 mg/mL Alhydrogel, as described previously.[10,13] GLA-AF is an aqueous solution supplied in multi-dose vials containing 25 µg/mL GLA without preservative. Doses of *Na*-APR-1(M74) on Alhydrogel were mixed with 5 µg GLA-AF within 24 h before vaccination. ENGERIX-B HBV was supplied in single-dose 1·0 mL vials containing 20 µg of recombinant HBsAg and 500 µg of aluminium as aluminium hydroxide. Injections were administered intramuscularly in the deltoid of each arm. Children were followed up for 9 months after their final vaccinations.

Safety data for the 14 days following administration of the first vaccinations to the first cohort were reviewed by a safety monitoring committee before initiating vaccinations in the second cohort. A review of safety data from the second cohort was also completed before initiating vaccinations in the third cohort. In addition, a physician based in Lambaréné served as an independent medical monitor overseeing participant safety.

Serum levels of *Na*-APR-1(M74)-specific and *Na*-GST-1-specific IgG antibodies expressed in Arbitrary Units (AU) were measured using qualified indirect ELISAs, as described previously[11,12,15,16] and in appendix (pp 89–90).

Outcomes

The primary outcome was safety and reactogenicity. This outcome was evaluated by the frequency and severity of: first, serious adverse events (SAEs) occurring from the time of the first study vaccination to 9 months after the last study vaccination; second, solicited adverse events (injection site reactions and systemic reactions [appendix p 58]) occurring within 14 days after each study vaccination; third, clinical safety laboratory adverse events occurring within 14 days of each study vaccination; fourth, unsolicited adverse events occurring within 28 days after each evaluation; fifth, new-onset chronic medical conditions occurring within 9 months of the last study vaccination; and sixth, adverse events of special interest (AESIs) occurring within 9 months from the last study vaccination. The secondary outcome was level of antigen-specific antibody responses to the vaccine antigens, measured by the highest IgG levels against the antigens generated approximately 14 days after the third vaccination.

Solicited injection site and systemic reactions, unsolicited adverse events, and safety laboratory test results were graded as mild, moderate, or severe (appendix pp 58–62). The occurrence of AESIs was monitored throughout the trial given use of GLA-AF; these are listed in appendix (p 64).[17]

Statistical analysis

The number and percentage of study participants experiencing each adverse event were tabulated by vaccine group. Given the small group sizes and no apparent differences between the incidence or severity of adverse events at each antigen dose level, results from the month 0, 2, and 4 and month 0, 2, and 6 vaccination schedules were combined for each dosage level. Data from participants in the comparator group were pooled, or combined from the three different cohorts. Logistic regression was used to test for differences in adverse event proportions between groups, with the outcome being occurrence of the adverse event and the exposure being the vaccine type with results stratified by timepoint. The trial was not powered to detect statistically significant differences between the groups.

IgG levels in response to *Na*-APR-1(M74) and *Na*-GST-1 were compared by study day among vaccine groups using Kruskal-Wallis tests. If a statistically significant result ($p<0·05$) was obtained, pair-wise comparisons were made using Wilcoxon rank sum tests, with significance determined using the Holm-Bonferroni adjustment. ELISA reactivity thresholds were used to define IgG seroresponders to each antigen as described previously.[15,18] Proportions of responders (with 95% CIs from the Wilson score) on each day were compared using Fisher's exact tests with Bonferroni correction for multiple comparisons.

Longitudinal models were constructed to assess changes in antibody levels over time by dose and schedule. A linear mixed effects model was fitted with log-transformed IgG responses as the dependent variable and antigen doses as independent variables. To assess differences in IgG responses over time between the two vaccination schedules, a linear mixed effects model was fitted with log-transformed IgG responses as the dependent variable and vaccination schedules as independent variables. SAS version 9.4 was used for all analyses.

Role of the funding source

The funder provided study oversight to ensure control over use of EU public funds, but played no role in study design, data collection, analysis, interpretation, or manuscript preparation. The authors had full access to all study data and had final responsibility for the decision to submit for publication.

Results

Between Jan 23 and Oct 3, 2017, 137 children were screened, of whom 76 were eligible. 16 children were eligible but were not enrolled due to reaching the enrolment threshold. The remaining 60 children were progressively enrolled into three study cohorts (figure 1). The mean age of enrolled children was 7·4 years (range, 6–10; SD 0·5). 26 (43%) were female and 34 (57%) were male (table 1).

Three children did not receive all vaccinations. The parents of one child withdrew consent for personal reasons after the first injections of 30 µg of the co-administered antigens (on the month 0, 2, 6 schedule). Two children in the 30 µg co-administered antigen group (on the month 0, 2, 4 schedule) did not receive all injections due to being away from the study area: one did not receive the third set of vaccinations, while the other missed the second and third vaccinations. These three participants were excluded from the immunology analyses after their first missed dose.

Co-administration of the antigens was well tolerated, regardless of antigen dose. None of the children withdrew because of significant reactogenicity, unsolicited adverse events, or abnormal clinical laboratory results. No AESIs or vaccine-related serious adverse events (SAEs) occurred. One unrelated SAE was reported in a child vaccinated with 30 µg of the two antigen preparations, and was a hospitalisation for surgical repair of a strangulated inguinal hernia that resolved without sequelae 4 months following final vaccination.

The most frequent solicited injection site reactions were mild tenderness and pain (table 2), which were observed in up to 11 (69%) of 16 participants per injection per vaccine group. Only one severe (grade 3) injection site reaction was reported: injection site pain in a child aged 8 years starting the day after second co-administration of 10 µg of the two antigen preparations, in the arm in which 10 µg *Na*-GST-1 on Alhydrogel was administered. This was accompanied by moderate

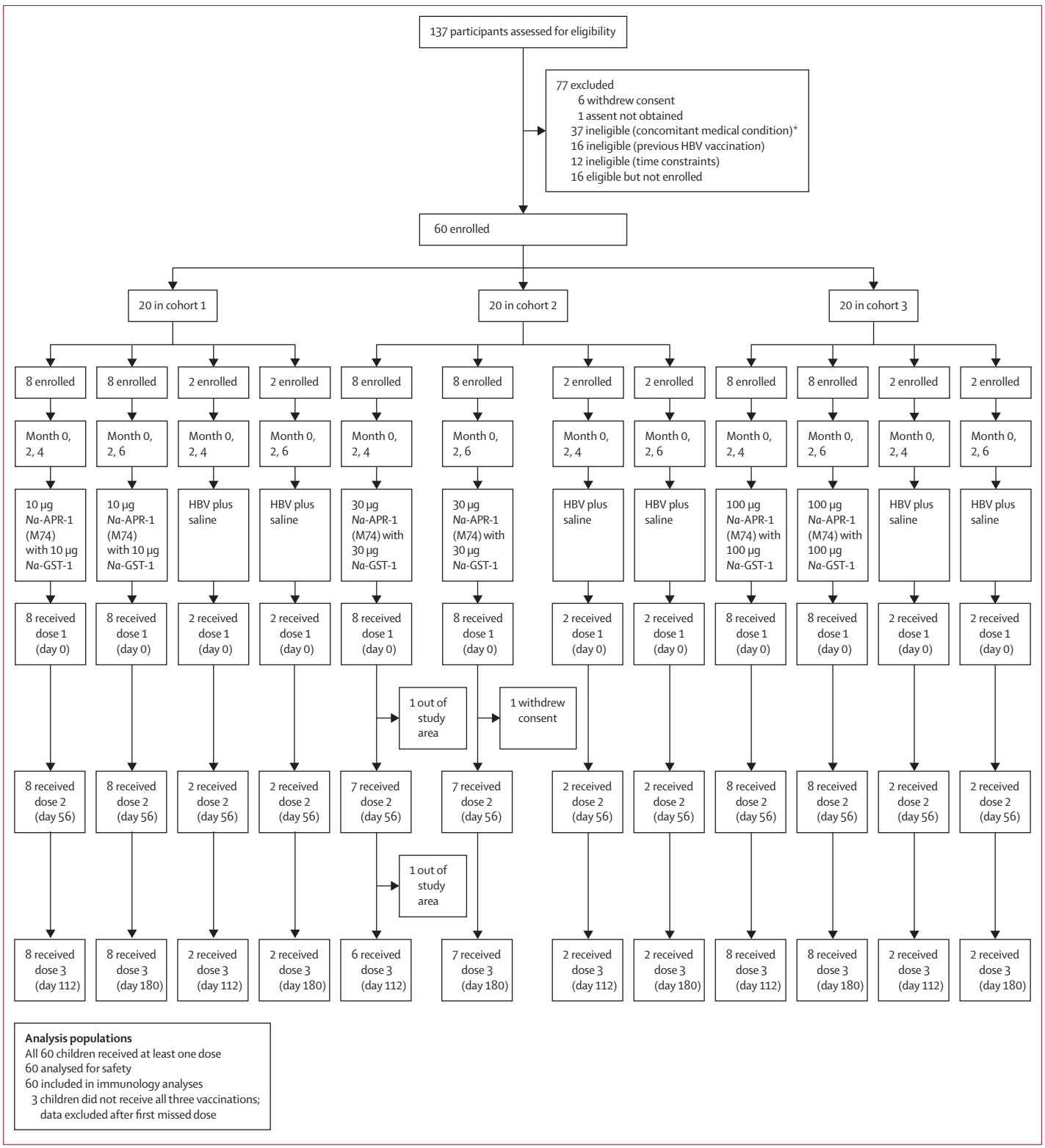

Figure 1: **CONSORT diagram**
HBV=hepatitis B vaccine. *Na*-APR-1=*Necator americanus* aspartic protease-1. *Na*-APR-1(M74)=modified enzymatically inactive *Na*-APR-1. *Na*-GST-1=*N americanus* glutathione S-transferase-1. *Children could have been ineligible due to more than one exclusion criterion.

	10 µg *Na*-APR-1 + 10 µg *Na*-GST-1 (N=8)	10 µg *Na*-APR-1 + 10 µg *Na*-GST-1 (N=8)	30 µg *Na*-APR-1 + 30 µg *Na*-GST-1 (N=8)	30 µg *Na*-APR-1 + 30 µg *Na*-GST-1 (N=8)	100 µg *Na*-APR-1 + 100 µg *Na*-GST-1 (N=8)	100 µg *Na*-APR-1 + 100 µg *Na*-GST-1 (N=8)	HBV plus saline (N=6)	HBV (N=6)
Vaccination schedule, months	0, 2, 4	0, 2, 6	0, 2, 4	0, 2, 6	0, 2, 4	0, 2, 6	0, 2, 4	0, 2, 6
Sex								
Male	7 (88%)	6 (75%)	4 (50%)	3 (37%)	6 (75%)	1 (12%)	3 (50%)	4 (67%)
Female	1 (12%)	2 (25%)	4 (50%)	5 (63%)	2 (25%)	7 (88%)	3 (50%)	2 (33%)
Race								
Black or African American	8 (100%)	8 (100%)	8 (100%)	8 (100%)	8 (100%)	8 (100%)	6 (100%)	6 (100%)
Ethnicity								
Not Hispanic or Latino	8 (100%)	8 (100%)	8 (100%)	8 (100%)	8 (100%)	8 (100%)	6 (100%)	6 (100%)
Age*, years	6·8 (0·7)	8·1 (1·1)	6·9 (1·0)	7·9 (1·1)	7·5 (1·6)	7·0 (0·9)	7·8 (1·5)	7·2 (1·6)

Data are n (%) or mean (SD) unless otherwise stated. *Na*-APR-1=*Necator americanus* aspartic protease-1 on Alhydrogel. *Na*-GST-1=*N americanus* glutathione S-transferase-1 on Alhydrogel. N=Number of participants in the safety population. HBV=hepatitis B vaccine. *At time of informed consent.

Table 1: **Demographic and baseline characteristics of study participants by vaccine group**

injection site swelling and mild tenderness in the same arm, but without systemic reactogenicity. The injection site pain lasted for 2 days and required management with oral ibuprofen.

Injection site pain and tenderness occurred substantially more frequently in the groups vaccinated with the co-administered antigens compared with the HBV and saline comparator group (table 2). However, when comparing the frequency and severity of injection site reactions between the hookworm vaccine dose groups (ie, 10 µg *vs* 30 µg *vs* 100 µg), there were no substantial differences in the incidence or severity of injection site reactions. The incidence of injection site reactions with the *Na*-APR-1(M74) preparation compared with the *Na*-GST-1 preparation show pain, tenderness, and swelling occurring more frequently with *Na*-APR-1(M74) ($p<0·001$). Injection site tenderness was only reported in deltoid muscles that were injected with the *Na*-APR-preparation, although in all cases this was mild in intensity. Moderate and severe administration site reactions occurred more commonly in participants who received the co-administered antigens: two (17%) of 12 children receiving HBV and saline reported moderate injection site pain; this increased to between five (31%) and nine (56%) of 16 for those vaccinated with the co-administered antigen preparations ($p=0·04$). After the third set of injections, there were no differences in injection site reactions between those allocated to the month 0, 2, 4 schedule and those on the month 0, 2, 6 schedule. Overall, injection site reactions resolved after a median of 1 day (range 0–4).

The most-frequent solicited systemic events were headache and fever, occurring in 15 (25%) and 12 (20%) of children, respectively (table 2). Most systemic reactions were mild, with only a few moderate reactions in each vaccine group. Three severe febrile reactions were reported: two in children vaccinated with 10 µg of the co-administered antigens after the first set of injections, and one in a child after the third set of vaccinations with 100 µg of the co-administered antigens. Although these severe fevers occurred during the 14 day post-vaccination period, the two events in the 10 µg group were considered unlikely to be related to vaccination (due to clinical judgement of the investigator at the site, mostly based on the timing of the fever and co-occurrence of symptoms indicating a potential alternate diagnosis, eg, infection) while the one from the 100 µg group was deemed unrelated to vaccination given a concomitant infection with *Plasmodium falciparum*.

Neither incidence nor severity of solicited systemic reactions differed between the three dosage levels of the co-administered antigens. However, the occurrence of systemic events was more frequent in those vaccinated with any dose of the co-administered antigen preparations compared to the HBV vaccine with saline. Solicited systemic events occurred with similar frequency after each of the three sets of injection, although statistical comparisons between the frequencies of solicited systemic adverse events could not be made due to their low numbers.

118 (80 mild; 33 moderate; five severe) unsolicited adverse events occurred in 47 (78%) of 60 participants (appendix pp 93–101). Of these, 28 (20 mild; seven moderate; one severe) were vaccine-related. The severe unsolicited adverse event assessed as being possibly related to the study vaccinations was a decrease in absolute neutrophil count from $2·48×10^3$/mm³ on day 56, before receiving the second doses of 30 µg of the co-administered antigens, to $0·39×10^3$/mm³ on Day 70. It recovered to $1·48×10^3$/mm³ on day 84. Neither a peripheral smear nor a manual white blood cell differential was performed on day 70, so it is not possible to determine if the low absolute neutrophil count value on that day was due to sample handling, laboratory error, or a true value possibly related to the study vaccine. No differences were found in the incidence of vaccine-related unsolicited adverse events between vaccine groups, although no formal statistics were performed to detect any differences, given the small number of events.

	10 μg *Na*-APR-1 + 10 μg *Na*-GST-1 (N=16)			30 μg *Na*-APR-1 + 30 μg *Na*-GST-1 (N=16)			100 μg *Na*-APR-1 + 100 μg *Na*-GST-1 (N=16)			HBV + Saline (N=12)		
	Mild	Moderate	Severe	Mild	Moderate	Severe	Mild	Moderate	Severe	Mild	Moderate	Severe
Injection site events												
Injection site pain	9 (56%)	5 (31%)	1 (6%)	1 (6%)	9 (56%)	0	12 (75%)	5 (31%)	0	5 (42%)	2 (17%)	0
Na-GST-1	11 (69%)	1 (6%)	0	5 (31%)	4 (25%)	0	8 (50%)	2 (13%)	0	NA	NA	NA
Na-APR-1(M74)	8 (50%)	5 (31%)	1 (6%)	9 (56%)	6 (38%)	0	9 (56%)	4 (25%)	0	NA	NA	NA
HBV	NA	NA	NA	NA	NA	NA	NA	NA	NA	4 (33%)	2 (17%)	0
Saline	NA	NA	NA	NA	NA	NA	NA	NA	NA	3 (25%)	0	0
Injection site tenderness	4 (25%)	0	0	0	0	0	2 (13%)	0	0	0	0	0
Na-GST-1	0	0	0	0	0	0	0	0	0	NA	NA	NA
Na-APR-1(M74)	4 (25%)	0	0	0	0	0	2 (13%)	0	0	NA	NA	NA
HBV	NA	NA	NA	NA	NA	NA	NA	NA	NA	0	0	0
Saline	NA	NA	NA	NA	NA	NA	NA	NA	NA	0	0	0
Injection site swelling	2 (13%)	1 (6%)	0	1 (6%)	0	0	8 (50%)	1 (6%)	0	2 (17%)	0	0
Na-GST-1	1 (6%)	0	0	0	0	0	2 (13%)	0	0	NA	NA	NA
Na-APR-1(M74)	1 (6%)	1 (6%)	0	1 (6%)	0	0	6 (38%)	1 (6%)	0	NA	NA	NA
HBV	NA	NA	NA	NA	NA	NA	NA	NA	NA	2 (17%)	0	0
Saline	NA	NA	NA	NA	NA	NA	NA	NA	NA	0	0	0
Systemic events												
Fever	2 (13%)	0	2 (13%)	0	1 (6%)	0	3 (19%)	2 (13%)	1 (6·3)	1 (8%)	0	0
Headache	5 (31%)	1 (6%)	0	1 (6%)	0	0	4 (25%)	1 (6%)	0	2 (17%)	1 (8%)	0
Myalgia	0	0	0	0	0	0	1 (6%)	0	0	0	0	0
Arthralgia	0	1 (6%)	0	0	0	0	1 (6%)	0	0	0	0	0
Vomiting	1 (6%)	0	0	0	0	0	0	1 (6%)	0	1 (8%)	0	0
Nausea	0	0	0	1 (6%)	0	0	0	0	0	0	0	0
Clinical laboratory events												
Decreased haemoglobin	1 (6%)	0	0	1 (6%)	3 (19%)	0	7 (44%)	2 (13%)	0	2 (17%)	0	0
Decreased platelet count	0	0	1 (6%)	0	1 (6%)	0	1 (6%)	0	2 (13%)	0	0	0
Decreased white blood cell count	0	0	0	0	0	0	1 (6%)	1 (6%)	0	1 (8%)	0	0
Increased white blood cell count	1 (6%)	0	0	0	0	0	1 (6%)	1 (6%)	0	0	0	0
Decreased absolute neutrophil count	0	0	0	0	0	2 (13%)	1 (6%)	0	0	0	0	0

Participants are counted only once if they experienced more than one of the same event. Data from the two vaccination schedules (month 0, 2, and 4 and month 0, 2, and 6) at each dosage level are combined. Severity levels for each laboratory parameter were defined according to standard toxicity grading tables for clinical vaccine trials. *Na*-APR-1=*Necator americanus* aspartic protease-1. *Na*-GST-1=*N americanus* glutathione S-transferase-1. HBV=hepatitis B vaccine. NA=not applicable. *Na*-APR-1(M74)=modified enzymatically inactive *Na*-APR-1.

***Table 2*: Number and percentage of participants experiencing solicited injection site, systemic, and clinical laboratory adverse events after any dose of vaccine by event, maximum severity, and vaccine group**

Although several adverse events related to abnormal haematology and clinical chemistry parameters were observed, no concerning differences between groups vaccinated with the co-administered antigens were identified, nor were major differences observed between the co-administered antigens and HBV and saline recipients (table 2). All laboratory adverse events were asymptomatic and resolved spontaneously. The most common clinical laboratory adverse events were mild or moderate decreases in haemoglobin concentration (21 events). There were no severe decreases in haemoglobin concentration, and none of the decreases were considered either definitely or probably related to vaccination. All cases resolved without sequelae.

The second most common haematologic laboratory adverse event was thrombocytopaenia. Five events were observed, three of which were severe, the first after the second administration of 10 μg of the co-administered antigens, and two in children in the 100 μg co-administered antigen group, one after the second set of vaccinations and the other after the third set of vaccinations. These severe reactions were assessed as being unrelated to the study vaccines. Four events resolved without sequelae; one was ongoing at the end of the study since follow-up measurements were not performed.

Three mild increases in serum alanine aminotransferase were observed in two children assigned to the 30 μg co-administered antigen group and one in the HBV plus saline comparator group. These abnormalities were assessed as being possibly related to vaccination, but were asymptomatic and resolved without sequelae.

No increases in creatinine concentration were observed during the study.

Tables 3 and 4 summarise anti-*Na*-APR-1(M74) and anti-*Na*-GST-1 IgG antibody levels respectively, by day and vaccine group. Longitudinal trends in geometric mean IgG levels (GML) by vaccine group are shown in figure 2. For both *Na*-APR-1(M74) and *Na*-GST-1, peak IgG levels were observed 2 weeks after the third injections,

	10 μg *Na*-APR-1(M74) + 10 μg *Na*-GST-1		30 μg *Na*-APR-1(M74) + 30 μg *Na*-GST-1		100 μg *Na*-APR-1(M74) + 100 μg *Na*-GST-1		HBV + saline
	0, 2, 4 months	0, 2, 6 months	0, 2, 4 months	0, 2, 6 months	0, 2, 4 months	0, 2, 6 months	
Baseline (pre-dose 1)							
N	8	8	6	7	8	8	12
GML (95% CI)	2·46 (0·96–6·29)	2·25 (1·19–4·26)	1·86 (0·8–4·31)	2·09 (0·99–4·40)	2·11 (1·22–3·67)	2·44 (1·15–5·17)	1·88 (1·35–2·60)
Responders, n (% [95% CI])	2 (25% [7–59])	2 (25% [7–59])	1 (17% [3–56])	2 (28·6, 8–64)	2 (25% [7–59])	3 (38% [14–69])	1 (8% [0–35])
Day 7							
N	8	8	6	7	8	8	12
GML (95% CI)	2·73 (1·13–6·59)	2·79 (1·18–6·57)	1·34 (NA)	2·05 (1·03–4·06)	2·15 (1·21–3·83)	2·64 (1·19–5·88)	1·82 (1·26–2·62)
Responders, n (% [95% CI])	3 (38% [14–69])	3 (38% [14–69])	0 (0% [0–39])	2 (29% [8–64])	2 (25% [7–59])	3 (38% [14–69])	2 (17% [5–45])
Day 14							
N	8	8	6	7	8	8	12
GML (95% CI)	2·70 (1·11–6·54)	3·08 (1·36–6·96)	5·03 (3·49–7·26)	3·09 (1·45–6·59)	5·83 (3·07–11·10)*	5·27 (1·88–14·74)	1·66 (1·20–2·30)*
Responders, n (% [95% CI])	3 (38% [14–69])	3 (38% [14–69])	5 (83% [44–97])	4 (57% [25–84])	6 (75% [41–93])	5 (63% [31–86])	2 (17% [5–45])
Day 28							
N	8	8	6	7	8	8	12
GML (95% CI)	2·25 (0·96–5·29)	2·73 (1·36–5·47)	4·06 (1·58–10·43)	3·15 (1·42–7·03)	8·12 (5·30–12·43)*	7·93 (2·04–30·80)	2·32 (1·50–3·60)*
Responders, n (% [95% CI])	2 (25% [7–59])	3 (38% [14–69])	4 (67% [30–90])	3 (43% [16–75])	8 (100% [68–100])	5 (63% [31–86])	5 (42% [19–68])
Day 56 (pre-dose 2)							
N	Not measured	7	6	7	8	8	10
GML (95% CI)	Not measured	2·43 (1·20–4·95)	2·51 (1·19–5·29)	2·61 (1·27–5·36)	5·20 (2·87–9·41)	6·05 (2·09–17·51)	2·37 (1·48–3·79)
Responders, n (% [95% CI])	Not measured	3 (43% [16–75])	3 (50% [19–82])	2 (29% [8–64])	6 (75% [41–93])	5 (63% [31–86])	4 (40% [17–69])
Day 63 (7 days post-dose 2)							
N	8	8	6	7	8	8	12
GML (95% CI)	1·90 (0·83–4·32)	2·89 (1·42–5·91)	4·86 (2·20–10·72)	3·99 (1·98–8·01)	22·35 (6·83–73·15)*	43·02 (6·41–288·58)*	1·90 (1·33–2·70)*
Responders, n (% [95% CI])	1 (13% [2–47])†	4 (50% [22–79])	5 (83% [44–97])	5 (71% [36–92])	8 (100% [68–100])†‡	7 (88% [53–98])	2 (17% [5–45])‡
Day 70 (14 days post-dose 2)							
N	7	8	6	7	8	8	11
GML (95% CI)	4·28 (1·05–17·36)	3·82 (1·82–8·00)	38·01 (9·46–152·69)	57·67 (9·37–354·84)	360·29 (64·83–2002·22)*	243·72 (52·53–1130·80)*	2·23 (1·47–3·39)*
Responders, n (% [95% CI])	3 (43% [16–75])	5 (63% [31–86])	6 (100% [61–100])	7 (100% [65–100])	8 (100% [68–100])	8 (100% [68–100])	3 (27% [10–57])
Day 84 (28 days post-dose 2)							
N	8	8	6	7	8	8	11
GML (95% CI)	2·55 (1·02–6·36)	3·38 (1·74–6·57)	20·31 (6·41–64·39)	11·81 (3·95–35·34)	177·81 (59·17–534·31)*	174·11 (33·90–894·24)*	2·30 (1·35–3·92)*
Responders, n (% [95% CI])	2 (25% [7–59])	5 (63% [31–86])	6 (100% [61–100])	6 (86% [49–97])	8 (100% [68–100])	8 (100% [68–100])	4 (36% [15–65])

(Table 3 continues on next page)

	10 µg *Na*-APR-1(M74) + 10 µg *Na*-GST-1		30 µg *Na*-APR-1(M74) + 30 µg *Na*-GST-1		100 µg *Na*-APR-1(M74) + 100 µg *Na*-GST-1		HBV + saline
	0, 2, 4 months	0, 2, 6 months	0, 2, 4 months	0, 2, 6 months	0, 2, 4 months	0, 2, 6 months	
(Continued from previous page)							
Day 112 or 180 (pre-dose 3)							
N	8	8	1§	7	8	8	10
GML (95% CI)	2·61 (0·92–7·43)	2·70 (1·33–5·45)	9·34 (NA)	6·82 (3·03–15·32)	44·90 (18·61–108·33)*	10·82 (5·53–21·15)	3·10 (1·97–4·89)*
Responders, n (% [95% CI])	2 (25% [7–59])	3 (38% [14–69])	1 (100% [21–100])	6 (86% [49–97])	8 (100% [68–100])	7 (88% [53–98])	5 (50% [24–76])
Day 119 or 187 (7 days post-dose 3)							
N	8	8	6	7	8	8	12
GML (95% CI)	4·73 (1·64–13·68)	5·71 (2·50–13·00)	113·92 (14·14–917·99)*	24·75 (10·68–57·37)*	61·32 (27·23–138·09)*	505·69 (176·82–1446·28)*	2·50 (1·60–3·89)*
Responders, n (% [95% CI])	5 (63% [31–86])	6 (75% [41–93])	6 (100% [61–100])	7 (100% [65–100])	8 (100% [68–100])	8 (100% [68–100])	4 (33% [14–61])
Day 126 or 194 (14 days post-dose 3)							
N	8	8	5	7	8	1§	10
GML (95% CI)	9·52 (4·36–20·78)	9·08 (3·81–21·64)	219·53 (13·05–3694·51)*	266·38 (84·44–840·35)*	726·89 (186·95–2826·33)*	2295·97 (NA)	2·36 (1·48–3·74)*
Responders, n (% [95% CI])	6 (75% [41–93])	6 (75% [41–93])	5 (100% [57–100])	7 (100% [65–100])	8 (100% [68–100])	1 (100% [21–100])	3 (30% [11–60])
Day 140 or 208 (28 days post-dose 3)							
N	8	8	6	6	8	8	11
GML (95% CI)	8·27 (3·52–19·45)	7·18 (2·82–18·28)	109·22 (15·14–787·63)	88·69 (41·91–187·67)	43·73 (19·70–97·05)*	859·68 (276·44–2673·40)*	3·98 (1·69–9·36)*
Responders, n (% [95% CI])	7 (88% [53–98])	6 (75% [41–93])	6 (100% [61–100])	6 (100% [61–100])	8 (100% [68–100])	8 (100% [68–100])	7 (64% [35–85])
Day 200 or 268 (follow-up)							
N	8	8	6	7	8	8	12
GML (95% CI)	4·82 (1·99–11·64)	6·24 (2·93–13·32)	10·19 (5·58–18·60)	11·43 (5·21–25·08)	25·01 (16·51–37·89)*	42·96 (20·18–91·45)*	4·08 (2·59–6·43)*
Responders, n (% [95% CI])	4 (50% [22–79])	5 (63% [31–86])	6 (100% [61–100])	6 (86% [49–97])	8 (100% [68–100])	8 (100% [68–100])	7 (58% [32–81])
Day 290 or 358 (follow-up)							
N	8	8	6	7	8	8	12
GML (95% CI)	3·60 (1·43–9·02)	2·84 (1·57–5·14)	10·75 (6·38–18·13)	9·06 (4·00–20·54)	12·32 (9·04–16·77)	19·21 (9·29–39·74)*	3·03 (1·70–5·42)*
Responders, n (% [95% CI])	4 (50% [22–79])	2 (25% [7–59])	6 (100% [61–100])	6 (86% [49–97])	8 (100% [68–100])	8 (100% [68–100])	5 (42% [19–68])

Na-APR-1=*Necator americanus* aspartic protease-1. *Na*-APR-1(M74)=modified enzymatically inactive *Na*-APR-1. *Na*-GST-1=*N americanus* glutathione S-transferase-1. HBV=hepatitis B vaccine. GML=geometric mean level. *p<0·05 for comparison with HBV/saline comparator group (Wilcoxon rank sum test). †p<0·01 for comparison with HBV plus saline comparator group (Fisher's exact test). ‡p<0·01 for comparison between groups (Fisher's exact test). §At some timepoints, either samples were not collected, or there was an issue with sample processing, storage, or shipment (usually due to temperature excursions and shipment of samples to the USA for testing). IgG levels were measured in arbitrary units by qualified ELISA. Seropositivity was defined as having an antibody level about the reactivity threshold for the assay. Participants in the comparator group (HBV plus saline) vaccinated according to the two schedules were combined.

***Table 3*: Seroresponders and anti-*Na*-APR-1(M74) IgG antibody levels by vaccine group, dose schedule, and study day**

regardless of vaccination schedule. Minimal IgG responses to either antigen were observed with the 10 µg doses.

For *Na*-APR-1(M74), peak responses were seen in children vaccinated with 100 µg of the co-administered antigens on the month 0, 2, 6 vaccination schedule, with a GML of 2295·97 AU on day 194, compared with 726·89 AU (95% CI 186·95–2826·33) on day 126 for those vaccinated with the same 100 µg doses using the month 0, 2, 4 schedule (table 3). By comparison, those vaccinated with HBV plus saline had a GML of 2·36 AU (1·48–3·74) at the equivalent timepoint (ie, 2 weeks after third injections).

A significant difference in anti-*Na*-APR-1(M74) IgG levels between groups was first observed on day 14 between those who received 100 µg of the co-administered antigens on the month 0, 2, and 4 schedule and those who received the HBV plus saline (p=0·04) and this difference remained statistically significant except on days 56 and 290 (table 3; figure 2). Significantly higher anti-*Na*-APR-1(M74)

	10 μg *Na*-APR-1(M74) + 10 μg *Na*-GST-1		30 μg *Na*-APR-1(M74) + 30 μg *Na*-GST-1		100 μg *Na*-APR-1(M74) + 100 μg *Na*-GST-1		HBV + saline
	0, 2, 4 months	0, 2, 6 months	0, 2, 4 months	0, 2, 6 months	0, 2, 4 months	0, 2, 6 months	
Baseline (pre-dose 1)							
N	8	8	6	7	8	8	12
GML (95% CI)	3·52 (NA)	3·84 (3·11–4·75)	3·52 (NA)	3·52 (NA)	5·11 (3·25–8·03)	4·57 (2·82–7·39)	3·52 (NA)
Responders, n (% [95% CI])	0 (0% [0–32])	0 (0% [0–32])	0 (0% [0–39])	0 (0% [0–35])	1 (13% [2–47])	1 (13% [2–47])	0 (0% [0–24])
Day 7							
N	8	8	6	7	8	8	12
GML (95% CI)	3·52 (NA)	3·52 (NA)	3·52 (NA)	3·52 (NA)	4·09 (2·85–5·87)	4·51 (3·07–6·64)	3·52 (NA)
Responders, n (% [95% CI])	0 (0% [0–32])	0 (0% [0–32])	0 (0% [0–39])	0 (0% [0–35])	1 (13% [2–47])	1 (13% [2–47])	0 (0% [0–24])
Day 14							
N	8	8	6	7	8	8	12
GML (95% CI)	3·52 (NA)	3·52 (NA)	3·52 (NA)	3·52 (NA)	4·83 (2·84–8·24)	6·15 (3·69–10·26)	3·52 (NA)
Responders, n (% [95% CI])	0 (0% [0–32])	0 (0% [0–32])	0 (0% [0–39])	0 (0% [0–35])	1 (13% [2–47])	3 (38% [14–69])	0 (0% [0–24])
Day 28							
N	8	8	6	7	8	8	12
GML (95% CI)	3·52 (NA)	3·52 (NA)	3·52 (NA)	3·52 (NA)	9·22 (5·15–16·51)*	7·52 (3·77–15·03)	3·83 (3·17–4·64)*
Responders, n (% [95% CI])	0 (0% [0–32])	0 (0% [0–32])	0 (0% [0–39])	0 (0% [0–35])	4 (50% [22–79])	4 (50% [22–79])	1 (8% [2–35])
Day 56 (pre-dose 2)							
N	Not measured	7	6	7	8	8	10
GML (95% CI)	Not measured	3·52 (NA)	3·52 (NA)	3·52 (NA)	5·68 (3·23–9·98)	6·53 (3·72–11·47)	3·52 (NA)
Responders, n (% [95% CI])	Not measured	0 (0% [0–32])	0 (0% [0–39])	0 (0% [0–35])	2 (25% [7–59])	3 (38% [14–69])	0 (0% [0–28])
Day 63 (7 days post-dose 2)							
N	8	8	6	7	8	8	12
GML (95% CI)	3·52 (NA)	3·52 (NA)	6·61 (2·34–18·65)	7·64 (2·19–26·71)	19·37 (8·88–42·28)*	20·45 (10·84–38·58)*	3·52 (NA)*
Responders, n (% [95% CI])	0 (0% [0–32])	0 (0% [0–32])	2 (33% [10–70])	2 (29% [8–64])	7 (88% [53–98])	7 (88% [53–98])†	0 (0% [0–24])†
Day 70 (14 days post-dose 2)							
N	7	8	6	7	8	8	11
GML (95% CI)	5·36 (2·54–11·33)	4·29 (2·68–6·86)	9·55 (4·05–22·50)	7·40 (3·59–15·25)	17·34 (8·43–35·67)*	31·81 (22·42–45·14)*	3·75 (3·24–4·35)*
Responders, n (% [95% CI])	1 (14% [3–51])	1 (13% [2–47])	4 (67% [30–90])	4 (57% [25–84])	6 (75% [41–93])	8 (100% [78–100])†	0 (0% [0–26])†
Day 84 (28 days post-dose 2)							
N	8	8	6	7	8	8	11
GML (95% CI)	3·52 (NA)	3·96 (2·99–5·23)	7·83 (2·90–21·18)	4·91 (2·79–8·63)	19·02 (8·83–40·97)*	26·35 (17·40–39·92)*	3·52 (NA)*
Responders, n (% [95% CI])	0 (0% [0–32])‡	0 (0% [0–32])§	3 (50% [19–81])	1 (14% [3–51])	7 (88% [53–98])†	8 (100% [78–100]) †‡§	0 (0% [0–26])†
Day 112 or 180 (pre-dose 3)							
N	8	8	1¶	7	8	8	10
GML (95% CI)	3·52 (NA)	3·52 (NA)	11·12 (NA)	4·64 (2·99–7·20)	8·03 (4·33–14·91)	14·56 (11·19–18·94)*	3·52 (NA)*
Responders, n (% [95% CI])	0 (0% [0–32])‡	0 (0% [0–32])§	1 (100% [21–100])	0 (0% [0–35])‖	4 (50% [22–79])	8 (100% [78–100])†‡§‖	0 (0% [0–28])†

(Table 4 continues on next page)

	10 μg *Na*-APR-1(M74) + 10 μg *Na*-GST-1		30 μg *Na*-APR-1(M74) + 30 μg *Na*-GST-1		100 μg *Na*-APR-1(M74) + 100 μg *Na*-GST-1		HBV + saline
	0, 2, 4 months	0, 2, 6 months	0, 2, 4 months	0, 2, 6 months	0, 2, 4 months	0, 2, 6 months	
(Continued from previous page)							
Day 119 or 187 (7 days post-dose 3)							
N	8	8	6	7	8	8	12
GML (95% CI)	5·56 (3·19–9·70)	4·53 (2·48–8·27)	24·82 (6·39–96·41)*	16·10 (6·39–40·55)*	16·06 (11·82–21·82)*	100·62 (43·30–233·79)*	3·84 (3·16–4·68)*
Responders, n (% [95% CI])	2 (25% [7–59])	1 (13% [2–47])	4 (67% [30–90])	5 (71% [36–92])	7 (88% [53–98])	8 (100% [78–100])†	1 (8% [2–35])†
Day 126 or 194 (14 days post-dose 3)							
N	8	8	5	7	8	1¶	10
GML (95% CI)	4·83 (2·93–7·96)	6·70 (3·55–12·63)	126·59 (41·43–386·77)	26·26 (6·74–102·33)	21·38 (11·00–41·52)	331·19 (NA)	5·32 (2·86–9·91)
Responders, n (% [95% CI])	2 (25% 7–59)	3 (38% 14–69)	5 (100% 57–100)	6 (86% 49–97)	7 (88% 53–98)	1 (100% 21–100)	1 (10% 2–40)
Day 140 or 208 (28 days post-dose 3)							
N	8	8	6	6	8	8	11
GML (95% CI)	3·85 (3·10–4·79)	5·90 (3·01–11·54)	24·58 (4·66–129·61)	24·63 (6·77–89·67)	17·81 (9·67–32·81)*	76·59 (52·06–112·68)*	4·02 (2·98–5·43)*
Responders, n (% [95% CI])	0 (0% [0–32])‡	2 (25% [7–59])	4 (67% [30–90])	5 (83% [44–97])	7 (88% [53–98])	8 (100% [78–100])†‡	1 (9% [2–38])†
Day 200 or 268 (follow-up)							
N	8	8	6	7	8	8	12
GML (95% CI)	3·52 (NA)	4·99 (2·88–8·65)	11·78 (3·98–34·83)	7·82 (2·89–21·14)	12·37 (6·19–24·69)*	17·89 (9·26–34·58)*	3·75 (3·26–4·31)*
Responders, n (% [95% CI])	0 (0% [0–32])	2 (25% [7–59])	4 (67% [30–90])	3 (43% [16–75])	6 (75% [41–93])	7 (88% [53–98])	0 (0% [0–24])
Day 290 or 358 (follow-up)							
N	8	8	6	7	8	8	12
GML (95% CI)	6·96 (3·58–13·51)	3·52 (NA)	10·74 (3·72–30·96)	6·24 (2·52–15·45)	5·52 (2·74–11·12)	11·50 (5·97–22·15)	3·93 (3·07–5·03)
Responders, n (% [95% CI])	3 (38% [14–69])	0 (0% [0–32])	3 (50% [19–81])	2 (29% [8–64])	2 (25% [7–59])	6 (75% [41–93])	1 (8% [2–35])

Na-APR-1=*Necator americanus* aspartic protease-1. *Na*-APR-1(M74)=modified enzymatically inactive *Na*-APR-1. *Na*-GST-1=*N americanus* glutathione S-transferase-1. HBV=hepatitis B vaccine. GML=geometric mean level. IgG levels were measured in arbitrary units by qualified ELISA. Seropositivity was defined as having an antibody level about the reactivity threshold for the assay. Participants in the comparator arm (HBV plus saline) vaccinated according to the two schedules were combined. *p<0.05 for comparison with HBV plus saline comparator group (Wilcoxon rank sum test). †p<0·01 for comparison with HBV plus saline comparator group (Fisher's exact test) ‡§||p<0·01 for comparison between groups (Fisher's exact test) as two-way tests between co-administered antigen groups. ¶At some timepoints, either samples were not collected, or there was an issue with sample processing, storage, or shipment (eg, due to temperature excursions and shipment of samples to the USA for testing).

Table 4: **Number and percentage of seroresponders and anti-*Na*-GST-1 IgG antibody responses by vaccine group, dose schedule, and study day**

IgG levels were also observed between the group given 100 μg co-administered antigens on the month 0, 2, 6 schedule compared with those who received the HBV plus saline injections on day 63 (p=0·04), and on most subsequent days except days 180 and 194 (figure 2). Anti-*Na*-APR-1(M74) IgG levels were significantly higher in the 30 μg groups compared with the HBV plus saline groups starting on days 119 and 187 for the two vaccination schedules, respectively (ie, seven days after the third vaccinations). This difference was maintained at 2 weeks following the third vaccinations (ie, days 126 and 194 for the month 0, 2, 4 and month 0, 2, 6 schedules, respectively), but not at subsequent timepoints.

Similar patterns were observed for anti-*Na*-GST-1 IgG responses: the highest level was seen in those vaccinated with 100 μg of the antigens according to the month 0, 2, 6 schedule, with a GML of 331·19 (95% CI NA) AU on day 194, compared with 21·38 AU (6·74–102·33) on day 126 for those vaccinated on the month 0, 2, 4 schedule (table 4). By comparison, the HBV plus saline comparator group had a GML of 5·32 AU (2·86–9·91) at the equivalent timepoint. In those vaccinated according to the month 0, 2, 4 schedule, the highest anti-*Na*-GST-1 IgG levels were in those vaccinated with 30 μg of both antigens on day 126 (14 days after the third dose), 126·59 AU (95% 41·43–386·77; table 4).

Significant differences in anti-*Na*-GST-1 IgG levels were first observed on day 28 between those who received 100 μg of hookworm antigens at 0, 2, and 4 months and HBV plus saline recipients (p=0·049); this difference remained significant except on days 56, 112, 126, and 290 (table 4). Significantly higher anti-*Na*-APR-1(M74)

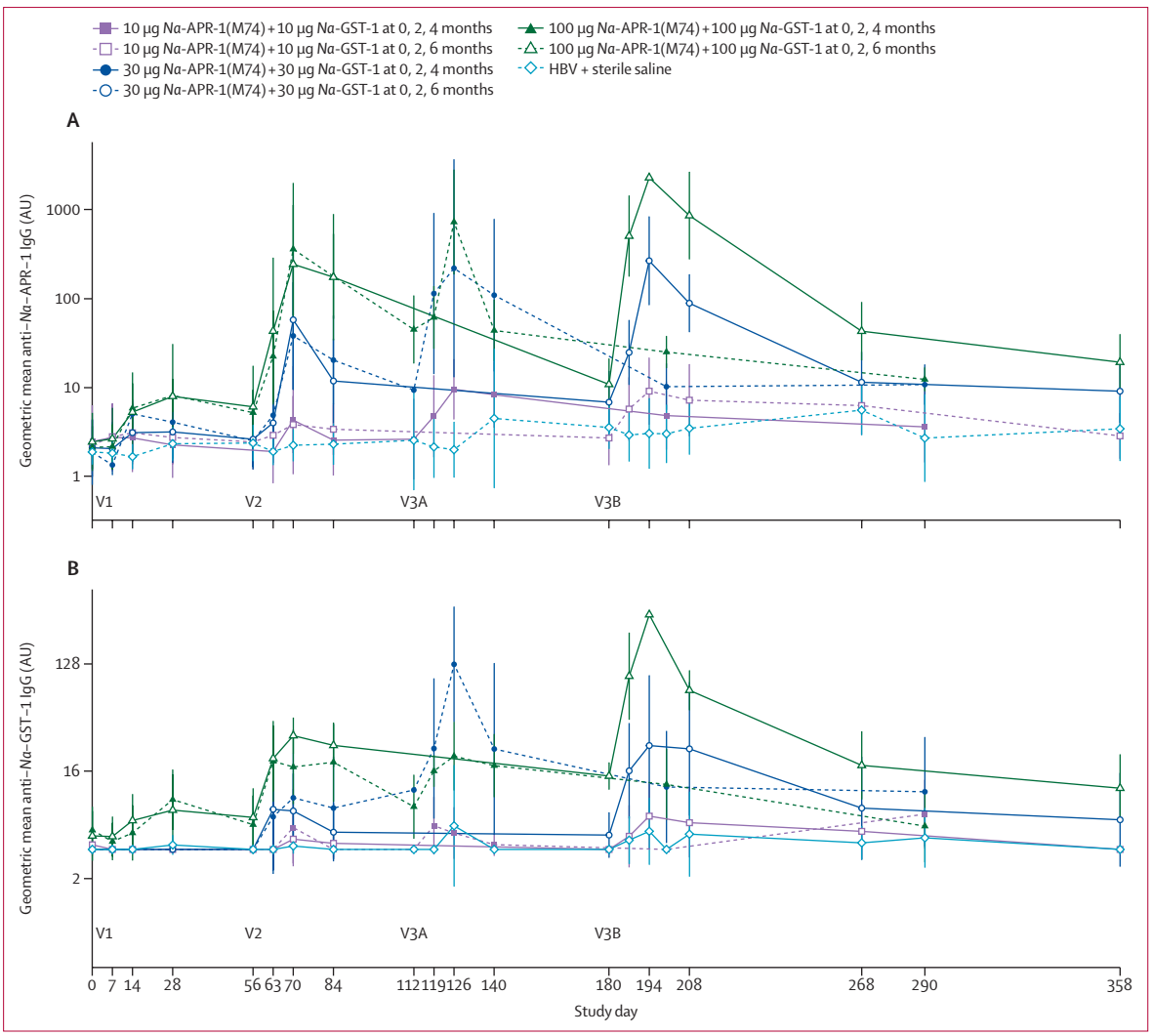

Figure 2: **Geometric mean levels of IgG against recombinant *Na*-APR-1(M74) (A) and *Na*-GST-1 (B) as measured by ELISA by vaccine group and study day**
Per-protocol immunogenicity population (N=57). Error bars represent 95% CIs. Note: the y-axes are on a logarithmic scale. *Na*-APR-1=*Necator americanus* aspartic protease-1. *Na*-APR-1(M74)=modified enzymatically inactive *Na*-APR-1. *Na*-GST-1=*N americanus* glutathione S-transferase-1. AU=arbitrary units. HBV=hepatitis B vaccine. V1=first vaccination; V2=second vaccination. V3A=third vaccination for 0, 2, 4 month schedule. V3B=third vaccination for 0, 2, 6 month schedule.

IgG levels were also observed in the 100 µg co-administered antigen group (month 0, 2, 6 schedule) compared with the comparator group on day 63 (p=0·003) and on most following days, except days 194 and 358. Anti-*Na*-GST-1 IgG levels were significantly higher in those vaccinated with 30 µg of the antigens according to both schedules on day 119 or 187 (ie, seven days after third vaccinations; p=0·04 and p=0·02, respectively).

A linear mixed effects model fitted with log-transformed IgG responses identified statistically significant antigen dose-response effects for both *Na*-APR-1(M74) and *Na*-GST-1 (p<0·0001 and p<0·0001, respectively). Longitudinal analyses of log-transformed IgG levels using a linear mixed effects model identified significant vaccination schedule effects for both *Na*-APR-1(M74) and *Na*-GST-1 (p=0·01 and p=0·04, respectively), with higher IgG levels in the month 0, 2, 6 schedule versus the month 0, 2, 4 schedule.

Tables 3 and 4 summarise the number and proportion of IgG responders to each antigen by study day, vaccine group, and dose schedule. The highest proportions of responders were in the 100 µg co-administered antigen groups, regardless of vaccination schedule.

For anti-*Na*-APR-1, in the 100 µg co-administered antigen groups response rates were mostly 100% after day 63. There were significantly more responders in the 100 µg co-administered antigen group vaccinated on the month 0, 2, 4 schedule on day 63 compared to those vaccinated with HBV plus saline (eight; 100% [95% CI 68–100] *vs* two; 17% [5–45]; table 3).

For anti-*Na*-GST-1, there were significantly more responders in the 100 µg co-administered antigen

groups, for both vaccination schedules on days 63, 70, and 84 compared to the comparator group. For those vaccinated at 0, 2, and 4 months there were significantly more responders in those vaccinated with 100 µg of the antigens on days 119, 140, and 200 compared with the HBV vaccine (table 4), while the same dose on the month 0, 2, and 6 schedule had significantly more responders on days 180, 187, and 268 compared with HBV. In this group, six (75%) of eight children were still responders at the final study visit.

Discussion

In this phase 1 study, co-administration of *Na*-APR-1(M74) on Alhydrogel and GLA-AF plus *Na*-GST-1 on Alhydrogel was safe and well tolerated in children aged six to ten years living in the hookworm-endemic region of Lambaréné, Gabon, and as such, had probably been previously exposed to the *N americanus* hookworm.[14] Vaccine-related adverse events were observed in most children. Although adverse events–both solicited and unsolicited–were more frequent in those who received the co-administered antigens compared with HBV, these were mostly mild, well tolerated, and short-lived. Mild-to-moderate injection-site pain, as well as mild headache and fever, were the most common solicited symptoms. The incidence of the events did not substantially differ between the 10 µg, 30 µg, and 100 µg doses of co-administered *Na*-GST-1 and *Na*-APR-1(M74). No vaccine-related SAEs or AESIs were observed, and vaccine-related clinical laboratory adverse events were mostly rare; furthermore, these abnormalities were asymptomatic, mild, and resolved without intervention.

Injection-site reactions were more frequent and intense with *Na*-APR-1(M74) on Alhydrogel and GLA-AF than with *Na*-GST-1 on Alhydrogel, which might relate to the greater number of children with pre-vaccination antibodies to *Na*-APR-1(M74). Data from other vaccines demonstrate that pre-existing immunity increases the likelihood of administration-site reactions.[19,20] Alternatively, increased reactogenicity may be due to the use of GLA-AF, since other MPL-based adjuvants such as $AS01_B$ have been shown to increase administration-site reactogenicity.[21,22] Supporting the latter hypothesis, hookworm-naive adults given *Na*-APR-1(M74) on Alhydrogel in combination with GLA-AF had a trend towards increased reactogenicity compared to *Na*-APR-1(M74) on Alhydrogel alone.[12]

Antigen-specific IgG responses to *Na*-APR-1(M74) and *Na*-GST-1 were induced in a dose-dependent manner, with peak levels observed 2 weeks following the third set of vaccinations, regardless of the vaccination schedule. Additionally, significant dose responses were observed for each antigen, with the 100 µg dosage levels producing the highest IgG responses, and the 30 µg and 100 µg groups having IgG responses significantly above those in the comparator group. Increasingly higher peaks of antibody levels were observed with successive vaccinations. Although the 100 µg vaccine recipients had the highest IgG responses, there were several moderate and severe laboratory adverse events in this group (decreases in haemoglobin and thrombocytopenia), a phenomenon of potential clinical relevance that will require attention in subsequent larger-scale, phase 2 and 3 clinical trials.

Although both the month 0, 2, and 4 and month 0, 2, and 6 vaccination schedules have been tested in previous clinical trials of *Na*-APR-1(M74) on Alhydrogel and *Na*-GST-1 on Alhydrogel, this was the first time they were directly compared. Significant schedule effects were identified, with improved IgG responses to both antigens observed on the month 0, 2, 6 schedule. Previous studies of other vaccines, including the SARS-CoV-2 vaccine and recombinant protein-based HBVs,[23,24] have shown improved immune responses when the interval between vaccinations is extended, probably because of enhanced affinity maturation in germinal centres of peripheral lymphoid tissues. Children vaccinated in the current study according to the month 0, 2, 6 schedule had peak antibody levels that were similar, although not superior in the case of *Na*-GST-1 on Alhydrogel, to the responses that were observed in healthy Gabonese adults who received the same co-administered vaccines according to the same schedule.[13]

Similar to a phase 1 trial of the same co-administered antigens in adults living in the same hookworm-endemic area where the current study was conducted, a substantial number of participating children had low baseline levels of IgG antibodies to *Na*-APR-1(M74), in contrast to the low numbers of participants with baseline IgG to this antigen in a phase 1 trial of *Na*-APR-1(M74) on Alhydrogel in previously unexposed adults living in Washington, DC (USA).[12] The baseline prevalence of seropositivity to *Na*-APR-1(M74) in the current study was similar to what was seen in a phase 1 trial in adults conducted in the same hookworm-endemic region of Gabon, in which just over half of participants had detectable IgG to the recombinant protein.[13] Given this difference in background prevalence of anti-*Na*-APR-1(M74) antibodies in endemic versus non-endemic populations, it is therefore more probable that these low levels of antibody are due to previous exposure or infection rather than cross-reactivity with similar proteins. In contrast to this finding with *Na*-APR-1(M74), few children or adults in Lambaréné had detectable pre-vaccination anti-*Na*-GST-1 IgG, an observation also seen in a hookworm-endemic area of Brazil.[11]

Encouragingly, the presence of pre-existing antibody responses to *Na*-APR-1(M74) did not interfere with the humoral response to vaccination in the paediatric trial reported here. IgG responses to *Na*-APR-1(M74) were substantially higher in this paediatric trial compared with those observed in adults in the same region.[16] Since the same laboratory and assay were used in the two trials, the observed difference in immunogenicity to *Na*-APR-1(M74)

is more probable to be related to the ages of the participants. Improved antibody responses in children compared with older individuals have been observed for other vaccines.[25,26]

The dose-dependent production of IgG to both *Na*-GST-1 and *Na*-APR-1(M4) in children vaccinated with these co-administered antigens is encouraging, given that the putative mechanism of action of the human hookworm vaccine as currently designed is to neutralise the action of the native enzymes by vaccine-induced IgG antibodies that will block the blood-feeding pathway of *N americanus* hookworms.

An important limitation of our study is that it is unknown if the levels of IgG to these antigens observed in vaccinated children will be sufficient to protect against infection, although protection from challenge infection in animal models has been demonstrated for both *Na*-GST-1 and *Na*-APR-1(M74).[27,28] Furthermore, the immunological profile that will be required of an effective human hookworm vaccine will be fundamentally different than that induced by natural infection, given that infection does not induce protection against re-infection. The efficacy of the current components of the human hookworm vaccine therefore must be tested empirically. To evaluate the protective efficacy of the human hookworm vaccine and at the same time assess potential antibody-based correlates of protection, the traditional next step in the clinical development of this vaccine would be to conduct field studies in children, in which infection or anaemia would be the primary efficacy endpoint. However, based on an evaluation of background rates of re-infection post-treatment, it is estimated that for hookworm such field trials would require large sample sizes and significant investment, even in areas of high hookworm transmission.[29] To provide additional justification for embarking on such ambitious field trials, a controlled human hookworm infection model has been developed that is currently being used to evaluate the efficacy of the proposed components of the human hookworm vaccine.[30]

In conclusion, in this seminal paediatric phase 1 hookworm vaccine trial, co-administration of the *Na*-APR-1(M74) on Alhydrogel and GLA-AF plus *Na*-GST-1 on Alhydrogel vaccines was safe, well tolerated and immunogenic in school-aged children living in a hookworm-endemic region of Gabon. Children in hookworm-endemic regions bear the highest burden of infection and disease, and may also develop physical and intellectual impairments that continue to affect them into adulthood. Given the results presented here, and if the results of proof-of-concept controlled human hookworm infection studies are positive, future paediatric efficacy trials of these two candidate vaccines will be initiated.

Contributors

DJD, MPG, AAA, JMB, MEB, PJH, and RvL conceived the study. DJD, GL, and JMB did the formal analysis. RvL, PJH, JMB, MEB, DJD, MPG, and PGK acquired the funding. AAA, JFZ, YJH, JCD-A, BRA, and KGV did the clinical investigation. MML, JMB, and RB did the laboratory investigation. DJD, JMB, PJH, MEB, and MPG devised the methodology. AAA, DJD, MPG, and PGK supervised the study. JMB and GL prepared the figures. DJD and JFZ wrote the original draft of the manuscript. All authors reviewed and edited the manuscript, contributed to the final version, and approved it for publication.

Declaration of interests

PJH, MEB, JMB, and DJD are named as inventors on a patent for a multivalent helminth vaccine (US8211438B2). All other authors declare no competing interests.

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Data sharing

De-identified individual participant data that underlie the results reported in this Article will be made available to anyone who wishes to access the data from the time of publication with no end date, for any purpose. The data will be available indefinitely online.

For the **underlying data** see https://clinicaltrials.gov/study/NCT02839161

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