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Population differences in vaccine responses (POPVAC): scientific rationale and cross-cutting analyses for three linked, randomised controlled trials assessing the role, reversibility and mediators of immunomodulation by chronic infections in the tropics

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ABSTRACT

Introduction Vaccine-specific immune responses vary between populations and are often impaired in low income, rural settings. Drivers of these differences are not fully elucidated, hampering identification of strategies for optimising vaccine effectiveness. We hypothesise that urban–rural (and regional and international) differences in vaccine responses are mediated to an important extent by differential exposure to chronic infections, particularly parasitic infections.

Methods and analysis Three related trials sharing core elements of study design and procedures (allowing comparison of outcomes across the trials) will test the effects of (1) individually randomised intervention against schistosomiasis (trial A) and malaria (trial B), and (2) Bacillus Calmette-Guérin (BCG) revaccination (trial C), on a common set of vaccine responses. We will enrol adolescents from Ugandan schools in rural high-schistosomiasis (trial A) and rural high-malaria (trial B) settings and from an established urban birth cohort (trial C). All participants will receive BCG on day '0'; yellow fever, oral typhoid and human papilloma virus (HPV) vaccines at week 4; and HPV and tetanus/diphtheria booster vaccine at week 28. Primary outcomes are BCG-specific IFN-γ responses (8 weeks after BCG) and for other vaccines, antibody responses to key vaccine antigens at 4 weeks after immunisation. Secondary analyses will determine effects of interventions on correlates of protective immunity, vaccine response waning, priming versus boosting immunisations, and parasite infection status and intensity. Overarching analyses will compare outcomes between the three trial settings. Sample archives will offer opportunities for exploratory evaluation of the role of immunological and 'trans-kingdom' mediators in parasite modulation of vaccine-specific responses.

Ethics and dissemination Ethics approval has been obtained from relevant Ugandan and UK ethics committees. Results will be shared with Uganda Ministry of Health, relevant district councils, community leaders and study participants. Further dissemination will be done through conference proceedings and publications.

Strengths and limitations of this study

- This will be the first well-powered programme of work to investigate effects of schistosomiasis treatment, of malaria treatment, and of Bacillus Calmette-Guérin revaccination on vaccine responses in adolescents.
- A major strength of this work is the opportunity to synthesise findings from three different study settings with differential parasite exposure using causal mediation analysis to obtain a comprehensive understanding of how parasitic infections influence vaccine responses in human populations.
- The results will provide insight into effects of parasites on infectious disease susceptibility: immunisation, notably with live vaccines, offers a surrogate for infection challenge in human subjects.
- The sample archives developed will provide a major asset for exploration of new leads arising from this hypothesis-driven work, or for an alternative, 'systems biology' approach investigating, for example, transcriptome, microbiome and virome.
- One limitation is that observational analyses of parasite effects are subject to potential unmeasured confounding; this will be mitigated by cautious interpretation of results and our intervention studies will address causality rigorously.

Trial registration numbers ISRCTN60517191, ISRCTN62041885, ISRCTN10482904.

INTRODUCTION

Population differences in vaccine responses

Effective vaccines are key weapons against infectious diseases,[1] but are still lacking for many poverty-related, neglected, emerging and re-emerging infections. Vaccine responses vary between populations and are often impaired in low income, rural settings.[2–6] A notable example is Bacillus Calmette-Guérin (BCG): both vaccine response and efficacy against tuberculosis differ internationally[3 4] and regionally.[6 7] Among other vaccines, yellow fever vaccine induced lower neutralising antibody levels, and responses waned faster, in Uganda compared with Switzerland.[5] Oral rotavirus and polio vaccines are also affected.[2] Within country, influenza and tetanus responses differed between urban and rural Gabon.[8 9] Responses to candidate tuberculosis,[10] malaria[11] and Ebola[12] vaccines are lower in Africa than in Europe or America. Prior exposure to the pathogen targeted by the vaccine, or to related organisms, may contribute to this phenomenon, but recent analyses implicate broader 'environmental sensitisation',[6] the drivers of which have not been determined. Prior exposure cannot explain results for vaccines against rare organisms, such as Ebola. Thus, drivers of POPulation differences in VACcine responses (POPVAC) are not fully elucidated; improved understanding is important for effective vaccine development and implementation.

The POPVAC programme comprises three trials, A, B and C, designed to address this challenge. The individual trial protocols are presented separately in this journal (bmjopen-2020-040426, bmjopen-2020-040427 and bmjopen-2020-040430). This 'Protocol X' provides an overview of our hypotheses and objectives.

Immunomodulation by parasitic infections

Parasitic infections are important in tropical low-income countries (LICs),[13–15] and long proposed as modulators of vaccine responses.[16–19] As detailed in POPVAC A (bmjopen-2020-040426) and POPVAC B (bmjopen-2020-040427) protocols, animal models[20–22] and observational human studies[23–25] support this hypothesis, but no well-powered trials have been conducted to evaluate causality and reversibility of parasite effects on vaccine responses in adolescents or adults.[26]

Trained immunity

Exposure to other unrelated infections or microbial antigens may also contribute to 'environmental sensitisation', modulating vaccine responses through training of the innate immune system.[27] BCG immunisation is a key model for this effect,[28 29] as considered in Protocol C (bmjopen-2020-040430).

The 'transkingdom' concept

The 'transkingdom' concept[30] emphasises that mammals support a complex ecosystem of multicellular organisms, such as helminths, as well as bacteria, fungi, protozoa and viruses, and suggests that these interact in their effects on the mammalian immune system, rather than acting alone, as individual agents.[30] For example, in a mouse model, infection with the gut helminth *Heligmosomoides polygyrus*, or exposure to schistosome eggs, activated latent herpesvirus infection via alternative macrophage activation and the IL-4/Stat6 pathway.[31] In a contrasting study, *H. polygyrus* caused enhanced responses to respiratory syncytial virus in the mouse lung through interaction with the gut microbiome, translocation of microbial products to the circulation and enhanced systemic type I interferon expression.[32] Interestingly, in these studies, the same helminth resulted in opposite outcomes for latent viruses (for which it induced activation), versus exogenous viruses (for which it improved control).

Little has been done to evaluate these phenomena in human populations but, regarding latent herpesviruses our studies show that parasite exposure associates with elevated Kaposi's Sarcoma Herpesvirus antibody prevalence and titre (indicating viral activation).[33–35] The impact of malaria on Epstein-Barr virus, promoting induction of Burkitt's lymphoma, is well recognised.[36] Cytomegalovirus has major immunological effects, including impact on vaccine responses.[37]

In humans, evidence of enhanced microbial translocation (MT) has been found during *Schistosoma mansoni*,[38] hookworm[39] and *Strongyloides*[40] infection, with altered expression of parameters such as toll-like receptor expression, but without the level of immune activation associated with septic shock or with MT in HIV infection. Nevertheless, the second mouse model discussed above shows that this may have profound effects on responses to infectious agents at remote sites.[32]

Thus, herpesvirus activation and, or, MT may mediate, in part, parasite-induced modulation of vaccine responses.

Differences in immunological characteristics between populations

Whatever the key exposures and mechanisms involved, immunological characteristics differ markedly between populations internationally,[5 41 42] and between urban and rural settings.[41 43 44] Characteristics that differ include gene methylation and expression (not solely attributable to population genetics)[43 45]; responses to innate stimuli[42 45]; frequency and activation of innate immune cells, T and B cells, and memory cell pools.[5 41] Understanding the immunological predictors of vaccine response, and factors that drive them, will contribute to strategies for improving vaccine efficacy for rural, tropical settings.

HYPOTHESIS AND OBJECTIVES

The overarching goal of the 'POPVAC' is to understand POPVAC, in order to identify strategies through which vaccine effectiveness can be optimised for low income, tropical settings where they are especially needed. We

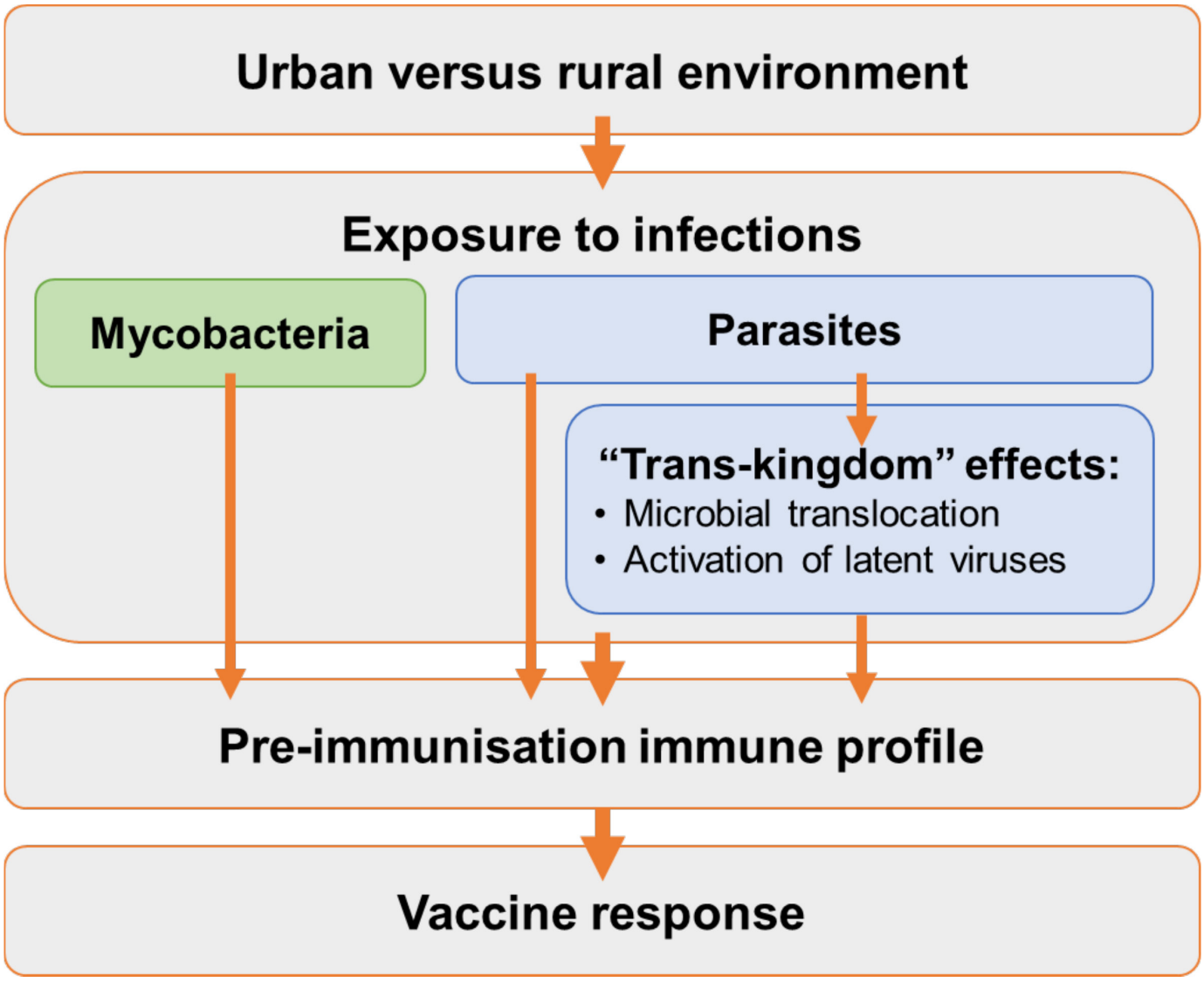

Figure 1 Hypothesised pathways to population differences in vaccine responses.

focus on the hypothesis (figure 1) that geographical differences in vaccine responses are mediated to an important extent by differential exposure to chronic, particularly parasitic, infections; that parasites act in part via 'transkingdom' effects; and that these exposures impact the preimmunisation immune profile and hence vaccine response (and efficacy).

We will address this hypothesis in three, linked trials (POPVAC A, B and C; detailed protocols published separately in this journal: bmjopen-2020-040426, bmjopen-2020-040427 and bmjopen-2020-040430, respectively) which share core elements of study design and procedures, allowing comparison of outcomes across the trials. Each trial will test effects of a different randomised intervention on a common set of vaccine responses. POPVAC A will determine the effect of intensive schistosomiasis treatment on vaccine responses among rural island adolescents. POPVAC B will determine the effect of intensive malaria treatment on vaccine responses among rural adolescents. POPVAC C will determine the effect of BCG revaccination on responses to unrelated vaccines.

This paper describes background and methods common to all three trials, summarises objectives linking the trials, and details planned approaches to cross-cutting objectives which will use data and samples from all trials. Standard Protocol Items: Recommendations for Interventional Trials reporting guidelines[46] are used.

Our objectives are to

1. Determine whether there are reversible effects of chronic parasitic infection on vaccine response (POPVAC A and B).
2. Determine whether BCG 'preimmunisation' enhances responses to unrelated vaccines (POPVAC C).
3. Determine which life-course exposures influence vaccine responses in adolescence (using data from POPVAC C).
4. Compare vaccine response profiles between three Ugandan settings (using data from all trials): rural,

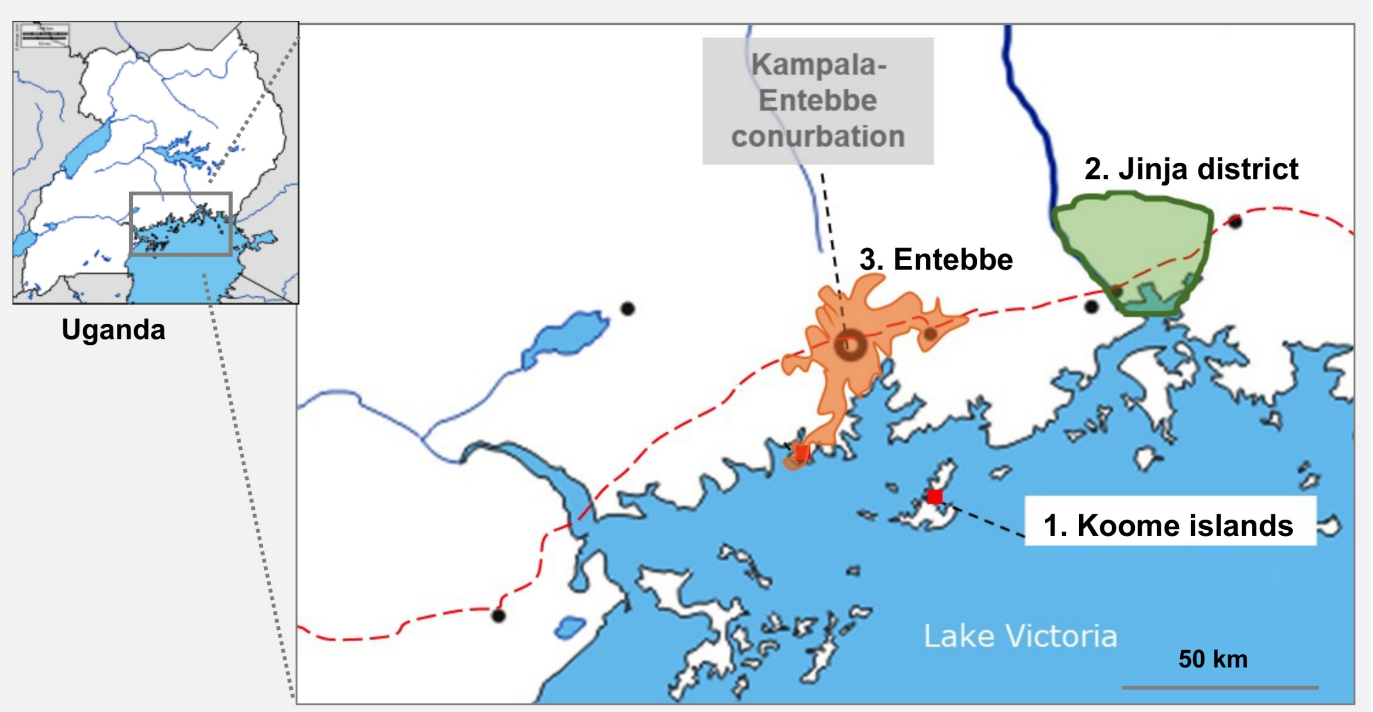

Figure 2 Study sites.

high schistosomiasis exposure; rural, high malaria exposure and urban.

5. Explore the role of 'transkingdom' interactions in determining vaccine responses (using samples and data from all Trials).
6. Investigate preimmunisation immunological parameters associated with vaccine responses and determine whether these are driven by parasite or microbial exposure (using samples and data from all trials).

METHODS AND ANALYSIS

Setting

Uganda still experiences high schistosomiasis[47 48] and malaria burdens.[49–51] Our study sites (figure 2) will be Lake Victoria Koome Islands (high schistosomiasis),[47 52] Jinja district rural subcounties (high malaria)[51] and Entebbe (urban; low schistosomiasis and low malaria).[52] These settings provide ideal opportunities to investigate effects of schistosomiasis and malaria on vaccine responses. Geohelminths are less common in our settings[47 53]: hookworm, especially, has declined dramatically following government intervention programmes; therefore, geohelminths will be considered as potential confounders, where appropriate, but not prioritised.

Cohorts

This work will involve adolescents from two rural settings and one urban cohort (figure 3). Rural trials will recruit participants aged 9–17 years from primary schools in Koome islands (POPVAC A) and Jinja district (POPVAC B), selected purposefully to assure schistosomiasis and malaria prevalence appropriate to our design. This will allow us to investigate our two major infections separately, averting the risk that a potent effect of one infection masks an impact of the other, or of its treatment.

POPVAC C will recruit members of the Entebbe Mother and Baby Study (EMaBS) birth cohort[54]: 2500 women were recruited between 2003 and 2005 in a trial of anthelminthic treatment during pregnancy, investigating effects on infants' vaccine responses.[54] Children from the EMaBS birth cohort will be aged 13 to 17 years during recruitment to this study; about 300 individuals are expected to take part.

Interventions

In the high-schistosomiasis cohort (trial A), we will individually randomise participants to intensive or standard praziquantel (PZQ) treatment, in a 1:1 ratio, in an open-label, parallel group trial.

In the high-malaria cohort (trial B), we will individually randomise participants to monthly dihydroartemisinin piperaquine (DP) versus placebo in a double-blind, placebo-controlled trial.

In the urban (EMaBS) cohort (trial C), we will individually randomise participants to BCG revaccination, or no BCG revaccination, as the first component of the vaccine schedule, in a controlled, open-label, parallel-group trial.

Recruitment criteria, interventions, randomisation and treatment allocation procedures are detailed in protocols for trials A, B and C (bmjopen-2020-040426, bmjopen-2020-040427 and bmjopen-2020-040430, respectively; published in this journal).

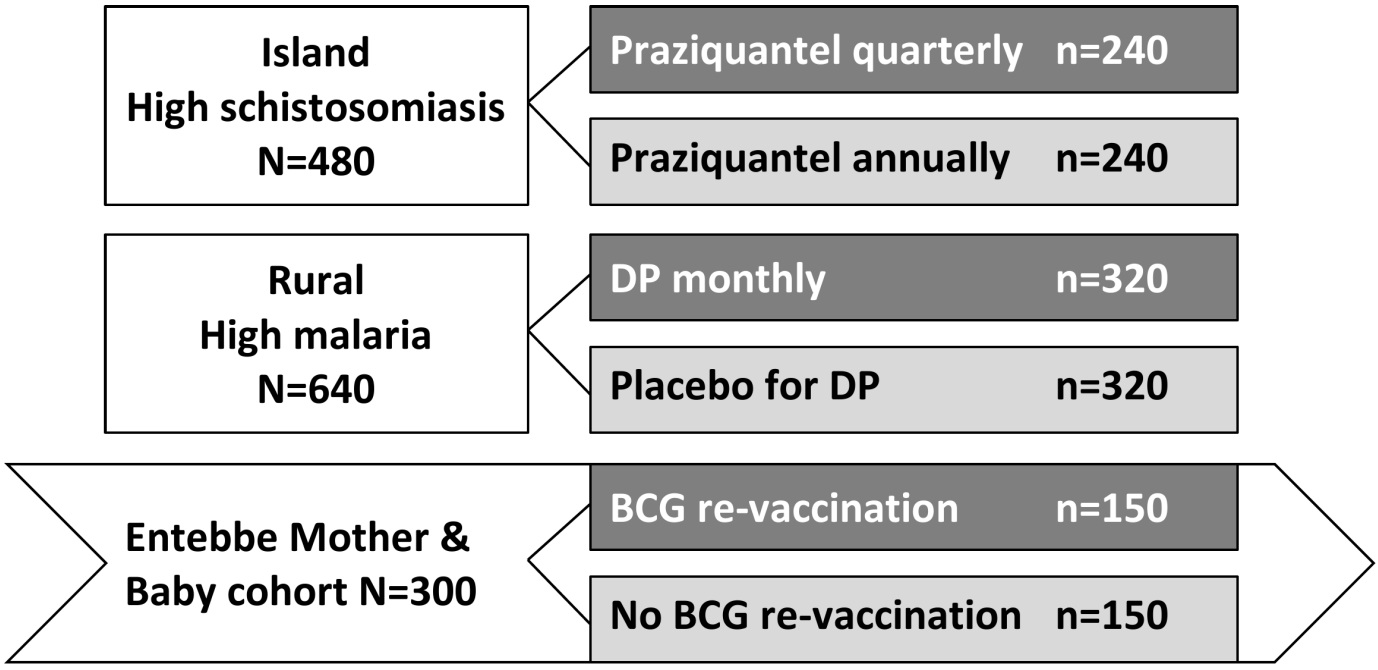

Figure 3 Overall programme design and sample sizes. BCG, bacillus calmette-guérin; DP, dihydroartemisinin.

Immunisations

We have previously highlighted the complexity of helminth effects on vaccine responses.[26] Differences in parasite effects on live and non-live, oral and parenteral, priming and boosting vaccines may contribute to this. Activated innate responses may kill live vaccines and suppress subsequent adaptive responses,[5 55] but bias, or enhance, responses to toxoids or proteins[17 23 56]; intestinal inflammation may impair responses to live oral vaccines[57]; priming may be more vulnerable than boosting.[58 59] Thus, results from a single-vaccine study would not be generalisable.

Therefore, we will study a portfolio of licensed vaccines expected to be beneficial (some already given) to adolescents in Uganda: live parenteral (BCG, yellow fever), live oral (typhoid) and non-live (HPV) (a viral particle vaccine) and tetanus/diphtheria (Td; toxoid vaccines). This will allow us to compare effects of interventions and exposures between vaccine types. Each cohort will receive the same vaccine portfolio (table 1), comprising three main immunisation days (weeks 0, 4 and 28). Additional HPV immunisation will be provided for girls aged 14 years or above, and a second Td boost will be given after study completion, to accord with national Expanded Programme on Immunisation (EPI) routines, but responses to these will not specifically be addressed. Further rationale for vaccine selection is detailed in online supplemental material 1. Our schedule has been developed in consultation with the EPI programme (see online supplemental table 1) and is cognizant of potential interference between vaccines (see online supplemental material 1, online supplemental table 2).

Although optimal timings for outcome measures vary between vaccines, sampling (for primary endpoints)

Table 1 Immunisation schedule

	Immunisation week 0	Immunisation week 4	(Immunisation week 8)	Immunisation week 28	(Immunisation week 52)
Live vaccines	BCG vaccination/ revaccination*, †, ‡	Yellow fever (YF-17D) Oral typhoid (Ty21a)			
Non-live vaccines		HPV prime	HPV boost for girls aged ≥14 years§, ¶	HPV boost and tetanus/diphtheria boost	Tetanus/diphtheria boost¶, **

*All participants in the urban (Entebbe Mother and Baby Study, EMaBS) cohort received BCG at birth, so within EMaBS this will berevaccination; prior BCG status may vary for the rural cohorts (data on history and documentation of prior BCG, and presence of a BCG scar, will be documented although these approaches have limitations for determining BCG status).
†EMaBS participants will be randomised to receive BCG 'preimmunisation' or not as part of trial C.
‡All participants in rural cohorts will receive BCG (trials A and B).
§The National EPI programme recommends three doses of HPV vaccine for older girls.
¶These doses will be given to comply with guidelines but outcomes specifically relating to these doses will not be assessed.
**Priming by immunisation in infancy is assumed.
BCG, bacillus calmette-guérin; HPV, human papilloma virus.

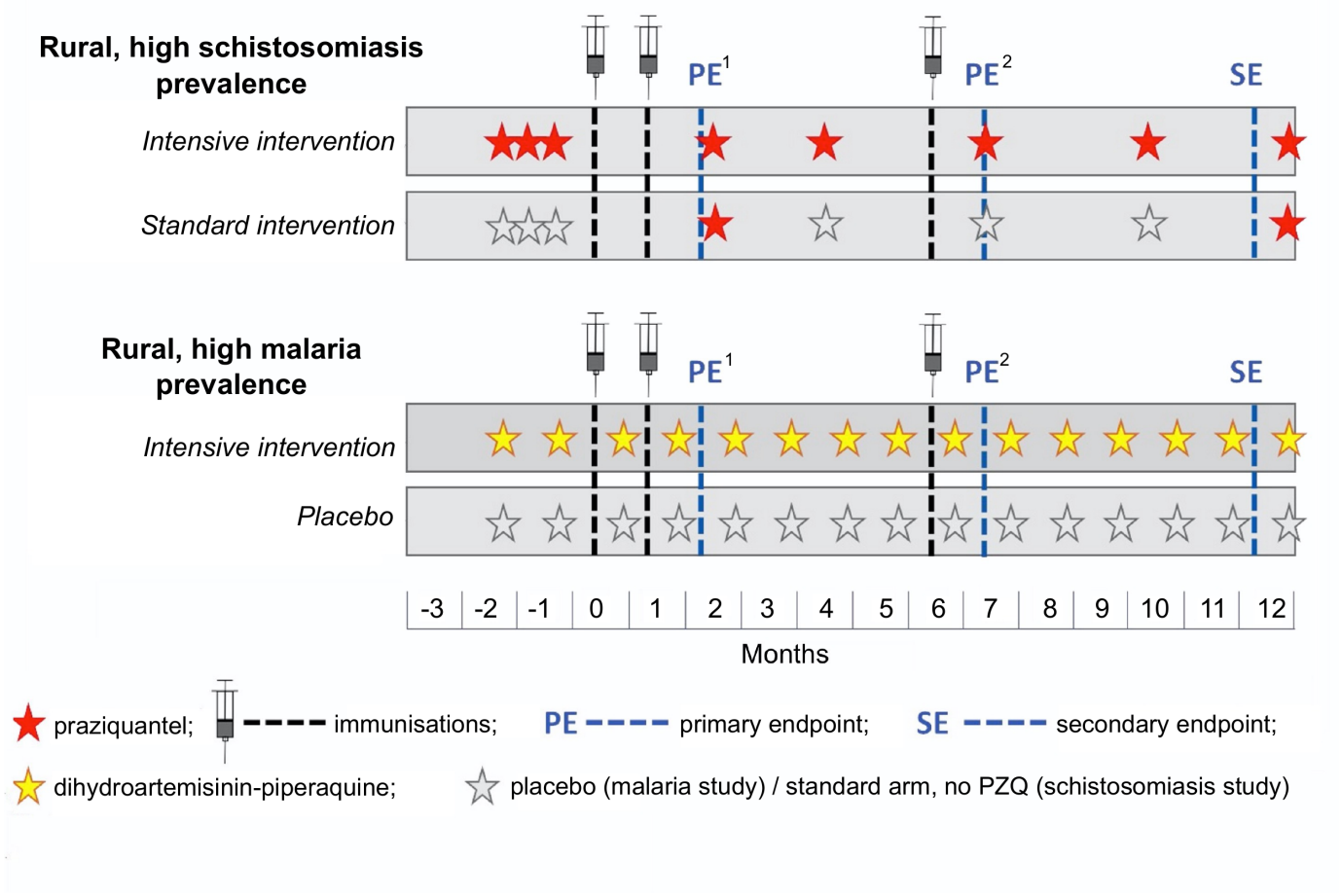

Figure 4 Outline of immunisations and interventions.[1]Primary endpoints will be at 8 weeks post-BCG and 4 weeks postyellow fever (YF-17D), oral typhoid (Ty21a), human papilloma virus and tetanus/diptheria vaccination.[2]Primary endpoint for responses to td given at 28 weeks. BCG, Bacillus Calmette-Guérin.

will be done at 8 weeks post BCG, 4 weeks post YF-17D, Ty21a, HPV and Td (figure 4), targeting establishment of memory responses and antibody response peaks. A secondary endpoint at 1 year will assess waning. Analyses will take baseline measurements into account.

Outcomes

Primary outcomes, assessed in all participants, will be

1. BCG: BCG-specific IFN-γ ELISpot response 8 weeks post-BCG immunisation: this response is associated with decreased risk of tuberculosis disease postimmunisation in infants.[60]
2. YF-17D: neutralising antibody titres (plaque-reduction neutralisation test) 4 weeks post-YF immunisation.
3. Ty21a: Salmonella typhi lipopolysaccharide-specific IgG concentration 4 weeks post-Ty21a immunisation.
4. HPV: IgG specific for L1-proteins of HPV-16/18 4 weeks post-HPV priming immunisation.
5. Td: tetanus and diphtheria toxoid-specific IgG concentration 4 weeks post-Td immunisation.

Secondary outcomes, assessed in all participants, will further investigate estimates of protective immunity (for vaccines where these are available) and dynamics of the vaccine responses, as well as the impact of interventions on parasite clearance.

1. *Protective immunity*. Proportions with protective neutralising antibody (YF); protective IgG levels (TT)[61]; seroconversion rates (Ty21a) 4 weeks postimmunisation.
2. *Response waning*. Primary outcome measures (all vaccines) repeated at week 52, and area under the curve analyses. Parasites may accelerate,[58] and interventions delay, waning.
3. *Priming versus boosting*. Effects on priming versus boosting will be examined for HPV, comparing outcomes 4 weeks after the first and second vaccine doses.

Our sample collection will offer opportunities for an array of exploratory immunological evaluations, focusing on vaccine antigen specific outcomes. Exploratory assessments will provide detail on immune response characteristics over the study time-course, and on the role of immunological profiles and trans-kingdom effects in mediating modulation of vaccine-specific responses.

Sample size

Our sample size estimates focus on primary outcomes for the main comparisons for objectives i, ii and iv.

Based on literature,[5 60 62] we anticipate SDs of primary outcome measures lying between 0.3 and 0.6 $\log_{10}$; responses in rural, high-parasite settings 0.3–0.4 $\log_{10}$ smaller than in the urban setting[63 64] and effective treatment restoring responses by approximately 0.2 $\log_{10}$.[63] We, therefore, power our study to detect differences of this magnitude (0.2 $\log_{10}$) or smaller. We assume *S. mansoni* prevalence of ≥80% in the high-schistosomiasis setting[47] and malaria infection prevalence of ≥60% in the high-malaria setting.

Our planned sample sizes are as follows:

High-schistosomiasis setting (objective i, trial A): 480 (240 quarterly PZQ, 240 annual PZQ); of whom we anticipate 384 will be *S. mansoni* infected, giving 192 participants in each trial arm with *S. mansoni* infection at baseline.

High-malaria setting (objective i, trial B): 640 (320 DP, 320 placebo) of whom we anticipate 384 will be malaria infected, giving 192 participants with malaria in each trial arm at baseline.

Urban setting (objective ii, trial C): 300 EMaBS participants (150 BCG 'preimmunisation', 150 no BCG immunisation).

Urban versus rural and urban versus island comparisons (objective iv): 150 urban EMaBS, 240 rural high-schistosomiasis control group participants and 320 rural high-malaria control group participants will be included. Allowing 20% lost to follow-up in rural cohorts and 10% in the EMaBS cohort, this will give >80% power to detect a difference of 0.14$\log_{10}$ or more in vaccine responses in urban compared with each rural setting at 5% significance level assuming vaccine response SD of 0.4$\log_{10}$.

Table 2 shows power estimates for objective i, ii and iv. Sample size considerations for additional analyses are detailed in the protocol papers for the individual trials (bmjopen-2020-040426, bmjopen-2020-040427 and bmjopen-2020-040430).

Approach to trial objectives

Approaches to objective i (trials A and B) and objective ii (trial C) are detailed in the focused papers for these trials (bmjopen-2020-040426, bmjopen-2020-040427 and bmjopen-2020-040430). Here we present approaches to objectives iii–vi.

Objective iii: life course exposures that influence vaccine responses in adolescence (Trial C)

Data collected in the EMaBS over the participants' life course (and as part of this protocol) will be used in regression analyses to investigate associations between infection exposure in utero, infancy, childhood and adolescence on vaccine responses. Sociodemographic variables will be considered as potential confounders. We will include the following important variables: age, sex, sociodemographic variables, BCG strain received at birth (and other vaccines received), helminth-related exposures, malaria and documented illness events.

Regression analyses will be used to evaluate associations between infections and outcomes, with adjustment for confounders. We will use a hierarchical statistical modelling approach, so that for analysis of early life exposures we will not adjust for later life exposures that may be on the causal pathway, but for associations

Table 2 Power estimates for objectives I, II and iv (5% significance level)

	$\log_{10}$ difference						
SD ($\log_{10}$)	**0.08**	**0.10**	**0.12**	**0.14**	**0.16**	**0.18**	**0.20**
Objective i: 192 high intensity vs 192 low intensity (infected only)							
0.3	65%	83%	94%	98%	>99%	>99%	>99%
0.4	42%	59%	75%	87%	94%	98%	99%
0.5	29%	42%	56%	69%	80%	88%	94%
0.6	21%	31%	42%	53%	65%	75%	83%
Objective ii: 150 BCG 'preimmunisation' vs 150 no BCG vaccination							
0.3	59%	78%	91%	97%	99%	>99%	>99%
0.4	37%	53%	69%	82%	91%	96%	98%
0.5	26%	37%	50%	63%	75%	84%	91%
0.6	19%	28%	37%	48%	59%	69%	78%
Objective iv: 240 rural vs 150 urban*							
0.3	66%	84%	94%	99%	>99%	>99%	>99%
0.4	43%	60%	76%	87%	94%	98%	99%
0.5	30%	43%	57%	80%	81%	89%	94%
0.6	22%	32%	43%	54%	66%	76%	84%

Cells highlighted in grey correspond to >80% power.
*Numbers shown for rural high schistosomiasis versus urban setting. Power will be greater for rural high malaria versus urban setting.
BCG, bacillus calmette-guérin.

between later childhood or current exposures and vaccine responses we will adjust for early life exposures as potential confounders. Linear regression will be used for the primary outcomes and for continuous secondary outcomes. Outcome distributions are likely to be positively skewed; where necessary, we will apply log transformations to normalise outcome distributions before linear regression analysis. Logistic regression will be used for the protective immunity secondary outcomes, which are binary. We shall also investigate whether multiple infection exposures combine multiplicatively in their effect and test for interaction.

Genetic factors will also be considered: genetic data is already available for EMaBS based on earlier approvals for work on genetic polymorphisms and vaccine responses in infancy. It will be of interest to determine whether genetic factors also have a strong influence on adolescents' responses.

Objective iv: urban–rural comparisons in vaccine response

We hypothesise that environmental (especially parasite) exposures are key drivers of POPVAC. Hence, we predict differences between urban and rural settings within Uganda (with urban vaccine responses stronger, as observed in Gabon[8 9] and Senegal[64] and that these differences will be related to parasite exposure.

The key exposure for this objective is 'setting'. We will compare outcomes between urban EMaBS and (1) rural high-schistosomiasis and (2) rural high-malaria participants. For these comparisons we shall include in the analysis only the urban EMaBS participants who were randomised to receive BCG 'preimmunisation,' such that their immunisation schedule is identical to that in the other two settings. We will include only control groups from the rural high-schistosomiasis and high-malaria settings, since these will have received minimal anti-parasite treatment (figures 3 and 4). In the primary analysis, we will adjust for age and sex, but not for factors likely to be on the causal pathway between setting and vaccine response.

Although our settings are purposely chosen based on parasite prevalence, there will be overlap in this, and other, exposures. Therefore, we will undertake exploratory analyses using causal mediation modelling, a statistical approach which aims to identify factors that mediate an observed association[65] (here, between setting and vaccine response). We will focus on current *S. mansoni and Plasmodium falciparum* infection, and prior exposure (assessed by anti-schistosome and antimalaria antibody), as key potential mediators of interest (see online supplemental figure 1). Explanatory factors for urban versus rural setting, and for vaccine responses, will be included in the causal diagram and adjusted for in analyses. Although nutrition may impact vaccine responses,[66] we expect this to be less important in healthy adolescents than might be the case in young children; however, data on anthropometric parameters and diet will be collected and adjusted for.

One limitation is that observational analyses of parasite effects are beset with potential unmeasured confounding factors. Results will be interpreted cautiously and objective i (trials A and B) will address causality rigorously. The sample archive will allow future investigation of additional potential mediators. Another potential limitation is that in Uganda, rural to urban migration for schooling is common. However, in the EMaBS birth cohort we have data on residence. Urban to rural migration for schooling is relatively unlikely but will be documented.

With objective iv, we expect to confirm differences in vaccine responses between settings; and to obtain insights into environmental factors that mediate this. By addressing the portfolio of vaccines and array of outcomes presented above, we will identify categories of vaccine most affected; we will distinguish effects on waning and (for HPV) on priming versus boosting. Vaccine response data from urban EMaBS adolescents will provide a reference, suggesting the extent to which changes in infection exposure and lifestyle are likely to influence responses to vaccines (and to infectious diseases) as Africa's urbanisation advances.

Objective v: the role of 'transkingdom' interactions in determining vaccine responses

We will address the hypothesis that parasites also impact vaccine responses through 'transkingdom' effects, mediated by other components of the host ecosystem. We propose that herpesvirus activation and MT are likely to be determinants of vaccine response, and they are driven by parasitic infections. Samples from selected participants from each setting and study arm will be examined. To optimise the precision of our comparisons these will be selected among those (for the high-schistosomiasis and high-malaria settings) who had the parasitic infection of interest at baseline (and, if possible, no other parasitic infection detected) and complied with the intended treatment. To test our hypothesis, we will measure markers of viral activation and MT in plasma/serum and/or stool. Initial analyses (figure 5) will investigate associations between these markers and vaccine responses (arrow F); and between settings (arrow D) or parasite infection and treatment (arrow E) and viral activation or MT. Objective vi will link herpesvirus activation, MT and parasitic infections to immune activation and regulation.

Objective vi: preimmunisation immunological parameters

There is strong evidence that preimmunisation immunological status impacts vaccine responses[5 60] but the factors that determine this status have not been identified. We hypothesise that the environmental, parasitic, viral and microbial exposures addressed in objectives i–v are key (figure 5). We aim to investigate this by identifying immunological parameters that specifically link the distal exposures to vaccine response. These include the circulating cytokine and chemokine milieu, innate cell responses (which govern adaptive responses), and frequencies and phenotypes of both innate and adaptive cells. Our

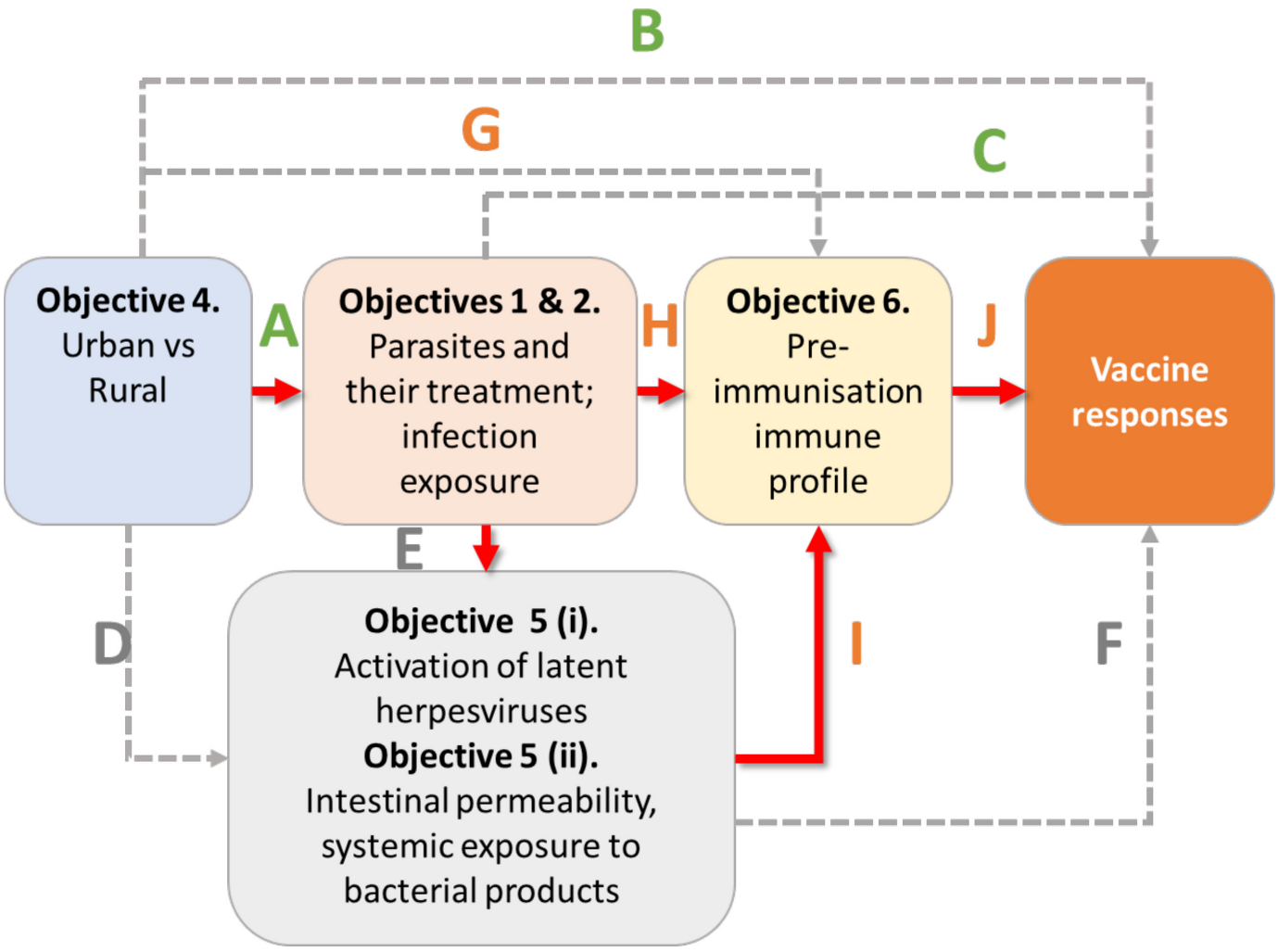

Figure 5 Synthesised analysis of study objectives. Red arrows represent our principal hypotheses. Arrows A–C are considered in objectives I and iv; Arrows D–F in objective v; Arrows G–J, in objective vi; as well as the fully synthesised analysis.

principal focus will be on pre-immunisation measurements although, in selected groups of most interest, it will also be possible to examine samples obtained post-immunisation to identify which biomarkers or cell populations change.

Initial analyses will confirm hypothesised, and identify new, preimmunisation immunological parameters associated with responses to vaccines in our portfolio (figure 5, arrow J). If we observe associations between rural versus urban location (arrow B) or parasites and their treatment (arrow C) and vaccines responses, we will explore whether these effects are mediated by viral and MT variables and preimmunisation immune profiles using causal mediation analyses.[65]

Operational considerations

A programme steering committee has been set up to guide progress across all projects. A data and safety monitoring board has also been appointed to provide real-time safety oversight. Details of these and other operational activities can be found in online supplemental material 1.

Ethics and dissemination

Ethical and regulatory approval has been granted from the Research Ethics Committees of Uganda Virus Research Institute and the London School of Hygiene and Tropical Medicine, the Uganda National Council for Science and Technology and the Uganda National Drug Authority; details are given in online supplemental material 1 and in each trial paper. Given the importance of the data and sample archive as a resource for mechanistic studies on the determinants of vaccine responses, assent and consent processes will include storage of samples for future and genetic studies, and anonymised data and sample sharing. Any protocol amendments will be submitted to ethics committees and regulatory bodies for approval before implementation.

Study findings will be published through open access peer-reviewed journals, presentations at local, national and international conferences and to the local community through community meetings. Anonymised participant level datasets generated will be available on request.

Patient and public involvement

Concepts involved in this work have been discussed with colleagues at the Vector Control Division and EPI in the Ministry of Health (Uganda) and with relevant District Councils, community leaders and Village Health Teams. We also have held meetings to explain the proposed work to teachers, parents, participants and village members, and to address their questions. Study findings will be shared with these stakeholders.

Data management and analysis

Sociodemographic information and clinical and laboratory measurements will be recorded and managed using Research Electronic Data Capture tools,[67 68] with paper-based forms as back-up. All data will be recorded under a unique study ID number. When paper forms must be used, data will be entered in a study-specific database, with standard checks for discrepancies. All data for analysis will be anonymised and stored on a secure and password-protected server, with access limited to essential research personnel. Anonymised participant-level datasets generated will be available for sharing on request.

DISCUSSION

This will be the first well-powered set of studies to investigate effects of schistosomiasis and malaria treatment, and of BCG revaccination, on vaccine responses in adolescents. The results will add to understanding of POPVAC and of interventions that may enhance them. The sample archives developed will provide a major asset for exploration of new leads arising from this hypothesis-driven work, or for an alternative, 'systems biology' approach investigating, for example, transcriptome, microbiome and virome.

Our focus is on the immunological effects of infection exposure in human participants. By understanding these effects, we aim to inform and promote vaccine design tailored to the challenging environment of LICs, and to inform the development of public health strategies (such as tailored immunisation regimens and combined parasite-control/immunisation interventions) that will optimise vaccine implementation in parasite-endemic settings.

Our strong immunoepidemiological design and nested immunological studies will address specific hypotheses regarding pathways of effects. Population immunology is useful for translation of findings to and from basic (especially animal) studies to human health. Our randomised design will determine causal, and reversible, effects of parasitic infections. Substantial sample sizes are needed because immune responses are highly variable in human populations.

We have several reasons for studying vaccine responses particularly among adolescents. In this study setting, they bear a heavy parasite burden.[69] As well, this age group is a target group for vaccines against tuberculosis and sexually transmitted infections (currently HPV—in future, it is hoped, for vaccines against HIV) and for booster immunisations. Also, they enter a period of increased risk of pulmonary tuberculosis after the relatively low-risk period of mid-childhood, and are thus a target group for improved vaccines for tuberculosis.

Study timeline

POPVAC A began recruiting in July 2019. Intervention will be up to 12 months, with completion of the project scheduled for September 2020. POPVAC B is scheduled begin recruiting in February 2021. Intervention will be up to 12 months, with completion of the project scheduled for April 2022. POPVAC C is scheduled to begin recruiting in May 2020. Intervention will be up to 12 months, with completion of the project scheduled for April 2022.

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Contributors AME conceived the study. AME, GN, EW, AN, SC, LZ, JN, AW, PK and HL contributed to study design. LZ, GO, CN, CZ and FA are site clinicians/nurses/clinical laboratory technicians providing valuable input on clinical considerations of the intervention. RK heads the team of field workers handling the organisational integration of the intervention. MA is the study internal monitor. AN and EW are involved in organisation of the databases, trial randomisation, treatment allocation and drawing up of analytical plans. GN, LZ, AN, SC, EW and AME drafted the manuscript. All authors reviewed the manuscript, contributed to it and approved the final version.

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SUPPLEMENTARY INFORMATION

Population differences in vaccine responses (POPVAC): scientific rationale and cross-cutting analyses for three linked, randomised controlled trials assessing the role, reversibility and mediators of immunomodulation by chronic infections in the tropics

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21 **Table S1. Uganda National Expanded Programme on Immunisation (EPI) schedule**

Vaccine/ antigen	Dosage and doses required	Minimum Interval Between Doses	Minimum Age to Start	Mode and site of Administration	Storage Temperatures
Infant vaccines					
BCG	Infants (0-11m) 0.05ml. ≥11 months and 0.1ml, 1 dose	Not applicable	At birth (or first contact)	*Intradermal*, right upper arm	+2^{0}C to +8^{0}C
DPT - HepB - Hib	0.5 ml, 3 doses	One month (4 weeks)	At 6 weeks or first contact after this age	*Intramuscular,* outer upper aspect of left thigh	+2^{0}C to +8^{0}C DO NOT FREEZE
PCV	0.5 mls, 3 doses	One month (4 weeks)	At 6 weeks or first contact after this age	*Intramuscular,* outer upper aspect of right thigh	+2^{0}C to +8^{0}C DO NOT FREEZE
Polio	2 drops, 3 doses	One month (4 weeks)	At birth or within the first 2 weeks (Polio 0) and at 6 weeks or first contact after 6 weeks (Polio 1)	*Orally*	+2^{0}C to +8^{0}C
IPV	0.5ml, 1 dose	Nil	At 14 weeks	*Intramuscular,* left upper thigh	+2^{0}C to +8^{0}C DO NOT FREEZE
Rotavirus	drops, 2 doses		6 weeks or 1st contact after this age	*Orally*	
Measles	0.5 ml, 1 doses	Nil	At 9 months (or first contact after that age).	*Subcutaneous,* left upper arm	+2^{0}C to +8^{0}C
Primary school/adolescent/adult vaccines					
Tetanus/Diphtheria	0.5 ml, 5 doses	Td1: First contact with a WCBA Td2: One month after TT1 Td3: Six months after TT2 Td4: One year after TT3 Td5: One year after TT4	At first contact with a pregnant woman or women of childbearing age (15-49 years)	*Intramuscular,* upper arm	+2^{0}C to +8^{0}C, DO NOT FREEZE
HPV	0.5 ml, 2 doses	HPV1: First contact with a girl in primary 4, or aged 10 years and out of school HPV2: Given at 6 months after HPV1[a]	Girls in primary 4 or 10-year-old girls who are out of school	*Intramuscular,* upper arm	+2^{0}C to +8^{0}C, DO NOT FREEZE

BCG: Bacillus Calmette Guerin. DPT: Diphtheria, Pertussis, Tetanus. Hep B: Hepatitis B. Hib: Haemophilus influenzae type B. PCV: pneumococcal conjugate vaccine. IPV: inactivated polio vaccine. HPV: Human Papilloma Virus. WCBA: woman of child-bearing age. [a]An additional dose of HPV, four weeks after the first dose, is recommended for girls aged 14 years or above receiving HPV immunisation for the first time.

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Further rationale for the selection of vaccines

Bacillus Calmette–Guérin (BCG)

BCG is a live, replicating parenteral vaccine, the only licensed vaccine against TB. BCG vaccine for these studies will be obtained from the Serum Institute of India either directly, or through a supplier in Uganda. The Serum Institute of India provides much of the BCG vaccine used in Uganda.

Worldwide, TB is among the top 10 causes of death; Uganda has an estimated incidence of 202/100,000 people.[1] Infectious, sputum positive, pulmonary TB classically emerges in adolescence, driving the on-going epidemic.[2] Thus adolescent booster immunisation is a key TB control strategy.[3] However, BCG vaccine response and efficacy are often impaired in tropical and rural settings[4-6] and new TB vaccines are similarly affected.[7] In the past, WHO has been hesitant to recommend BCG re-vaccination. However, in 2017 WHO's Strategic Advisory Group of Experts (SAGE) recommended: "Further research is warranted to explore whether certain sub-groups of age, geographic or *M. tuberculosis* exposure categories would benefit from re-vaccination."[8] Recent results suggest that, despite the variability of BCG efficacy between populations, BCG vaccination in adolescence offers benefit in some tropical settings, especially for individuals who are not yet infected with *Mycobacterium tuberculosis*, and may also be cost-effective.[5 9] Also, BCG vaccine is currently being used among adolescents in South Africa as a comparator in a trial of a novel TB vaccine (trial registration NCT02075203). To our knowledge, BCG efficacy in Ugandan adolescents, and differences in BCG vaccine responses between urban and rural Ugandan populations, have not been tested. Information obtained from this study is expected to further inform the use of BCG in adolescents, and also to inform the development of new vaccines for tuberculosis.

Yellow fever vaccine

Yellow fever vaccine YF-17D is a live replicating parenteral vaccine. The vaccine (Stamaril; Sanofi Pasteur) is available for purchase in Uganda. Yellow Fever (YF) causes outbreaks in Uganda and the wider region[10] and YF-17D is a candidate for Uganda's expanded programme on immunisation (EPI). Lower vaccine replication, lower neutralising antibody induction, and greater waning, are described in Uganda compared to Switzerland.[11] YF-17D is a potential vector for novel vaccine constructs,[12] adding relevance to vaccine development.

Typhoid vaccine Ty21a

Typhoid vaccine Ty21a is a live replicating oral vaccine and also a potential vector for new vaccine constructs.[13] Ty21a vaccine will be purchased from PaxVax, Redwood City, California. Substantial, multi-year typhoid outbreaks occur in Uganda and immunisation campaigns have been advocated as

cost effective.[14] Schistosomiasis has been associated with prolonged *S. typhi* infection[15] and impaired antibody responses to killed typhoid vaccines.[16]

Ty21a was developed in the 1970s. Although not routinely used in Uganda, it has been (and is currently) registered in many countries. It was first registered in the United States and United Kingdom in the 1980s, and is recommended by the WHO for both endemic and epidemic settings.[17] It has comparable efficacy to the parenteral Vi polysaccharide typhoid vaccine, good durability and minimal adverse effects.[17] It is proposed for use in this study to model effects of study exposures and intervention on the response to a live oral vaccine.

The Ty21a vaccine is given as a three-dose regimen on alternate days.

Human Papilloma Virus (HPV) vaccine

Human Papilloma Virus (HPV) vaccine is a protein virus-like particle. The quadrivalent HPV Vaccine Gardasil (Merck) is available for purchase in Uganda and is the vaccine used by the national EPI programme. Studies after three vaccine doses have found somewhat enhanced responses in the presence of malaria, but no effect of helminths.[18] No study has previously investigated parasite effects on the priming response, but recent results for tetanus suggest that priming may be more susceptible than boosting to adverse effects.[19] This will be important if forthcoming trials support single-dose HPV immunisation (NCT02834637). HPV immunisation is being rolled out among girls to prevent cervical neoplasia, the commonest cancer among Ugandan women and we will coordinate provision with the national HPV immunisation programme.[20] HPV immunisation is also beneficial for boys since HPV infection is associated with anogenital warts, anal cancer and oropharyngeal cancers in both males and females, and with penile cancer in men,[21] and we will include boys in these studies.

Tetanus and diphtheria vaccines

Tetanus and diphtheria vaccines comprise inert toxoids (Td). Schistosomiasis is associated with a Th2 biased response to tetanus toxoid[22] and with suppressed antibody responses among those with low pre-immunisation antibody levels.[19] Booster immunisation is recommended for young women to prevent maternal and neonatal tetanus. Recent evidence emphasises the need to protect young men also.[23] Uganda's EPI programme recommends tetanus boosters in adolescence and plans to change from tetanus alone to Td in 2018.

Additional considerations regarding the vaccine schedule

Live vaccines given in combination may influence the response to each other – a phenomenon described as "interference". Observations in the 1960s suggested that elevated circulating interferon(IFN)-γ after measles immunisation might interfere with the response to Vaccinia[24] and, to avoid such interference between live vaccines, it was recommended that live vaccines be given either together or three to four weeks apart.[25] However, with the introduction of new, live vaccines into use, Public Health England reviewed and revised this recommendation in 2014, limiting it to vaccines for which there was an evidence base (**Table S2**). We have adopted a four-week interval between BCG immunisation and the other proposed live vaccines (YF and Ty21a), which will be given together.[26]

Non-live vaccines can be given at the same time as live vaccines and there are no specific recommendations as to the number of non-live vaccines that can be given together. Two injections can be given into the same muscle although it is suggested that these should be at least 2.5 cm apart in case it is necessary to distinguish local adverse reactions to the two injections.[27] Our schedule avoids giving more than two injections on the same date but at week 4 it may be appropriate to give two into the deltoid muscle of the same arm if a BCG scar is developing on the other arm. Nevertheless, uncertainties remain regarding the effects of vaccines on responses to each other. Generalisations from this programme of work will need to take potential "interference" between vaccines into account.

Table S2: Public Health England recommendations for giving more than one live attenuated vaccine in current use in the UK[26]

Vaccine combinations	Recommendations
Yellow Fever and Measles, Mumps, Rubella (MMR)	A four-week minimum interval period should be observed between the administration of these two vaccines. Yellow Fever and MMR should not be administered on the same day.
Varicella (and zoster) vaccine and MMR	If these vaccines are not administered on the same day, then a four-week minimum interval should be observed between vaccines.
Tuberculin skin testing (Mantoux) and MMR	If a tuberculin skin test has already been initiated, then MMR should be delayed until the skin test has been read unless protection against measles is required urgently. If a child has had a recent MMR, and requires a tuberculin test, then a four-week interval should be observed.
All currently used live vaccines (BCG, rotavirus, live attenuated influenza vaccine (LAIV), oral typhoid vaccine, yellow fever, varicella, zoster and MMR) and tuberculin (Mantoux) skin testing.	Apart from those combinations listed above, these live vaccines can be administered at any time before or after each other. This includes tuberculin (Mantoux) skin testing.

Vaccine storage and transport

In order to maintain a reliable vaccine cold chain, the vaccines and diluents to be used will be stored and transported within the recommended temperature range of +2°C to +8°C. Care will be taken to ensure that the vaccines are not frozen. BCG, being sensitive to light, will be kept in the dark (normally within its secondary packaging) for as long as possible to protect it during storage and transportation. All vaccines will be kept in appropriate refrigeration equipment with a temperature monitoring device to ensure temperatures remain between +2°C and +8°C. Cold boxes/vaccines carriers with temperature monitors will be used to transport vaccines and the diluents from the MRC/UVRI and LSHTM Uganda Research Unit (Entebbe) to Koome island and while transporting vaccines to immunization sessions. Designated staff will be given responsibility for managing the vaccine cold chain. All cold chain equipment including the temperature monitoring devices used for this project will comply with relevant technical specifications as defined by the EPI standards. Basic routine maintenance will be regularly carried out on all cold chain equipment.

Additional laboratory measurements

Additional assays will comprise HIV serology, pregnancy testing and full blood counts. HIV testing and pregnancy testing will be accompanied by appropriate counselling by trained staff.

- HIV serology will be done on blood samples using rapid tests and according to prevailing national algorithms.[28] This will be done at baseline.
- Pregnancy testing will be done using urine samples and standard operating procedures for assessment of urine β-human chorionic gonadotropin (βhCG). This will be done at baseline and before immunisation on each immunisation day.
- Full blood counts will be conducted using a haematology analyser. Mild, moderate and severe anaemia will be defined according to WHO guidelines, by age.[29] This will be done at baseline to test for anaemia as part of the eligibility assessment, and pre-immunisation as part of the assessment of immunological profile.
- Information on *S. mansoni* and malaria diagnosis before treatment and throughout the trials is detailed in the focused papers for these trials (bmjopen-2020-040426, bmjopen-2020-040427 and bmjopen-2020-040430). Briefly, current *S. mansoni* infection status and intensity will be determined by serum/plasma levels of circulating anodic antigen (CAA). In Trial A, CAA will be assessed retrospectively on stored samples collected at baseline, on immunisation days, and on primary and secondary endpoint days. In Trial B, CAA will be assessed retrospectively on stored samples collected at baseline and at weeks 28 and 52. In

Trial C, CAA will be assessed retrospectively on stored samples collected at baseline. In all three trials, current malaria infection status and intensity will be assessed retrospectively by PCR on stored blood samples collected on immunisation days and at week 52.

Individuals found to be HIV positive or pregnant will be referred to appropriate providers for further care. Individuals with severe anaemia (haemoglobin <82g/L) will be excluded from the randomised intervention (since the intervention might be beneficial in management of anaemia). They will be treated for anaemia and for any underlying cause identified.

Sample handling and archive

Blood and other samples will be processed according to local laboratory standard operating procedures (SOPs). All samples will reach the laboratory in anonymised form.

A sample archive will be developed. Although our current programme of work plans to address specific hypotheses regarding pathways of effects of parasites and interventions, the sample archive will provide a major asset for exploration of new leads arising from this work, or for an alternative, "systems biology" approach employing (for example) proteomic, genomic, epigenetic and transcriptomic analyses, and investigating the microbiome and virome. Information provided to participants, and consent forms, will include considerations of sample storage, and the possibility of sample analysis in laboratories within and outside Uganda. Participants will be able to decide if they will permit such future use of any leftover samples. We plan to store the samples for up to 20 years. If further storage is needed after that time, permission will be requested from the Uganda Virus Research Institute and London School of Hygiene and Tropical Medicine review committees. If they elect not to permit this, all of those leftover samples will be discarded after the completion of the work included in the current protocol.

Operational considerations

Programme governance

A Programme Steering Committee has been set up to guide progress across all projects. This comprises the following:

- An independent chair
- Representatives from the Ministry of Health programmes for immunisation and for vector borne disease control
- Representatives of district authorities (Mukono and Jinja districts)
- Community representatives
- Principal investigator and co-investigators

- Project leader and post-doctoral immunologist
- Trial statistician
- Laboratory manager
- Medical Research Council observer

Informed consent

Both written informed assent from the participants and written informed consent from a parent or guardian will be required for participation, although these may not necessarily be obtained at the same time. Information will be provided in both English and the appropriate local language. For individuals who cannot speak the languages used, or who cannot read or write, a witness who can read the information sheet and translate the information to the participant or parent/guardian will be used. For trials A and B, two different types of age specific assent forms will be used for the group of participants aged 9 – 12 years and for the group aged 13 – 17 years. Informed consent by emancipated or mature minors will be obtained using a designated consent form for these categories of participants

The aims of the study, all tests, treatments and immunisations to be carried out and potential risks will be explained. The participant will be given the opportunity to ask about details of the trial, and will then have time to consider whether or not to participate. If they do decide to participate, they and their parent/guardian will sign and date two copies of the assent and consent forms, one for them to take away and keep, and one to be stored securely by the research team. Separate information and consent forms will be provided (i) for consent for storage of samples for future studies and for anonymous sharing of data from this study and (ii) for possible genetic studies (for the urban cohort genetic data are already available based on previous approval); the information sheet will explain that these data may be used in analyses related to this protocol.

Screening and Eligibility Assessment

Once the informed consent process has been completed, and consent (and assent) given, a baseline medical history (including concomitant medication) will be collected. Vital signs will be checked and a physical examination will be performed. Inclusion and exclusion criteria will be checked.

Participants will undergo pre- and post-test counselling for HIV and (for girls) pregnancy testing by a trained and experienced nurse- or clinician-counsellor. Blood, urine and stool samples will be obtained, for tests as specified in the schedule of procedures. These tests are to exclude the major, immunomodulating co-infection, HIV, and conditions that might impact safety (anaemia, pregnancy).

Enrolment

Participants who consent/assent, complete the screening processes, satisfy all the inclusion criteria and meet none of the exclusion criteria will be enrolled.

Discontinuation / withdrawal criteria

In accordance with the principles of the current revision of the Declaration of Helsinki and any other applicable regulations, a participant has the right to withdraw from the study at any time and for any reason, and is not obliged to give his or her reasons for doing so. The Investigator may withdraw the participant at any time in the interests of the participant's health and well-being. In addition, the participant may withdraw/be withdrawn for any of the following reasons:

- Ineligibility (either arising during the study or retrospectively, having been overlooked at screening)
- Administrative decision by the Investigator
- Significant protocol deviation
- Participant non-compliance with study requirements
- An adverse event which requires discontinuation of the study involvement or results in inability to continue to comply with study procedures.

Any participant who becomes pregnant during the trial will be followed up until the end of the pregnancy but no further immunisations will be given unless indicated during pregnancy (as is the case for tetanus toxoid). The trial allocation for this participant will be unblinded and the participant will only be given further treatment if clinically indicated. The babies will also be followed up and examined for any adverse effects. We will not routinely perform venipuncture in a pregnant participant.

The reason for withdrawal will be recorded in the case report form (CRF). If withdrawal is due to an AE, appropriate follow-up visits or medical care will be arranged, with the agreement of the participant, until the AE has resolved, stabilised or a non-trial related causality has been assigned.

If a participant withdraws from the study samples collected before their withdrawal from the trial will be used/ stored unless the participant specifically requests otherwise.

Trial discontinuation

The trial will be discontinued in the event of new scientific information that renders continuation futile or unethical, or for any other reason, at the discretion of the Programme Steering Committee.

End of study definition

The trial will be completed when the last participant enrolled into the trial has completed their final follow up visit.

Safety assessments and oversight

No new investigational drug or product will be used in the proposed trial. However, standard approaches for monitoring safety and reporting of serious adverse events will be followed.

Monitoring

The trial will be monitored by both internal and external monitors according to a pre-defined monitoring plan which will include a site initiation visit, monitoring visits at least annually, and a close-out visit. The monitors will assess patient safety, data integrity, and adherence to the protocol and to Good Clinical Research Practice procedures.

Considerations regarding standard of care for parasitic infections

Malaria and *S. mansoni* infection status will be determined retrospectively through assays conducted in bulk on stored samples. These results will not, therefore, be useful to determine management of individual participants.

Participants in the standard anthelminthic treatment (trial A) and the malaria placebo (trial B) arms will receive lower levels of treatment. However, all trial arms will receive a minimum of well-implemented national standard of care.

In trial A, standard of care will comprise annual praziquantel treatment. Our own results from the Lake Victoria Island Intervention Study on Worms and Allergy-related diseases (LaVIISWA),[30] which compared annual versus quarterly intervention for schistosomiasis at community level over three years, showed no advantage of quarterly treatment for morbidity outcomes attributed to schistosomiasis. Schistosomiasis can cause anaemia. To manage the expected differential benefits of the interventions for anaemia, a full blood count will be performed at baseline, as discussed above; anaemic children will be managed appropriately and severely anaemic children excluded. Albendazole will be provided twice a year to manage nematode infections (after collection of primary and secondary endpoint samples).

Dihydroartemisinin/piperaquine is considered an attractive option for preventive treatment and preventive chemotherapy for malaria because of the long half-life of piperaquine (approximately 23 days).[31] Monthly treatment with DP has been shown to reduce the prevalence of anaemia and reduce episodes of clinical malaria in Ugandan schools[32] but has not been adopted as standard of

care. Trial B is expected to add further evidence regarding the potential benefits of monthly DP for school children by determining the effect on vaccine responses, thereby further contributing to policy debate in this field. To manage the expected differential benefits of the interventions for anaemia, a full blood count will be performed at baseline, as discussed above; anaemic children will be managed appropriately and severely anaemic children excluded. Malaria standard of care will comprise provision of bed nets to minimise malaria exposure for all participants. Rapid diagnostic tests and treatment will be made readily available for participants who develop symptomatic malaria. Albendazole will be provided twice a year to manage nematode infections (after collection of primary and secondary endpoint samples).

Procedures to be followed in the event of abnormal findings

Abnormal clinical findings from medical history, examination or blood tests will be assessed as to their clinical significance throughout the trials. If an abnormal test result is deemed clinically significant, it may be repeated. If a test remains clinically significant, the participant will be informed and appropriate medical care arranged as appropriate and with the permission of the participant. Specific details regarding findings, discussion with participants and resulting actions will be recorded in the clinical records. Decisions to exclude the participant from enrolling in the trial or to withdraw a participant from the trial will be at the discretion of the Investigator.

Data and Safety Monitoring Board (DSMB)

The DSMB will be notified within 7 days of the Investigators' being aware of the occurrence of SAEs. The DSMB may recommend the Investigators to place the trial on hold if deemed necessary following an intervention-related SAE. The DSMB will be chaired by a clinician experienced in clinical trials. There will be a minimum of two other appropriately qualified committee members. In the case of events related to a blinded intervention, the DSMB can request unblinding. Membership will include a statistician, and at least one Ugandan member. All correspondence between Investigators and the DSMB will be conveyed by the Principal Investigator to the trial Sponsor. The Chair of the DSMB will be contacted for advice and independent review by the Investigator or trial Sponsor in the following situations:

- The occurrence of any SAE
- Any other situation where the Investigator or trial Sponsor feels independent advice or review is important

Ethical and regulatory considerations

Ethical approvals

Trial A

Ethical approval has been granted from the Research Ethics Committee of the Uganda Virus Research Institute (UVRI REC, reference: GC/127/19/05/664) and the London School of Hygiene and Tropical Medicine (LSHTM, reference: 16032), and from the Uganda National Council for Science and Technology (UNCST, reference: HS2486) and the Uganda National Drug Authority (NDA, reference: CTA0093).

Trial B

Ethical approval has been granted from the Research Ethics Committees of the Uganda Virus Research Institute (UVRI REC, reference: GC/127/19/05/681) and the London School of Hygiene and Tropical Medicine (LSHTM, reference: 16033), and from the Uganda National Council for Science and Technology (UNCST, reference: HS 2487) and the Uganda National Drug Authority (NDA, reference: CTC0117/2020).

Trial C

Ethical approval has been granted from the Research Ethics Committees of the Uganda Virus Research Institute (reference: GC/127/19/05/682), the London School of Hygiene and Tropical Medicine (reference: 16034), the Uganda National Council for Science and Technology (reference: HS 2491) and from the Uganda National Drug Authority (certificate number: CTA0094).

Further information regarding risks

The immunisations to be given have recognised side effects which are usually mild and resolve spontaneously in a few days to one week. Parenteral vaccines are likely to result in pain and swelling at the site of injection and mild fever; very occasionally pain and swelling can be severe and associated with difficulty in moving the shoulder. Sometimes headache and tiredness occur. Rarely a vaccine may cause a severe allergic reaction. For most vaccines this is estimated at less than one in a million doses (but 1 in 55,000 for Yellow Fever vaccine).[33] Individuals with a history of a possible allergic reaction to drugs or vaccines, or to vaccine components including eggs or chicken proteins, will be excluded from the studies. The research team will be trained and prepared to manage severe allergic reactions.

Adverse reactions to Yellow Fever vaccine include severe nervous system reaction (about 1 person in 125,000) and severe, life-threatening illness with organ failure (about 1 person in 250,000). The mortality for this severe, life-threatening adverse effect is reported as about 50%.[33]

BCG immunisation is likely to induce a scar in many cases. This may develop over several weeks, starting as a small papule at the injection site which may become ulcerated and then heal over a

period of 2 to 5 months; and lymphadenopathy may develop. Occasionally a more severe local reaction occurs (estimated at 1 per 1,000-10,000 doses): for example, an abscess develops and scars may develop into keloids. Rarely BCG can cause disseminated disease (1 per 230,000 to 640,000 doses), or disease in sites remote from the immunisation site. Disseminated BCG disease usually occurs in immunocompromised people: HIV positive people will be excluded from these studies.[34] BCG "pre-immunisation" may interfere with the response to the subsequent live vaccines; indeed our hypothesis, and published results, suggest that it may suppress replication of YF 17D vaccine.[35] However, this reduced replication has not been shown to correlate with, or result in, reduced levels of neutralising antibody titres (which are the desired protective outcome).[11 35]

Oral typhoid vaccine (Ty21a) may occasionally be associated with stomach pain, nausea, vomiting and (rarely) rash.[33]

Praziquantel has been in use for about 30 years. It has a well-recognised profile of side effects including dizziness, nausea, vomiting, abdominal pain, diarrhoea (sometimes with blood) and urticarial rash. The symptoms are considered to arise largely from the effects of killing worms and to be more severe in people with heavy infections. Symptoms are better tolerated when the drugs are given after food and we will provide treatment after a meal or snack. Simple medications, such as paracetamol and cetirizine, can alleviate symptoms and these will be available on treatment days.

More information on causal mediation analysis

Causal mediation analysis is a statistical approach that aims to assess the relative importance of intermediate variables (mediators) through which an exposure may affect an outcome. It uses a counterfactual framework. In essence, this investigates how, for each individual, values of the mediator and outcome might change if their main exposure status changed. For example, in Objective iv, it will be used in exploratory analysis to assess whether, and to what degree, any differences in vaccine response (the outcome) by setting (the exposure) may be explained by differences in current or previous *S. mansoni* and *P. falciparum* infection experience (the potential mediators). An alternative approach would be to use a path analytic approach such as structural equation modelling, but estimation of pathway-specific effects using this technique requires strong assumptions regarding linearity and normality and lack of pairwise confounding for all variables in the model. Causal mediation analysis is itself subject to key assumptions regarding confounding between exposure, mediator and outcomes, and we will assess the potential impact of violations in these assumptions using sensitivity analysis. Specifically, we will assess the degree of uncontrolled confounding that would substantially change conclusions regarding pathway-specific effects using techniques such as those described by Hafemen (2011).[36] Regarding the mediators for Objective iv,

359 we will consider *S. mansoni* and *P. falciparum* separately, under the assumption that there is no
360 direct causal link between these infections. For each infection, we will include both prior exposure
361 and current infection as mediators in the same set of models (**Figure S1**), allowing the possibility that
362 current infection depends on prior exposure, using the approach outlined in Steen *et al*.[37]

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Figure S1. Causal diagram for assessment of whether exposure to parasites mediates differences in vaccine response between urban and rural settings

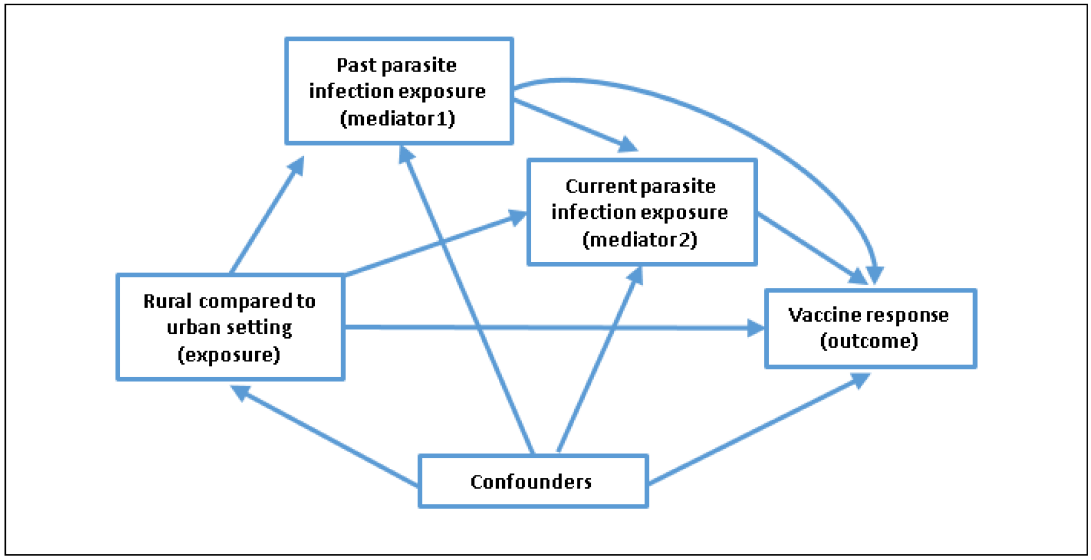

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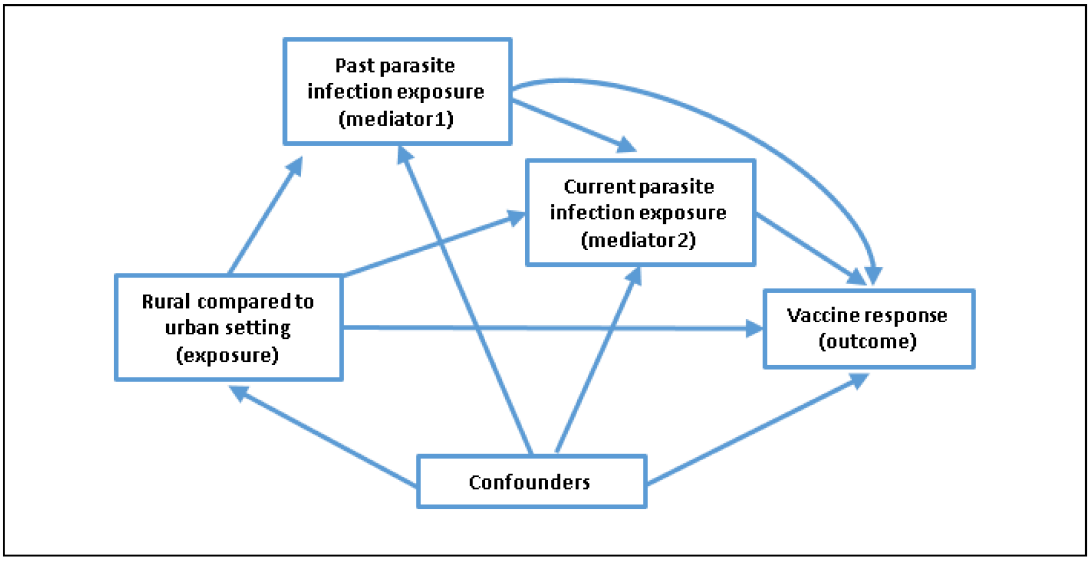
Figure S1. Causal diagram for assessment of whether exposure to parasites mediates differences in vaccine response between urban and rural settings



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21 **Table S1. Uganda National Expanded Programme on Immunisation (EPI) schedule**

Vaccine/ antigen	Dosage and doses required	Minimum Interval Between Doses	Minimum Age to Start	Mode and site of Administration	Storage Temperatures
Infant vaccines					
BCG	Infants (0-11m) 0.05ml. ≥11 months and 0.1ml, 1 dose	Not applicable	At birth (or first contact)	*Intradermal*, right upper arm	+2^{0}C to +8^{0}C
DPT - HepB - Hib	0.5 ml, 3 doses	One month (4 weeks)	At 6 weeks or first contact after this age	*Intramuscular,* outer upper aspect of left thigh	+2^{0}C to +8^{0}C DO NOT FREEZE
PCV	0.5 mls, 3 doses	One month (4 weeks)	At 6 weeks or first contact after this age	*Intramuscular,* outer upper aspect of right thigh	+2^{0}C to +8^{0}C DO NOT FREEZE
Polio	2 drops, 3 doses	One month (4 weeks)	At birth or within the first 2 weeks (Polio 0) and at 6 weeks or first contact after 6 weeks (Polio 1)	*Orally*	+2^{0}C to +8^{0}C
IPV	0.5ml, 1 dose	Nil	At 14 weeks	*Intramuscular,* left upper thigh	+2^{0}C to +8^{0}C DO NOT FREEZE
Rotavirus	drops, 2 doses		6 weeks or 1st contact after this age	*Orally*	
Measles	0.5 ml, 1 doses	Nil	At 9 months (or first contact after that age).	*Subcutaneous,* left upper arm	+2^{0}C to +8^{0}C
Primary school/adolescent/adult vaccines					
Tetanus/Diphtheria	0.5 ml, 5 doses	Td1: First contact with a WCBA Td2: One month after TT1 Td3: Six months after TT2 Td4: One year after TT3 Td5: One year after TT4	At first contact with a pregnant woman or women of childbearing age (15-49 years)	*Intramuscular,* upper arm	+2^{0}C to +8^{0}C, DO NOT FREEZE
HPV	0.5 ml, 2 doses	HPV1: First contact with a girl in primary 4, or aged 10 years and out of school HPV2: Given at 6 months after HPV1[a]	Girls in primary 4 or 10-yearold girls who are out of school	*Intramuscular,* upper arm	+2^{0}C to +8^{0}C, DO NOT FREEZE

BCG: Bacillus Calmette Guerin. DPT: Diphtheria, Pertussis, Tetanus. Hep B: Hepatitis B. Hib: Haemophilus influenzae type B. PCV: pneumococcal conjugate vaccine. IPV: inactivated polio vaccine. HPV: Human Papilloma Virus. WCBA: woman of child-bearing age. [a]An additional dose of HPV, four weeks after the first dose, is recommended for girls aged 14 years or above receiving HPV immunisation for the first time.

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Table S2: Public Health England recommendations for giving more than one live attenuated vaccine in current use in the UK[26]

Vaccine combinations	Recommendations
Yellow Fever and Measles, Mumps, Rubella (MMR)	A four-week minimum interval period should be observed between the administration of these two vaccines. Yellow Fever and MMR should not be administered on the same day.
Varicella (and zoster) vaccine and MMR	If these vaccines are not administered on the same day, then a four-week minimum interval should be observed between vaccines.
Tuberculin skin testing (Mantoux) and MMR	If a tuberculin skin test has already been initiated, then MMR should be delayed until the skin test has been read unless protection against measles is required urgently. If a child has had a recent MMR, and requires a tuberculin test, then a fourweek interval should be observed.
All currently used live vaccines (BCG, rotavirus, live attenuated influenza vaccine (LAIV), oral typhoid vaccine, yellow fever, varicella, zoster and MMR) and tuberculin (Mantoux) skin testing.	Apart from those combinations listed above, these live vaccines can be administered at any time before or after each other. This includes tuberculin (Mantoux) skin testing.

SUPPLEMENTARY INFORMATION

Population differences in vaccine responses (POPVAC): scientific rationale and cross-cutting analyses for three linked, randomised controlled trials assessing the role, reversibility and mediators of immunomodulation by chronic infections in the tropics

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21 **Table S1. Uganda National Expanded Programme on Immunisation (EPI) schedule**

Vaccine/ antigen	Dosage and doses required	Minimum Interval Between Doses	Minimum Age to Start	Mode and site of Administration	Storage Temperatures
Infant vaccines					
BCG	Infants (0-11m) 0.05ml. ≥11 months and 0.1ml, 1 dose	Not applicable	At birth (or first contact)	*Intradermal*, right upper arm	+2^{0}C to +8^{0}C
DPT - HepB - Hib	0.5 ml, 3 doses	One month (4 weeks)	At 6 weeks or first contact after this age	*Intramuscular,* outer upper aspect of left thigh	+2^{0}C to +8^{0}C DO NOT FREEZE
PCV	0.5 mls, 3 doses	One month (4 weeks)	At 6 weeks or first contact after this age	*Intramuscular,* outer upper aspect of right thigh	+2^{0}C to +8^{0}C DO NOT FREEZE
Polio	2 drops, 3 doses	One month (4 weeks)	At birth or within the first 2 weeks (Polio 0) and at 6 weeks or first contact after 6 weeks (Polio 1)	*Orally*	+2^{0}C to +8^{0}C
IPV	0.5ml, 1 dose	Nil	At 14 weeks	*Intramuscular,* left upper thigh	+2^{0}C to +8^{0}C DO NOT FREEZE
Rotavirus	drops, 2 doses		6 weeks or 1st contact after this age	*Orally*	
Measles	0.5 ml, 1 doses	Nil	At 9 months (or first contact after that age).	*Subcutaneous,* left upper arm	+2^{0}C to +8^{0}C
Primary school/adolescent/adult vaccines					
Tetanus/Diphtheria	0.5 ml, 5 doses	Td1: First contact with a WCBA Td2: One month after TT1 Td3: Six months after TT2 Td4: One year after TT3 Td5: One year after TT4	At first contact with a pregnant woman or women of childbearing age (15-49 years)	*Intramuscular,* upper arm	+2^{0}C to +8^{0}C, DO NOT FREEZE
HPV	0.5 ml, 2 doses	HPV1: First contact with a girl in primary 4, or aged 10 years and out of school HPV2: Given at 6 months after HPV1[a]	Girls in primary 4 or 10-year-old girls who are out of school	*Intramuscular,* upper arm	+2^{0}C to +8^{0}C, DO NOT FREEZE

BCG: Bacillus Calmette Guerin. DPT: Diphtheria, Pertussis, Tetanus. Hep B: Hepatitis B. Hib: Haemophilus influenzae type B. PCV: pneumococcal conjugate vaccine. IPV: inactivated polio vaccine. HPV: Human Papilloma Virus. WCBA: woman of child-bearing age. [a]An additional dose of HPV, four weeks after the first dose, is recommended for girls aged 14 years or above receiving HPV immunisation for the first time.

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Further rationale for the selection of vaccines

Bacillus Calmette–Guérin (BCG)

BCG is a live, replicating parenteral vaccine, the only licensed vaccine against TB. BCG vaccine for these studies will be obtained from the Serum Institute of India either directly, or through a supplier in Uganda. The Serum Institute of India provides much of the BCG vaccine used in Uganda.

Worldwide, TB is among the top 10 causes of death; Uganda has an estimated incidence of 202/100,000 people.[1] Infectious, sputum positive, pulmonary TB classically emerges in adolescence, driving the on-going epidemic.[2] Thus adolescent booster immunisation is a key TB control strategy.[3] However, BCG vaccine response and efficacy are often impaired in tropical and rural settings[4-6] and new TB vaccines are similarly affected.[7] In the past, WHO has been hesitant to recommend BCG re-vaccination. However, in 2017 WHO's Strategic Advisory Group of Experts (SAGE) recommended: "Further research is warranted to explore whether certain sub-groups of age, geographic or *M. tuberculosis* exposure categories would benefit from re-vaccination."[8] Recent results suggest that, despite the variability of BCG efficacy between populations, BCG vaccination in adolescence offers benefit in some tropical settings, especially for individuals who are not yet infected with *Mycobacterium tuberculosis*, and may also be cost-effective.[5 9] Also, BCG vaccine is currently being used among adolescents in South Africa as a comparator in a trial of a novel TB vaccine (trial registration NCT02075203). To our knowledge, BCG efficacy in Ugandan adolescents, and differences in BCG vaccine responses between urban and rural Ugandan populations, have not been tested. Information obtained from this study is expected to further inform the use of BCG in adolescents, and also to inform the development of new vaccines for tuberculosis.

Yellow fever vaccine

Yellow fever vaccine YF-17D is a live replicating parenteral vaccine. The vaccine (Stamaril; Sanofi Pasteur) is available for purchase in Uganda. Yellow Fever (YF) causes outbreaks in Uganda and the wider region[10] and YF-17D is a candidate for Uganda's expanded programme on immunisation (EPI). Lower vaccine replication, lower neutralising antibody induction, and greater waning, are described in Uganda compared to Switzerland.[11] YF-17D is a potential vector for novel vaccine constructs,[12] adding relevance to vaccine development.

Typhoid vaccine Ty21a

Typhoid vaccine Ty21a is a live replicating oral vaccine and also a potential vector for new vaccine constructs.[13] Ty21a vaccine will be purchased from PaxVax, Redwood City, California. Substantial, multi-year typhoid outbreaks occur in Uganda and immunisation campaigns have been advocated as

cost effective.[14] Schistosomiasis has been associated with prolonged *S. typhi* infection[15] and impaired antibody responses to killed typhoid vaccines.[16]

Ty21a was developed in the 1970s. Although not routinely used in Uganda, it has been (and is currently) registered in many countries. It was first registered in the United States and United Kingdom in the 1980s, and is recommended by the WHO for both endemic and epidemic settings.[17] It has comparable efficacy to the parenteral Vi polysaccharide typhoid vaccine, good durability and minimal adverse effects.[17] It is proposed for use in this study to model effects of study exposures and intervention on the response to a live oral vaccine.

The Ty21a vaccine is given as a three-dose regimen on alternate days.

Human Papilloma Virus (HPV) vaccine

Human Papilloma Virus (HPV) vaccine is a protein virus-like particle. The quadrivalent HPV Vaccine Gardasil (Merck) is available for purchase in Uganda and is the vaccine used by the national EPI programme. Studies after three vaccine doses have found somewhat enhanced responses in the presence of malaria, but no effect of helminths.[18] No study has previously investigated parasite effects on the priming response, but recent results for tetanus suggest that priming may be more susceptible than boosting to adverse effects.[19] This will be important if forthcoming trials support single-dose HPV immunisation (NCT02834637). HPV immunisation is being rolled out among girls to prevent cervical neoplasia, the commonest cancer among Ugandan women and we will coordinate provision with the national HPV immunisation programme.[20] HPV immunisation is also beneficial for boys since HPV infection is associated with anogenital warts, anal cancer and oropharyngeal cancers in both males and females, and with penile cancer in men,[21] and we will include boys in these studies.

Tetanus and diphtheria vaccines

Tetanus and diphtheria vaccines comprise inert toxoids (Td). Schistosomiasis is associated with a Th2 biased response to tetanus toxoid[22] and with suppressed antibody responses among those with low pre-immunisation antibody levels.[19] Booster immunisation is recommended for young women to prevent maternal and neonatal tetanus. Recent evidence emphasises the need to protect young men also.[23] Uganda's EPI programme recommends tetanus boosters in adolescence and plans to change from tetanus alone to Td in 2018.

Additional considerations regarding the vaccine schedule

Live vaccines given in combination may influence the response to each other – a phenomenon described as "interference". Observations in the 1960s suggested that elevated circulating interferon(IFN)-γ after measles immunisation might interfere with the response to Vaccinia[24] and, to avoid such interference between live vaccines, it was recommended that live vaccines be given either together or three to four weeks apart.[25] However, with the introduction of new, live vaccines into use, Public Health England reviewed and revised this recommendation in 2014, limiting it to vaccines for which there was an evidence base (**Table S2**). We have adopted a four-week interval between BCG immunisation and the other proposed live vaccines (YF and Ty21a), which will be given together.[26]

Non-live vaccines can be given at the same time as live vaccines and there are no specific recommendations as to the number of non-live vaccines that can be given together. Two injections can be given into the same muscle although it is suggested that these should be at least 2.5 cm apart in case it is necessary to distinguish local adverse reactions to the two injections.[27] Our schedule avoids giving more than two injections on the same date but at week 4 it may be appropriate to give two into the deltoid muscle of the same arm if a BCG scar is developing on the other arm. Nevertheless, uncertainties remain regarding the effects of vaccines on responses to each other. Generalisations from this programme of work will need to take potential "interference" between vaccines into account.

Table S2: Public Health England recommendations for giving more than one live attenuated vaccine in current use in the UK[26]

Vaccine combinations	Recommendations
Yellow Fever and Measles, Mumps, Rubella (MMR)	A four-week minimum interval period should be observed between the administration of these two vaccines. Yellow Fever and MMR should not be administered on the same day.
Varicella (and zoster) vaccine and MMR	If these vaccines are not administered on the same day, then a four-week minimum interval should be observed between vaccines.
Tuberculin skin testing (Mantoux) and MMR	If a tuberculin skin test has already been initiated, then MMR should be delayed until the skin test has been read unless protection against measles is required urgently. If a child has had a recent MMR, and requires a tuberculin test, then a four-week interval should be observed.
All currently used live vaccines (BCG, rotavirus, live attenuated influenza vaccine (LAIV), oral typhoid vaccine, yellow fever, varicella, zoster and MMR) and tuberculin (Mantoux) skin testing.	Apart from those combinations listed above, these live vaccines can be administered at any time before or after each other. This includes tuberculin (Mantoux) skin testing.

Vaccine storage and transport

In order to maintain a reliable vaccine cold chain, the vaccines and diluents to be used will be stored and transported within the recommended temperature range of +2°C to +8°C. Care will be taken to ensure that the vaccines are not frozen. BCG, being sensitive to light, will be kept in the dark (normally within its secondary packaging) for as long as possible to protect it during storage and transportation. All vaccines will be kept in appropriate refrigeration equipment with a temperature monitoring device to ensure temperatures remain between +2°C and +8°C. Cold boxes/vaccines carriers with temperature monitors will be used to transport vaccines and the diluents from the MRC/UVRI and LSHTM Uganda Research Unit (Entebbe) to Koome island and while transporting vaccines to immunization sessions. Designated staff will be given responsibility for managing the vaccine cold chain. All cold chain equipment including the temperature monitoring devices used for this project will comply with relevant technical specifications as defined by the EPI standards. Basic routine maintenance will be regularly carried out on all cold chain equipment.

Additional laboratory measurements

Additional assays will comprise HIV serology, pregnancy testing and full blood counts. HIV testing and pregnancy testing will be accompanied by appropriate counselling by trained staff.

- HIV serology will be done on blood samples using rapid tests and according to prevailing national algorithms.[28] This will be done at baseline.
- Pregnancy testing will be done using urine samples and standard operating procedures for assessment of urine β-human chorionic gonadotropin (βhCG). This will be done at baseline and before immunisation on each immunisation day.
- Full blood counts will be conducted using a haematology analyser. Mild, moderate and severe anaemia will be defined according to WHO guidelines, by age.[29] This will be done at baseline to test for anaemia as part of the eligibility assessment, and pre-immunisation as part of the assessment of immunological profile.
- Information on *S. mansoni* and malaria diagnosis before treatment and throughout the trials is detailed in the focused papers for these trials (bmjopen-2020-040426, bmjopen-2020-040427 and bmjopen-2020-040430). Briefly, current *S. mansoni* infection status and intensity will be determined by serum/plasma levels of circulating anodic antigen (CAA). In Trial A, CAA will be assessed retrospectively on stored samples collected at baseline, on immunisation days, and on primary and secondary endpoint days. In Trial B, CAA will be assessed retrospectively on stored samples collected at baseline and at weeks 28 and 52. In

Trial C, CAA will be assessed retrospectively on stored samples collected at baseline. In all three trials, current malaria infection status and intensity will be assessed retrospectively by PCR on stored blood samples collected on immunisation days and at week 52.

Individuals found to be HIV positive or pregnant will be referred to appropriate providers for further care. Individuals with severe anaemia (haemoglobin <82g/L) will be excluded from the randomised intervention (since the intervention might be beneficial in management of anaemia). They will be treated for anaemia and for any underlying cause identified.

Sample handling and archive

Blood and other samples will be processed according to local laboratory standard operating procedures (SOPs). All samples will reach the laboratory in anonymised form.

A sample archive will be developed. Although our current programme of work plans to address specific hypotheses regarding pathways of effects of parasites and interventions, the sample archive will provide a major asset for exploration of new leads arising from this work, or for an alternative, "systems biology" approach employing (for example) proteomic, genomic, epigenetic and transcriptomic analyses, and investigating the microbiome and virome. Information provided to participants, and consent forms, will include considerations of sample storage, and the possibility of sample analysis in laboratories within and outside Uganda. Participants will be able to decide if they will permit such future use of any leftover samples. We plan to store the samples for up to 20 years. If further storage is needed after that time, permission will be requested from the Uganda Virus Research Institute and London School of Hygiene and Tropical Medicine review committees. If they elect not to permit this, all of those leftover samples will be discarded after the completion of the work included in the current protocol.

Operational considerations

Programme governance

A Programme Steering Committee has been set up to guide progress across all projects. This comprises the following:

- An independent chair
- Representatives from the Ministry of Health programmes for immunisation and for vector borne disease control
- Representatives of district authorities (Mukono and Jinja districts)
- Community representatives
- Principal investigator and co-investigators

- Project leader and post-doctoral immunologist
- Trial statistician
- Laboratory manager
- Medical Research Council observer

Informed consent

Both written informed assent from the participants and written informed consent from a parent or guardian will be required for participation, although these may not necessarily be obtained at the same time. Information will be provided in both English and the appropriate local language. For individuals who cannot speak the languages used, or who cannot read or write, a witness who can read the information sheet and translate the information to the participant or parent/guardian will be used. For trials A and B, two different types of age specific assent forms will be used for the group of participants aged 9 – 12 years and for the group aged 13 – 17 years. Informed consent by emancipated or mature minors will be obtained using a designated consent form for these categories of participants

The aims of the study, all tests, treatments and immunisations to be carried out and potential risks will be explained. The participant will be given the opportunity to ask about details of the trial, and will then have time to consider whether or not to participate. If they do decide to participate, they and their parent/guardian will sign and date two copies of the assent and consent forms, one for them to take away and keep, and one to be stored securely by the research team. Separate information and consent forms will be provided (i) for consent for storage of samples for future studies and for anonymous sharing of data from this study and (ii) for possible genetic studies (for the urban cohort genetic data are already available based on previous approval); the information sheet will explain that these data may be used in analyses related to this protocol.

Screening and Eligibility Assessment

Once the informed consent process has been completed, and consent (and assent) given, a baseline medical history (including concomitant medication) will be collected. Vital signs will be checked and a physical examination will be performed. Inclusion and exclusion criteria will be checked.

Participants will undergo pre- and post-test counselling for HIV and (for girls) pregnancy testing by a trained and experienced nurse- or clinician-counsellor. Blood, urine and stool samples will be obtained, for tests as specified in the schedule of procedures. These tests are to exclude the major, immunomodulating co-infection, HIV, and conditions that might impact safety (anaemia, pregnancy).

Enrolment

Participants who consent/assent, complete the screening processes, satisfy all the inclusion criteria and meet none of the exclusion criteria will be enrolled.

Discontinuation / withdrawal criteria

In accordance with the principles of the current revision of the Declaration of Helsinki and any other applicable regulations, a participant has the right to withdraw from the study at any time and for any reason, and is not obliged to give his or her reasons for doing so. The Investigator may withdraw the participant at any time in the interests of the participant's health and well-being. In addition, the participant may withdraw/be withdrawn for any of the following reasons:

- Ineligibility (either arising during the study or retrospectively, having been overlooked at screening)
- Administrative decision by the Investigator
- Significant protocol deviation
- Participant non-compliance with study requirements
- An adverse event which requires discontinuation of the study involvement or results in inability to continue to comply with study procedures.

Any participant who becomes pregnant during the trial will be followed up until the end of the pregnancy but no further immunisations will be given unless indicated during pregnancy (as is the case for tetanus toxoid). The trial allocation for this participant will be unblinded and the participant will only be given further treatment if clinically indicated. The babies will also be followed up and examined for any adverse effects. We will not routinely perform venipuncture in a pregnant participant.

The reason for withdrawal will be recorded in the case report form (CRF). If withdrawal is due to an AE, appropriate follow-up visits or medical care will be arranged, with the agreement of the participant, until the AE has resolved, stabilised or a non-trial related causality has been assigned.

If a participant withdraws from the study samples collected before their withdrawal from the trial will be used/ stored unless the participant specifically requests otherwise.

Trial discontinuation

The trial will be discontinued in the event of new scientific information that renders continuation futile or unethical, or for any other reason, at the discretion of the Programme Steering Committee.

End of study definition

The trial will be completed when the last participant enrolled into the trial has completed their final follow up visit.

Safety assessments and oversight

No new investigational drug or product will be used in the proposed trial. However, standard approaches for monitoring safety and reporting of serious adverse events will be followed.

Monitoring

The trial will be monitored by both internal and external monitors according to a pre-defined monitoring plan which will include a site initiation visit, monitoring visits at least annually, and a close-out visit. The monitors will assess patient safety, data integrity, and adherence to the protocol and to Good Clinical Research Practice procedures.

Considerations regarding standard of care for parasitic infections

Malaria and *S. mansoni* infection status will be determined retrospectively through assays conducted in bulk on stored samples. These results will not, therefore, be useful to determine management of individual participants.

Participants in the standard anthelminthic treatment (trial A) and the malaria placebo (trial B) arms will receive lower levels of treatment. However, all trial arms will receive a minimum of well-implemented national standard of care.

In trial A, standard of care will comprise annual praziquantel treatment. Our own results from the Lake Victoria Island Intervention Study on Worms and Allergy-related diseases (LaVIISWA),[30] which compared annual versus quarterly intervention for schistosomiasis at community level over three years, showed no advantage of quarterly treatment for morbidity outcomes attributed to schistosomiasis. Schistosomiasis can cause anaemia. To manage the expected differential benefits of the interventions for anaemia, a full blood count will be performed at baseline, as discussed above; anaemic children will be managed appropriately and severely anaemic children excluded. Albendazole will be provided twice a year to manage nematode infections (after collection of primary and secondary endpoint samples).

Dihydroartemisinin/piperaquine is considered an attractive option for preventive treatment and preventive chemotherapy for malaria because of the long half-life of piperaquine (approximately 23 days).[31] Monthly treatment with DP has been shown to reduce the prevalence of anaemia and reduce episodes of clinical malaria in Ugandan schools[32] but has not been adopted as standard of

care. Trial B is expected to add further evidence regarding the potential benefits of monthly DP for school children by determining the effect on vaccine responses, thereby further contributing to policy debate in this field. To manage the expected differential benefits of the interventions for anaemia, a full blood count will be performed at baseline, as discussed above; anaemic children will be managed appropriately and severely anaemic children excluded. Malaria standard of care will comprise provision of bed nets to minimise malaria exposure for all participants. Rapid diagnostic tests and treatment will be made readily available for participants who develop symptomatic malaria. Albendazole will be provided twice a year to manage nematode infections (after collection of primary and secondary endpoint samples).

Procedures to be followed in the event of abnormal findings

Abnormal clinical findings from medical history, examination or blood tests will be assessed as to their clinical significance throughout the trials. If an abnormal test result is deemed clinically significant, it may be repeated. If a test remains clinically significant, the participant will be informed and appropriate medical care arranged as appropriate and with the permission of the participant. Specific details regarding findings, discussion with participants and resulting actions will be recorded in the clinical records. Decisions to exclude the participant from enrolling in the trial or to withdraw a participant from the trial will be at the discretion of the Investigator.

Data and Safety Monitoring Board (DSMB)

The DSMB will be notified within 7 days of the Investigators' being aware of the occurrence of SAEs. The DSMB may recommend the Investigators to place the trial on hold if deemed necessary following an intervention-related SAE. The DSMB will be chaired by a clinician experienced in clinical trials. There will be a minimum of two other appropriately qualified committee members. In the case of events related to a blinded intervention, the DSMB can request unblinding. Membership will include a statistician, and at least one Ugandan member. All correspondence between Investigators and the DSMB will be conveyed by the Principal Investigator to the trial Sponsor. The Chair of the DSMB will be contacted for advice and independent review by the Investigator or trial Sponsor in the following situations:

- The occurrence of any SAE
- Any other situation where the Investigator or trial Sponsor feels independent advice or review is important

Ethical and regulatory considerations

Ethical approvals

Trial A

Ethical approval has been granted from the Research Ethics Committee of the Uganda Virus Research Institute (UVRI REC, reference: GC/127/19/05/664) and the London School of Hygiene and Tropical Medicine (LSHTM, reference: 16032), and from the Uganda National Council for Science and Technology (UNCST, reference: HS2486) and the Uganda National Drug Authority (NDA, reference: CTA0093).

Trial B

Ethical approval has been granted from the Research Ethics Committees of the Uganda Virus Research Institute (UVRI REC, reference: GC/127/19/05/681) and the London School of Hygiene and Tropical Medicine (LSHTM, reference: 16033), and from the Uganda National Council for Science and Technology (UNCST, reference: HS 2487) and the Uganda National Drug Authority (NDA, reference: CTC0117/2020).

Trial C

Ethical approval has been granted from the Research Ethics Committees of the Uganda Virus Research Institute (reference: GC/127/19/05/682), the London School of Hygiene and Tropical Medicine (reference: 16034), the Uganda National Council for Science and Technology (reference: HS 2491) and from the Uganda National Drug Authority (certificate number: CTA0094).

Further information regarding risks

The immunisations to be given have recognised side effects which are usually mild and resolve spontaneously in a few days to one week. Parenteral vaccines are likely to result in pain and swelling at the site of injection and mild fever; very occasionally pain and swelling can be severe and associated with difficulty in moving the shoulder. Sometimes headache and tiredness occur. Rarely a vaccine may cause a severe allergic reaction. For most vaccines this is estimated at less than one in a million doses (but 1 in 55,000 for Yellow Fever vaccine).[33] Individuals with a history of a possible allergic reaction to drugs or vaccines, or to vaccine components including eggs or chicken proteins, will be excluded from the studies. The research team will be trained and prepared to manage severe allergic reactions.

Adverse reactions to Yellow Fever vaccine include severe nervous system reaction (about 1 person in 125,000) and severe, life-threatening illness with organ failure (about 1 person in 250,000). The mortality for this severe, life-threatening adverse effect is reported as about 50%.[33]

BCG immunisation is likely to induce a scar in many cases. This may develop over several weeks, starting as a small papule at the injection site which may become ulcerated and then heal over a

period of 2 to 5 months; and lymphadenopathy may develop. Occasionally a more severe local reaction occurs (estimated at 1 per 1,000-10,000 doses): for example, an abscess develops and scars may develop into keloids. Rarely BCG can cause disseminated disease (1 per 230,000 to 640,000 doses), or disease in sites remote from the immunisation site. Disseminated BCG disease usually occurs in immunocompromised people: HIV positive people will be excluded from these studies.[34] BCG "pre-immunisation" may interfere with the response to the subsequent live vaccines; indeed our hypothesis, and published results, suggest that it may suppress replication of YF 17D vaccine.[35] However, this reduced replication has not been shown to correlate with, or result in, reduced levels of neutralising antibody titres (which are the desired protective outcome).[11 35]

Oral typhoid vaccine (Ty21a) may occasionally be associated with stomach pain, nausea, vomiting and (rarely) rash.[33]

Praziquantel has been in use for about 30 years. It has a well-recognised profile of side effects including dizziness, nausea, vomiting, abdominal pain, diarrhoea (sometimes with blood) and urticarial rash. The symptoms are considered to arise largely from the effects of killing worms and to be more severe in people with heavy infections. Symptoms are better tolerated when the drugs are given after food and we will provide treatment after a meal or snack. Simple medications, such as paracetamol and cetirizine, can alleviate symptoms and these will be available on treatment days.

More information on causal mediation analysis

Causal mediation analysis is a statistical approach that aims to assess the relative importance of intermediate variables (mediators) through which an exposure may affect an outcome. It uses a counterfactual framework. In essence, this investigates how, for each individual, values of the mediator and outcome might change if their main exposure status changed. For example, in Objective iv, it will be used in exploratory analysis to assess whether, and to what degree, any differences in vaccine response (the outcome) by setting (the exposure) may be explained by differences in current or previous *S. mansoni* and *P. falciparum* infection experience (the potential mediators). An alternative approach would be to use a path analytic approach such as structural equation modelling, but estimation of pathway-specific effects using this technique requires strong assumptions regarding linearity and normality and lack of pairwise confounding for all variables in the model. Causal mediation analysis is itself subject to key assumptions regarding confounding between exposure, mediator and outcomes, and we will assess the potential impact of violations in these assumptions using sensitivity analysis. Specifically, we will assess the degree of uncontrolled confounding that would substantially change conclusions regarding pathway-specific effects using techniques such as those described by Hafemen (2011).[36] Regarding the mediators for Objective iv,

we will consider *S. mansoni* and *P. falciparum* separately, under the assumption that there is no direct causal link between these infections. For each infection, we will include both prior exposure and current infection as mediators in the same set of models (**Figure S1**), allowing the possibility that current infection depends on prior exposure, using the approach outlined in Steen *et al*.[37]

Figure S1. Causal diagram for assessment of whether exposure to parasites mediates differences in vaccine response between urban and rural settings

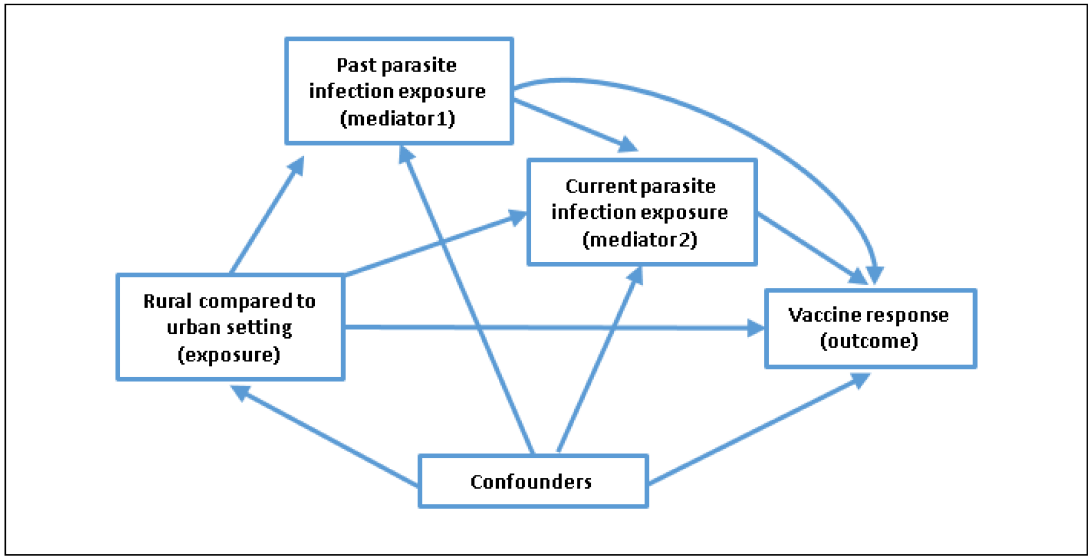

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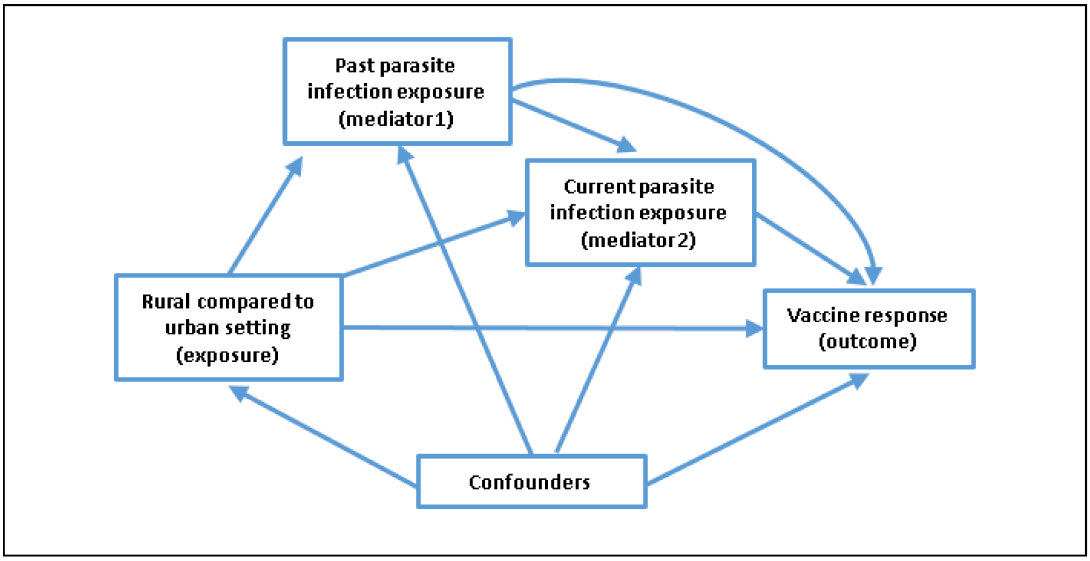
Figure S1. Causal diagram for assessment of whether exposure to parasites mediates differences in vaccine response between urban and rural settings



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21 **Table S1. Uganda National Expanded Programme on Immunisation (EPI) schedule**

Vaccine/ antigen	Dosage and doses required	Minimum Interval Between Doses	Minimum Age to Start	Mode and site of Administration	Storage Temperatures
Infant vaccines					
BCG	Infants (0-11m) 0.05ml. ≥11 months and 0.1ml, 1 dose	Not applicable	At birth (or first contact)	*Intradermal*, right upper arm	+2^{0}C to +8^{0}C
DPT - HepB - Hib	0.5 ml, 3 doses	One month (4 weeks)	At 6 weeks or first contact after this age	*Intramuscular,* outer upper aspect of left thigh	+2^{0}C to +8^{0}C DO NOT FREEZE
PCV	0.5 mls, 3 doses	One month (4 weeks)	At 6 weeks or first contact after this age	*Intramuscular,* outer upper aspect of right thigh	+2^{0}C to +8^{0}C DO NOT FREEZE
Polio	2 drops, 3 doses	One month (4 weeks)	At birth or within the first 2 weeks (Polio 0) and at 6 weeks or first contact after 6 weeks (Polio 1)	*Orally*	+2^{0}C to +8^{0}C
IPV	0.5ml, 1 dose	Nil	At 14 weeks	*Intramuscular,* left upper thigh	+2^{0}C to +8^{0}C DO NOT FREEZE
Rotavirus	drops, 2 doses		6 weeks or 1st contact after this age	*Orally*	
Measles	0.5 ml, 1 doses	Nil	At 9 months (or first contact after that age).	*Subcutaneous,* left upper arm	+2^{0}C to +8^{0}C
Primary school/adolescent/adult vaccines					
Tetanus/Diphtheria	0.5 ml, 5 doses	Td1: First contact with a WCBA Td2: One month after TT1 Td3: Six months after TT2 Td4: One year after TT3 Td5: One year after TT4	At first contact with a pregnant woman or women of childbearing age (15-49 years)	*Intramuscular,* upper arm	+2^{0}C to +8^{0}C, DO NOT FREEZE
HPV	0.5 ml, 2 doses	HPV1: First contact with a girl in primary 4, or aged 10 years and out of school HPV2: Given at 6 months after HPV1[a]	Girls in primary 4 or 10-yearold girls who are out of school	*Intramuscular,* upper arm	+2^{0}C to +8^{0}C, DO NOT FREEZE

BCG: Bacillus Calmette Guerin. DPT: Diphtheria, Pertussis, Tetanus. Hep B: Hepatitis B. Hib: Haemophilus influenzae type B. PCV: pneumococcal conjugate vaccine. IPV: inactivated polio vaccine. HPV: Human Papilloma Virus. WCBA: woman of child-bearing age. [a]An additional dose of HPV, four weeks after the first dose, is recommended for girls aged 14 years or above receiving HPV immunisation for the first time.

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Table S2: Public Health England recommendations for giving more than one live attenuated vaccine in current use in the UK[26]

Vaccine combinations	Recommendations
Yellow Fever and Measles, Mumps, Rubella (MMR)	A four-week minimum interval period should be observed between the administration of these two vaccines. Yellow Fever and MMR should not be administered on the same day.
Varicella (and zoster) vaccine and MMR	If these vaccines are not administered on the same day, then a four-week minimum interval should be observed between vaccines.
Tuberculin skin testing (Mantoux) and MMR	If a tuberculin skin test has already been initiated, then MMR should be delayed until the skin test has been read unless protection against measles is required urgently. If a child has had a recent MMR, and requires a tuberculin test, then a fourweek interval should be observed.
All currently used live vaccines (BCG, rotavirus, live attenuated influenza vaccine (LAIV), oral typhoid vaccine, yellow fever, varicella, zoster and MMR) and tuberculin (Mantoux) skin testing.	Apart from those combinations listed above, these live vaccines can be administered at any time before or after each other. This includes tuberculin (Mantoux) skin testing.