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The effect of intensive treatment for schistosomiasis on immune responses to vaccines among rural Ugandan island adolescents: randomised controlled trial protocol A for the 'POPulation differences in VACcine responses' (POPVAC) programme

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3 1 **The effect of intensive treatment for schistosomiasis on immune responses to vaccines among**
4 **rural Ugandan island adolescents: randomised controlled trial protocol A for the ‘POPulation**
5 **differences in VACCine responses’ (POPVAC) programme**
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3 20 **Abstract**

4
5 21 *Introduction*

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7 22 Several licensed and investigational vaccines have lower efficacy, and induce impaired immune
8
9 23 responses, in low-income versus high-income countries and in rural, versus urban, settings.
10
11 24 Understanding these population differences is essential to optimising vaccine effectiveness in the
12
13 25 tropics. We suggest that repeated exposure to and immunomodulation by chronic helminth
14
15 26 infections is crucial.

16 27 *Methods and analysis*

17
18 28 We have designed an individually randomised, parallel group trial of intensive versus standard
19
20 29 praziquantel (PZQ) intervention against schistosomiasis, to determine effects on vaccine response
21
22 30 outcomes among school-going adolescents (9 to 17 years) from rural *Schistosoma mansoni* (*Sm*)-
23
24 31 endemic Ugandan islands. Vaccines to be studied comprise BCG on day 'zero'; yellow fever, oral
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26 32 typhoid and Human Papilloma Virus (HPV) vaccines at week 4; and HPV and Tetanus/diphtheria
27
28 33 booster vaccine at week 28. The intensive arm will receive PZQ doses three times, each two weeks
29
30 34 apart, before BCG immunisation, followed by a dose at week 8 and quarterly thereafter. The
31
32 35 standard arm will receive PZQ at week 8 and week 52. We expect to enrol 480 participants, with 80%
33
34 36 *Sm* infected at the outset.

35
36 37 Primary outcomes are BCG-specific IFN- γ ELISpot responses eight weeks after BCG immunisation and
37
38 38 for other vaccines, antibody responses to key vaccine antigens at four weeks after immunisation.
39
40 39 Secondary analyses will determine effects of intensive anthelmintic treatment on correlates of
41
42 40 protective immunity, on vaccine response waning, on priming vs boosting immunisations, and on *Sm*
43
44 41 infection status and intensity. Exploratory immunology assays using archived samples will enable
45
46 42 assessment of mechanistic links between helminths and vaccine responses.

45 43 *Ethics and dissemination*

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47 44 Ethics approval has been obtained from relevant Ugandan and UK ethics committees. Results will be
48
49 45 shared with Uganda Ministry of Health, relevant district councils, community leaders and study
50
51 46 participants. Further dissemination will be done through conference proceedings and publications.

52 47 *Trial registration*

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54 48 Current Controlled Trials identifier: ISRCTN60517191.
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3 50 **Article summary**
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5 51 *Strengths and limitations of this study*
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- 7 52
- 8 • This will be the first well-powered intervention study to investigate effects of schistosomiasis
9 treatment on vaccine responses in adolescents.
10
 - 11 54 • Effects on both live-attenuated and inert vaccines will be studied.
12
 - 13 55 • Our strong immunoepidemiological design and nested immunological studies will address
14 specific hypotheses regarding pathways of effects.
15
 - 16 57 • The sample archives developed will provide a major asset for exploration of new leads arising
17 from this hypothesis-driven work, or for an alternative, “systems biology” approach
18 58 investigating (for example) transcriptome, microbiome and virome.
19 59
 - 20 60 • Even with intensive anthelmintic intervention, it may be difficult to “successfully” treat
21 *Schistosoma* infection in our endemic setting due to re-infections; however, we still expect a
22 substantial difference in intensity between the two trial arms.
23 61
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28 64 **Word count**
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30 65 3066
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32 66 **Keywords**
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34 67 Vaccine; Schistosomiasis; Praziquantel; Immunization
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68 Introduction

69 Vaccine-specific immune responses are often impaired, and vaccine efficacy lower, in tropical low-
70 income countries (LICs) compared to temperate high-income countries and in rural, compared to
71 urban, LIC settings.¹⁻⁸ This has been recognised for both live vaccines (such as BCG,^{2,3,5,9} polio,¹ yellow
72 fever⁴ vaccines) and non-live vaccines (such as influenza¹⁰ and tetanus¹¹). Investigational malaria⁷
73 and viral-vectored tuberculosis⁶ and Ebola¹² vaccines are also affected. Previous exposure to the
74 target pathogen (or related organisms) may mask the benefit of the vaccine.^{13,14} However, pre-
75 vaccination exposure does not explain UK-Senegal differences in Ebola trial vaccine-specific
76 responses in healthy adults,¹² as the target organism is rare. Therefore, “environmental
77 sensitisation” may play an important role.⁵

78 A long-held hypothesis is that parasites, particularly helminths, modulate vaccine responses through
79 profound pre- and post-immunisation bystander effects on immunological activation and
80 regulation.¹⁵⁻¹⁷ Helminths might also impact vaccines responses through interactions with the
81 complex ecosystem of mammalian gut bacteria, fungi, protozoa and viruses (the “trans-kingdom”
82 concept¹⁸ detailed elsewhere in this issue). Helminth-induced gut mucosa damage, the associated
83 translocation of microbial products into the systemic circulation¹⁹⁻²¹ and systemic immune activation
84 or regulation mediated by microbial products might contribute to modulation of responses to
85 vaccines and other infections.

86 Helminth-mediated modulation of vaccine responses has not been substantiated in human
87 populations. No well-powered trials have been conducted to evaluate reversibility of their effects. In
88 animal models, helminths generally impair priming and accelerate waning of vaccine responses,
89 although effects vary with helminth species, vaccine type and the timing of infection and
90 immunisation.²² Most observational studies in humans also suggest suppressed or biased responses
91 during helminth infection, especially during systemic infections, such as schistosomiasis and the
92 filariases. There is modest evidence that treating geohelminths in humans improves responses to
93 BCG^{23,24} or oral cholera vaccine²⁵ and we found that schistosomiasis treatment improved the
94 measles-booster response in pre-school children.²⁶ There is therefore a strong case for a
95 comprehensive assessment of the effects of helminths and their treatment on vaccine responses.

96 The extent to which helminths and related “trans-kingdom” mediators causally and reversibly
97 impact immunological characteristics associated with vaccine responses may best be determined by
98 intervention studies. This trial protocol A of the ‘Population differences in Vaccine responses’
99 programme (POPVAC A; Current Controlled Trials identifier: ISRCTN60517191) has been designed to
100 evaluate the effect of *Schistosoma mansoni* and its treatment on vaccine responses. This study is

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3 101 one of three parallel trials whose designs and cross-cutting analyses are described separately in this
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5 102 issue.
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3 103 **Hypothesis**
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5 104 The overarching goal of the POPVAC programme is to understand population differences in vaccine
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7 105 responses in Uganda, in order to identify strategies through which vaccine effectiveness can be
8
9 106 optimised for the low-income, tropical settings where they are especially needed. For this Trial A, we
10
11 107 focus on the hypothesis that *Schistosoma mansoni* infection suppresses responses to unrelated
12
13 108 vaccines; and that this effect can be reversed, at least in part, by intensive praziquantel treatment
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15 109 intervention.

16 110 **Objective**

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18 111 To determine whether there are reversible effects of chronic *Schistosoma mansoni* infection on
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20 112 vaccine response in adolescents, using an intervention study.
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113 **Methods and analysis**

114 ***Setting and participants***

115 SPIRIT reporting guidelines²⁷ have been used. We will conduct an individually randomised, parallel
116 group trial of intensive versus standard intervention against schistosomiasis (described below) in the
117 *S. mansoni*-endemic Koome islands of Lake Victoria, Mukono district, Uganda.²⁸ We aim to enroll
118 480 participants, randomising 240 to each intervention arm. The study cohort will recruit
119 participants aged 9 to 17 years in primary school years 1 to 6. Adolescents²⁹ in this study setting
120 bear a heavy parasite burden.³⁰ In addition, this age-group is a target group for vaccines against
121 sexually transmitted infections (currently human papilloma virus [HPV] – in future, it is hoped, for
122 vaccines against HIV) and for booster immunisations.

123 ***Recruitment criteria***

124 *Inclusion criteria*

- 125 i. Attending the selected school and planning to continue to attend the school for the duration
126 of the study
- 127 ii. Aged 9 to 17 years and enrolled in primary 1 to 6 (to avoid primary leaving examinations in
128 late year 7, and loss to follow up of children leaving after primary 7)
- 129 iii. Written informed assent by participant and consent by parent or guardian
- 130 iv. Females agree to avoid pregnancy for the duration of the trial
- 131 v. Willing to provide locator information and to be contacted during the course of the trial
- 132 vi. Able and willing (in the investigator's opinion) to comply with all the study requirements

133 *Exclusion criteria*

- 134 i. Clinically significant history of immunodeficiency (including HIV), cancer, cardiovascular
135 disease, gastrointestinal disease, liver disease, renal disease, endocrine disorder and
136 neurological illness
- 137 ii. History of serious psychiatric condition or disorder
- 138 iii. Moderate or severe acute illness characterised by any of the following symptoms: fever,
139 impaired consciousness, convulsions, difficulty in breathing, vomiting; or as otherwise
140 determined by the attending project clinician.
- 141 iv. Concurrent oral or systemic steroid medication or the concurrent use of other
142 immunosuppressive agents within 2 months prior to enrolment
- 143 v. History of allergic reaction to immunisation or any allergy likely to be exacerbated by any
144 component of the study vaccines including egg or chicken proteins

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3 145 vi. History of previous immunisation with Yellow Fever (YF), oral typhoid or Human Papillomavirus
4 146 (HPV) vaccine; previous immunisation with BCG or Tetanus and diphtheria vaccine (Td) at age
5 147 ≥ 5 years
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8 148 vii. Tendency to develop keloid scars
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10 149 viii. Haemoglobin less than 82g/L
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12 150 ix. Positive HIV serology
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14 151 x. Positive pregnancy test
15 152 xi. Female currently lactating, confirmed pregnancy or intention to become pregnant during the
16 153 trial period
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18 154 xii. Use of an investigational medicinal product or non-registered drug, live vaccine, or medical
19 155 device other than the study vaccines for 30 days prior to dosing with the study vaccine, or
20 156 planned use during the study period
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22 157 xiii. Administration of immunoglobulins and/or any blood products within the three months
23 158 preceding the planned trial immunisation date
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27 159 Further information on recruitment criteria can be found in Supplementary information.
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29 160 **Interventions**

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31 161 We will individually randomise participants to intensive or standard praziquantel (PZQ) treatment, in
32 162 a 1:1 ratio. The intensive arm will receive three doses of PZQ (approximately 40mg/kg, assessed by
33 163 height pole) each two weeks apart prior to the first immunisation (the last of these 2-3 weeks before
34 164 immunisation), followed by PZQ at 8 weeks (after primary endpoint sampling) and thereafter quarterly
35 165 PZQ (approximately; timings adjusted to accommodate school terms) during follow up. The standard
36 166 arm will receive their first dose of PZQ at week 8 (after immunisation and after primary endpoint
37 167 sampling) and a second dose at week 52 (to conform to Uganda Ministry of Health policy, which is
38 168 annual treatment) (**Figure 1**). No placebo will be used in this trial because all participants will be
39 169 treated (albeit at different frequencies) and participants are unlikely to seek additional treatments
40 170 outside the trial schedule: praziquantel treatment is not popular because of the recognised (although
41 171 temporary) adverse effects (described in Supplementary information).
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50 172 **Randomisation and allocation to treatment arm**

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52 173 A randomisation code will be generated by an independent statistician using a randomly permuted
53 174 block size. At enrolment, eligibility criteria will be checked and eligible participants will be allocated
54 175 sequentially to the next randomisation number. The randomisation code will be kept securely by
55 176 the trial statistician and made available only to those responsible for providing or preparing the trial
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177 interventions. A second copy will be held by a data manager or statistician not otherwise involved in
178 the trial at the MRC/UVRI and LSHTM Uganda Research Unit.

179 Implementation will be done by a clinician using sequentially numbered opaque sealed envelopes. A
180 set of envelopes will be prepared, labelled with the randomisation numbers and containing a card
181 indicating the allocation (to intensive or standard treatment). After the next randomisation number
182 in the sequence has been allocated, the envelope bearing that number will be opened to reveal the
183 allocation.

184 **Blinding**

185 Clinicians and participants will not be blinded to the treatment allocation since they will not
186 participate in outcome ascertainment; only immunology laboratory staff who are assessing trial
187 outcomes will be blinded.

188 **Immunisations**

189 We will study a portfolio of licensed vaccines (live and inert, oral and parental, priming and boosting)
190 expected to be beneficial (in some cases, already given) to adolescents in Uganda. Our schedule
191 (**Table 1**, supplementary **Table S1**) will comprise three main immunisation days (week 0, week 4 and
192 week 28). Additional HPV immunisation will be provided for girls aged 14 years or above, and a
193 second Tetanus/diphtheria boost will be given after completion of the study, to accord with the
194 national Expanded Programme on Immunisation (EPI) routines, but the response to these will not
195 specifically be assessed. Further rationale for the selection of vaccines is detailed in the
196 Supplementary information.

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Table 1. Immunisation schedule

	Immunisation week 0	Immunisation week 4	[Immunisation week 8]	Immunisation week 28	[Immunisation week 52]
Live vaccines	BCG vaccination / re-vaccination ¹	Yellow fever (YF-17D) Oral typhoid (Ty21a)			
Non-live vaccines		HPV prime	HPV boost for girls aged ≥ 14 years ^{2,3}	HPV boost and Tetanus/ diphtheria (Td) boost	Tetanus/ diphtheria (Td) boost ^{3,4}
1. Prior BCG status may vary (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) 2. The National EPI programme recommends three doses of HPV vaccine for older girls 3. These doses will be given to comply with guidelines but outcomes specifically relating to these doses will not be assessed 4. Priming by immunisation in infancy is assumed					

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Schedule of immunisation and sampling

The schedule of immunisation and sampling is outlined in **Figure 1** and **Table S1**. While optimal timings for outcome measures vary between vaccines, sampling at 8 weeks post BCG and 4 weeks post YF-17D, Ty21a, HPV and Td is proposed for the primary endpoints, targeting the establishment of memory responses and approximate peak of antibody responses. A secondary endpoint at one year will assess waning. All analyses will take baseline measurements into account. Immunisation postponement criteria are detailed in Supplementary information.

Outcomes*Primary outcomes*

These will be assessed in all participants.

- i. **BCG:** BCG-specific IFN- γ ELISpot response eight weeks post BCG immunisation.
- ii. **YF-17D:** neutralising antibody titres (plaque-reduction neutralisation test) at four weeks post YF immunisation.
- iii. **Ty21a:** *Salmonella typhi* lipopolysaccharide (LPS)-specific immunoglobulin (Ig)G concentration at four weeks post Ty21a immunisation.
- iv. **HPV:** IgG specific for L1-proteins of HPV-16/18 at four weeks post HPV priming immunisation.
- v. **Td:** Tetanus and diphtheria toxoid-specific IgG concentration at four weeks post Td immunisation.

Secondary outcomes

These will be assessed in all participants and 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 the interventions on parasite clearance.

- i. **Protective immunity.** Proportions with protective neutralising antibody (YF); protective IgG levels (TT);³¹ seroconversion rates (Ty21a) at four weeks post the corresponding immunisation.
- ii. **Response waning.** Primary outcome measures (all vaccines) repeated at week 52, and area-under-the curve (AUC) analyses. Parasitic infection may accelerate,³² and anti-parasitic interventions delay, waning.
- iii. **Priming versus boosting.** Effects on priming versus boosting will be examined for HPV only, comparing outcomes four weeks after the first, and four weeks after the second vaccine dose.

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2
3 229 iv. **Current *S. mansoni* infection status and intensity** will be determined by serum/plasma levels
4 230 of circulating anodic antigen (CAA). The method is quantitative, highly specific for *Schistosoma*
5 231 infection, and much more sensitive than the conventional Kato Katz method.³³ CAA will be
6 232 assessed retrospectively on stored samples collected at baseline, on immunisation days, and
7 233 on primary and secondary endpoint days.

11 234 Furthermore, our sample collection will offer opportunities for an array of exploratory
12 235 immunological evaluations on stored samples, focusing mainly on vaccine antigen specific outcomes.
13 236 Exploratory assays will provide further detail on the role of immunological profiles and trans-
14 237 kingdom effects in mediating helminth modulation of vaccine-specific responses.

19 238 *Additional evaluation of parasite infection exposure*

- 21 239 i. **Prior exposure to schistosomiasis** will be evaluated by ELISA for IgG to schistosome egg
22 240 antigen using stored blood samples collected at baseline.
- 23 241 ii. **The presence of other helminth infections** will be determined retrospectively using stool
24 242 PCR of samples collected at baseline and at weeks 28 and 52.³⁰ In accordance with national
25 243 guidelines, all participants will be treated with albendazole or mebendazole after collection
26 244 of samples for primary endpoints at week 8 and 28, and after collection of samples for
27 245 secondary endpoints at week 52.
- 28 246 iii. **Current malaria infection status and intensity** will be assessed retrospectively by PCR on
29 247 stored samples collected on immunisation days and at week 52. Individuals presenting with
30 248 fever will be investigated using rapid diagnostic tests for malaria and treated based on the
31 249 results and according to prevailing national guidelines.
- 32 250 iv. **Prior malaria exposure** will be evaluated by ELISA for IgG to malaria antigen using stored
33 251 samples collected at baseline.

34 252 *Sample size considerations*

35 253 Based on the literature^{4 34 35} and preliminary data, we anticipate that standard deviations (SDs) of
36 254 primary outcome measures will lie between 0.3 and 0.6 log₁₀, and that effective treatment may
37 255 increase responses by approximately 0.2 log₁₀ (based on Tweyongyere *et al.*²⁶). We have therefore
38 256 powered our study to detect differences of this magnitude (0.2 log₁₀) or (in some cases) smaller. We
39 257 assume *S. mansoni* prevalence of $\geq 80\%$.

40 258 Based on these assumptions, we plan to include 480 participants in total (240 quarterly PZQ, 240
41 259 annual PZQ); of whom 384 are expected to be *S. mansoni* infected, giving 192 participants in each
42 260 trial arm who are infected at baseline.

261 **Table 2** shows power estimates, for 5% significance level and assuming 20% loss to follow-up.

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Table 2. Power estimates (5% significance level)

Standard deviation (\log_{10})	Log ₁₀ difference						
	0.08	0.10	0.12	0.14	0.16	0.18	0.20
192 intensive PZQ vs 192 standard PZQ (<i>S. mansoni</i> 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%

Cells highlighted in grey correspond to >80% power.

263

264 ***Ethical and regulatory considerations***

265 Ethical approval has been granted from the Research Ethics Committee of the Uganda Virus
 266 Research Institute (UVRI REC, reference: GC/127/19/05/664) and the London School of Hygiene and
 267 Tropical Medicine (LSHTM, reference: 16032), and from the Uganda National Council for Science and
 268 Technology (UNCST, reference: HS2486) and the Uganda National Drug Authority (NDA, reference:
 269 CTA0093). Any protocol amendments will be submitted to ethics committees and regulatory bodies
 270 for approval before implementation.

271 Participants are adolescents and therefore a vulnerable human population. Care will be taken to
 272 provide adequate, age and education-status appropriate information and to ensure that it is
 273 understood; and to emphasise that participation is voluntary. Participants will be enrolled only when
 274 they have given their own assent and when consent has been given by the parent or guardian. No
 275 major risks to the participants are anticipated since all the treatments and vaccines to be given are
 276 licensed and known to be safe. The main risk to participants will be time lost from school work: we
 277 will work with teachers and parents to minimise disruption to classes, and will avoid enrolment of
 278 primary 7 students since these classes are involved in national examinations. Further risks are
 279 discussed in Supplementary information.

280 ***Patient and public involvement***

281 Concepts involved in this work have been discussed with colleagues at the Vector Control Division
 282 and Expanded Programme on Immunisation in the Ministry of Health (Uganda), with the Mukono
 283 District Council and with community leaders and Village Health Teams from Koome subcounty. We
 284 also have held meetings to explain the proposed work to teachers, parents, participants and village
 285 members, and to address their questions about issues such as study length, the study's ethical

1
2
3 286 approval status, if boys would also receive the HPV vaccine, and why adults were excluded from the
4
5 287 study. Study findings will be shared with these stakeholders and with participants.
6

7 288 ***Dissemination***

8
9 289 Study findings will be published through open access peer-reviewed journals, presentations at local,
10
11 290 national and international conferences and to the local community through community meetings.
12
13 291 Anonymised participant level datasets generated will be available upon request.
14

15 292 ***Data management and analysis***

16 293 Socio-demographic information and clinical and laboratory measurements will be recorded and
17
18 294 managed using Research Electronic Data Capture (REDCap) tools,^{36 37} with paper-based forms as
19
20 295 back-up. All data will be recorded under a unique study ID number. When paper forms must be
21
22 296 used, data will be double entered in a study-specific database, with standard checks for
23
24 297 discrepancies. All data for analysis will be anonymised and stored on a secure and password-
25
26 298 protected server, with access limited to essential research personnel.

27 299 The effect of intensive (compared to standard) praziquantel treatment on the outcomes will be
28
29 300 analysed. Information on infection status will only be available after randomisation. The primary
30
31 301 analysis will be done on individuals identified as infected at baseline (through randomisation, these
32
33 302 will be balanced between treatment arms); this will test the hypothesis that treating the infection
34
35 303 (and subsequent reinfections) removes the parasite's effects. Secondary analyses will include all
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37 304 randomised individuals; this will provide insight into the potential benefit of the interventions as
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39 305 public health measures.
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306 Discussion

307 This will be the first well-powered intervention study to investigate the effect of schistosomiasis
308 treatment on vaccine responses in adolescents. This study will determine whether *S. mansoni*
309 infection has a causal, reversible, impact on the response to live or inert vaccines, including effects
310 on vaccine replication, immune response profile, priming, boosting and waning. The results will add
311 to understanding of population differences in vaccine responses and on interventions that may
312 enhance responses. If treating helminths improves vaccine responses in adolescents, combined
313 parasite-control/immunisation programmes offer an attractive, practical public health intervention
314 for schools and communities.

315 There are risks associated with our approach to addressing the trial objective. **First**, there is a risk of
316 failure to clear *S. mansoni* infections, and repeated reinfection during the trial. This issue can be
317 challenging because of incomplete cure or maturation of immature worms after treatment, and
318 lifestyles in endemic communities that result in repeated exposure. To mitigate this, we will
319 administer three PZQ treatments over a six-week period before the first immunisations, and
320 continuing quarterly treatment in the intensive arm. **Second**, there is a risk that *S. mansoni* infection
321 has long-term effects, not removed by treatment, mediated, for example, by epigenetic change.³⁸
322 However, studies show that parasite treatment results in immunological changes,^{39,40} and our data
323 suggest at least partial recovery of the measles vaccine response among young children treated for
324 schistosomiasis.²⁶ By initiating intervention six to eight weeks before the first immunisations, and
325 providing repeated intervention in the intensive arms, we hope to achieve significant resolution of *S.*
326 *mansoni* effects.

327 We are interested in the effects of removing *S. mansoni*. Treating parasites can induce acute
328 immunological change due to release of previously hidden antigens. To minimise such effects,
329 immunisations will be given at least 2 weeks after PZQ (the longest practicable interval; **Figure 1**).

330 Laboratory analyses will also highlight immune parameters and cellular populations that link
331 environmental exposures to vaccine responses. Identifying processes associated with poor or good
332 outcomes will inform strategies in vaccine design (for example, the genetic modification of vaccines,
333 or innovative use of adjuvants, to counter any adverse immunological milieu, currently an area of
334 intense research for cancer vaccines⁴¹); ultimately supporting the development of effective vaccines
335 tailored to the low-income settings that most need them.

336

337 Study timeline

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3 338 Applications for ethical approval were submitted in May 2018, with approval received in September
4
5 339 2018 (Uganda Virus Research Institute Research Ethics Committee), May 2019 (National Drug
6
7 340 Authority and Uganda National Council for Science and Technology), June 2019 (London School of
8
9 341 Hygiene and Tropical Medicine). Collaborator/investigator/trial steering committee meetings were
10
11 342 also held during the initial 12-month planning period. The study began recruitment in July 2019.
12
13 343 Intervention will be up to 12 months, with completion of the project scheduled for September 2020.
14

15 344

16 345 **Competing interests**

17
18 346 Alison Elliott reports a grant from the Medical research Council, UK (POPVAC programme funding).
19
20 347 The rest of the authors declare that they have no conflicts of interest.

21 22 348 **Author contributions**

23
24 349 AME conceived the study. AME, GN, ELW, AN, JN, SC, LZ and JK contributed to study design. LZ, GO,
25
26 350 PNK, EN, GK, RA, CN, CO, MN, SA and FA are site clinicians/nurses/clinical laboratory technicians
27
28 351 providing valuable input on clinical considerations of the intervention. MS, SK and RK are field
29
30 352 workers handling the organisational integration of the intervention. AN, AM and ELW are involved in
31
32 353 organisation of the databases, trial randomization, treatment allocation and drawing up of analytical
33
34 354 plans. GN, LZ, JN, AN, SC, ELW and AME drafted the manuscript. All authors reviewed the
35
36 355 manuscript, contributed to it and approved the final version.

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38
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40
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42
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44
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46
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48
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56
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369
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9
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11 377 study design; collection, management, analysis, and interpretation of data; writing of the protocol;
12 378 and the decision to submit the protocol for publication.

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19 379 **POPVAC trial team**

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21 380 **Principal investigator:** Alison Elliott; **Project leader:** Ludoviko Zirimenya; **laboratory staff:** Gyaviira
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24 383 **managers:** Emily Webb, Agnes Natukunda, Helen Akurut, Alex Mutebe; **clinicians:** Anne Wajja, Milly
25 384 Namutebi, Christopher Zziwa, Joel Serubanja; **nurses:** Caroline Onen, Esther Nakazibwe, Josephine
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27 386 Akello; **field workers:** Robert Kizindo, Moses Sewankambo, Denis Nsubuga, Samuel Kiwanuka, Fred
28 387 Kiwudhu; **boatman:** David Abiriga; **administrative management:** Moses Kizza, Samsi Nansukusa;
29 388 **internal and external collaborators:** Pontiano Kaleebu, Hermelijn Smits, Maria Yazdanbakhsh,
30 389 Govert van Dam, Paul Corstjens, Sarah Staedke, Henry Luzze, James Kaweesa, Edridah Tukahebwa,
31 390 Elly Tumushabe, Moses Muwanga
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3 516 **FIGURE LEGENDS**
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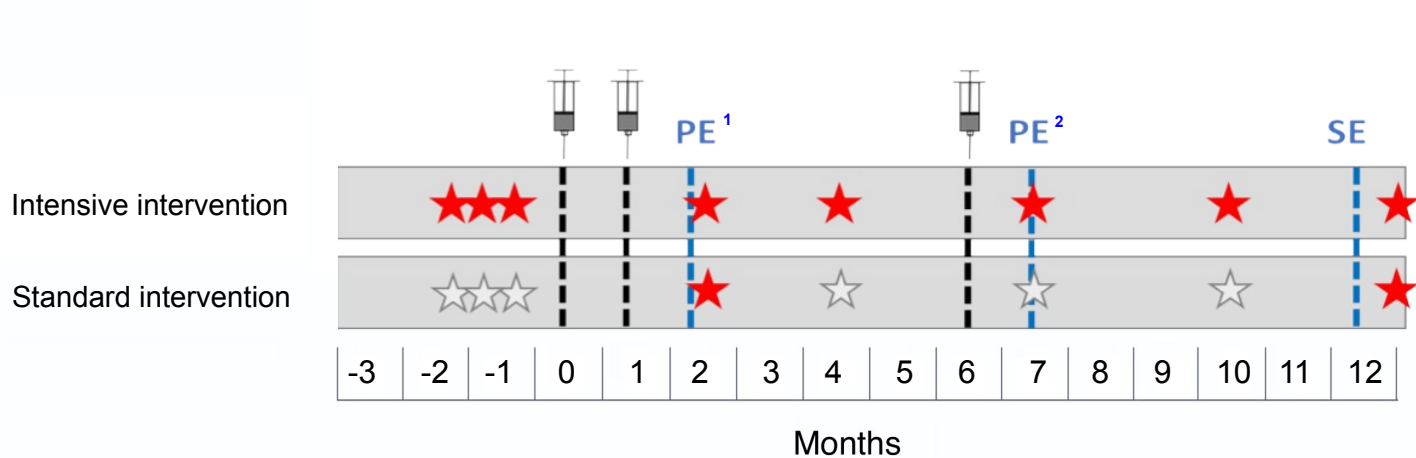
6 517 **Figure 1. Outline of immunisations and anthelmintic intervention**
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8 518 ¹Primary endpoints will be at eight weeks post BCG and four weeks post Yellow fever (YF-17D), oral
9 519 typhoid (Ty21a), Human Papilloma Virus (HPV) and Tetanus/diphtheria (Td) vaccination.

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11 520 ²Primary endpoint for responses to Td given at 28 weeks.
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For peer review only



★ praziquantel;  - - - immunisations; PE - - - primary endpoint; SE - - - secondary endpoint

☆ standard arm, no praziquantel

For peer review only - <http://bmjopen.bmj.com/site/about/guidelines.xhtml>

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2
3 1 [SUPPLEMENTARY INFORMATION](#)
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7 3 **The effect of intensive treatment for schistosomiasis on immune responses to vaccines among**
8 **rural Ugandan island adolescents: randomised controlled trial protocol A for the ‘POPulation**
9 **differences in VACCine responses’ (POPVAC) programme**
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12 6 Gyaviira Nkurunungi^{1,†,*}, Ludoviko Zirimenya^{1,†}, Jacent Nassuuna^{1,†}, Agnes Natukunda^{1,†}, Prossy N
13 7 Kabuubi¹, Emmanuel Niwagaba¹, Gloria Oduru¹, Grace Kabami¹, Rebecca Amongin¹, Alex Mutebe¹,
14 8 Milly Namutebi¹, Christopher Zziwa¹, Susan Amongi¹, Caroline Ninsiima¹, Caroline Onen¹, Florence
15 9 Akello¹, Moses Sewankambo¹, Samuel Kiwanuka¹, Robert Kizindo¹, James Kaweesa², Stephen Cose^{1,3},
16 10 Emily L Webb⁴, Alison M Elliott^{1,3} for the POPVAC trial team
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Table S1. Schedule of visits and procedures

VISIT NUMBER	1	2 & 3	4	5 ⁹	5.2	6	7	8	9	10	11
WEEKS FROM 1 ST IMMUNISATION	-8 ¹	-6 ¹⁰ , -4, -2	0	4	4 weeks +4 days	8	20	28	32	44	52
	Screening	Treatment (Rx) only	Immunisations	Immunisations		Primary Endpoint (PE), Immunisations	Rx only	Immunisations	PE/Secondary Endpoint (SE) ²	Rx only	SE, Immunisations
RANDOMISED INTENSIVE VS STANDARD PRAZIQUANTEL INTERVENTION											
PZQ intensive arm (x)		x				x ³	x		x ³	x	x ³
PZQ standard arm						x ³					x ³
Albendazole						x ³			x ³		x ³
VACCINES											
BCG			x								
YF-17D				x							
Ty21a				x							
HPV				x		[x] ⁴		x			
Td								x			[x] ⁵
INVESTIGATIONS/PROCEDURES											
Inclusion/exclusion criteria	x										
Informed consent	x										
Questionnaire	x		x	x	x	x		x	x		x
Examination	x		(x)	(x)	(x)	(x)		(x)	(x)		(x)
Urine β-HCG test (female only) 1mL	x		x	x				x			
Urine YF viral load					x						
Stool for PCR and storage	x							x			x
Stool for coproantibody and storage	x					x					
BLOOD SAMPLES											
Malaria PCR (1ml)			x	x				x			x
Serology for HIV, prior malaria and <i>S. mansoni</i> (0.5ml)	x										
Mansonella perstans (1ml)	x										
Serum/plasma CAA (1ml)	x		x	x		x		x	x		x
Hb ⁸ / Full blood count (0.5ml)	x		x	x				x			
Assessments of pre-immunisation responses, and/or vaccine response outcomes and/or exploratory immunology; storage ⁷ (10 – 20 mls)			x	x		x		x	x		x
Blood for gene expression (2mls)			x	x				x			
Blood vol (mL)	4		27	17		10-20		27	10		14
Cumulative blood vol (mL) ⁷	4		31	48		68		95	105		119

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PE: primary endpoint; **SE:** secondary endpoint; **Rx only:** treatment only
 Immunisation days highlighted in green, primary end point days in red, days for treatment only in grey
 (x) performed if clinically indicated

1. Screening and enrolment into Project A will take place about 8 weeks before immunisation 0 to allow initiation of the praziquantel intervention.
2. Week 32 is primary endpoint for responses to Td given at 28 weeks; secondary endpoint for HPV booster
3. Treatments given after sampling when schedules coincide
4. Week 8 HPV dose will be given for previously-unvaccinated girls aged ≥ 14 years
5. Week 52 Td booster dose will be provided as a service
6. Exploratory immunology blood volume will be guided by guidelines from Harvard Mass General, where a maximum of 3ml/kg body weight is taken at any one time point and not more than 3ml/kg is taken over any 8-week period (ref http://www.drgreene.com/21_1616.html.) These guidelines have been followed in a previous study vaccinating adolescents with investigational tuberculosis vaccine MVA85A (in Uganda).¹ The total blood volume planned is 68 ml over the initial intensive sampling period of 8 weeks. Revision of sample volumes based on weight will only be required for participants who weigh less than 22 kg; the average weight of children aged 9 years is expected to be 28kg (with 21kg the 3rd centile) with greater weights for older children.²
7. At baseline, it will only be Hb estimation by Haemocue
8. Oral typhoid vaccine doses will be administered on three alternate days namely visit 5, 5.1 and 5.2.
9. The first PZQ treatment at week -6 will be administered at the end of the screening visit

23 **Further information on recruitment criteria**

- 24 • Participants who are excluded from the trial because they have been discovered (during
25 screening procedures) to be suffering from a previously undiagnosed condition thought to
26 require further medical attention will be referred appropriately for further investigation and
27 treatment.
- 28 • Participants discovered to have severe anaemia will be excluded from the trial and treated
29 for anaemia
- 30 • Participants discovered to be HIV-positive will be counselled and offered a CD4 T-cell count
31 and referred to a provider of antiretroviral treatment (“Test and Treat” – i.e. initiation of
32 treatment regardless of CD4 count is recommended for these high-risk communities).
- 33 • Participants discovered to be pregnant will be counselled and referred to an antenatal clinic
34 of their choice.

35 This trial proposes to recruit all participants within a short time-frame. It will not, therefore, be
36 possible to reconsider enrolment of potential participants with temporary exclusion criteria after
37 treatment and resolution of the condition.

38 **Further rationale for the selection of vaccines**

39 *Bacillus Calmette–Guérin (BCG)*

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

44 Worldwide, TB is among the top 10 causes of death; Uganda has an estimated incidence of
45 202/100,000 people.³ Infectious, sputum positive, pulmonary TB classically emerges in adolescence,
46 driving the on-going epidemic.⁴ Thus adolescent booster immunisation is a key TB control strategy.⁵
47 However, BCG vaccine response and efficacy are often impaired in tropical and rural settings⁶⁻⁸ and
48 new TB vaccines are similarly affected.⁹ In the past, the WHO has been hesitant to recommend BCG
49 re-vaccination. However, in 2017 WHO’s Strategic Advisory Group of Experts (SAGE) recommended:
50 “Further research is warranted to explore whether certain sub-groups of age, geographic or *M.*
51 *tuberculosis* exposure categories would benefit from re-vaccination.”¹⁰ Recent results suggest that,
52 despite the variability of BCG efficacy between populations, BCG vaccination in adolescence offers
53 benefit in some tropical settings, especially for individuals who are not yet infected with
54 *Mycobacterium tuberculosis*, and may also be cost-effective.^{7,11} Also, BCG vaccine is currently being
55 used among adolescents in South Africa as a comparator in a trial of a novel TB vaccine (trial

1
2
3 56 registration NCT02075203). BCG efficacy in Ugandan adolescents, and differences in BCG vaccine
4
5 57 responses between urban and rural Ugandan populations, have not been tested. Information
6
7 58 obtained from this study is expected to further inform the use of BCG in adolescents, and also to
8
9 59 inform the development of new vaccines for tuberculosis.

10 60 *Yellow fever vaccine*

11
12 61 Yellow fever vaccine YF-17D is a live replicating parenteral vaccine. The vaccine (Stamaril; Sanofi
13
14 62 Pasteur) is available for purchase in Uganda. Yellow Fever (YF) causes outbreaks in Uganda and the
15
16 63 wider region¹² and YF-17D is a candidate for Uganda's expanded programme on immunisation (EPI).
17
18 64 Lower vaccine replication, lower neutralising antibody induction, and greater waning, are described
19
20 65 in Uganda compared to Switzerland.¹³ YF-17D is a potential vector for novel vaccine constructs,¹⁴
21
22 66 adding relevance to vaccine development.

23 67 *Typhoid vaccine Ty21a*

24
25 68 Typhoid vaccine Ty21a is a live replicating oral vaccine and also a potential vector for new vaccine
26
27 69 constructs.¹⁵ The Ty21a vaccine will be purchased from PaxVax, Redwood City, California.
28
29 70 Substantial, multi-year typhoid outbreaks occur in Uganda and immunisation campaigns have been
30
31 71 advocated as cost effective.¹⁶ Schistosomiasis has been associated with prolonged *S. typhi* infection¹⁷
32
33 72 and impaired antibody responses to killed typhoid vaccines.¹⁸

34
35 73 Ty21a was developed in the 1970s. Although not routinely used in Uganda, it has been (and is
36
37 74 currently) registered in many countries. It was first registered in the United States and United
38
39 75 Kingdom in the 1980s, and is recommended by the WHO for both endemic and epidemic settings.¹⁹
40
41 76 It has comparable efficacy to the parenteral Vi polysaccharide typhoid vaccine, good durability and
42
43 77 minimal adverse effects.¹⁹ It is proposed for use in this study to model effects of study exposures
44
45 78 and intervention on the response to a live oral vaccine.

46
47 79 The Ty21a vaccine is given as a three-dose regimen on alternate days.

48 80 *Human Papilloma Virus (HPV) vaccine*

49
50 81 The Human Papilloma Virus (HPV) vaccine is a protein virus-like particle. The quadrivalent HPV
51
52 82 Vaccine Gardasil (Merck) is available for purchase in Uganda and is the vaccine used by the national
53
54 83 EPI programme. Studies after three vaccine doses have found somewhat enhanced responses in the
55
56 84 presence of malaria, but no effect of helminths.²⁰ No study has previously investigated parasite
57
58 85 effects on the priming response, but recent results for tetanus suggest that priming may be more
59
60 86 susceptible than boosting to adverse effects.²¹ This will be important if forthcoming trials support
87
88 89 single-dose HPV immunisation (NCT02834637). HPV immunisation is being rolled out among girls to

1
2
3 88 prevent cervical neoplasia, the most common cancer among Ugandan women and we will
4
5 89 coordinate provision with the national HPV immunisation programme.²² HPV immunisation is also
6
7 90 beneficial for boys since HPV infection is associated with anogenital warts, anal cancer and
8
9 91 oropharyngeal cancers in both males and females, and with penile cancer in men,²³ and we will
10
11 92 include boys in these studies.

12 93 *Tetanus and diphtheria vaccines*

14 94 Tetanus and diphtheria (Td) vaccines comprise inert toxoids. Schistosomiasis is associated with a Th2
15
16 95 biased response to tetanus toxoid²⁴ and with suppressed antibody responses among those with low
17
18 96 pre-immunisation antibody levels.²¹ Booster immunisation is recommended for young women to
19
20 97 prevent maternal and neonatal tetanus. Recent evidence emphasises the need to protect young
21
22 98 men also.²⁵

23 99 ***Immunisation Postponement Criteria***

25 100 If any one of the following is identified at the time scheduled for immunisation, the participant may
26
27 101 be immunised at a later date, or withdrawn, at the discretion of the Investigator. The participant
28
29 102 must be followed until resolution of the event as with any adverse event:

- 31 103 • Acute disease at the time of immunisation. Acute disease is defined as the presence of a
32
33 104 moderate or severe illness with or without fever. All vaccines can be administered to persons
34
35 105 with a minor illness such as diarrhoea or mild upper respiratory infection with or without low-
36
37 106 grade fever, i.e. temperature of $\leq 37.5^{\circ}\text{C}$ (99.5°F)
- 38 107 • Temperature of $>37.5^{\circ}\text{C}$ (99.5°F) at the time of immunisation
- 40 108 • Taking antibiotics or antimalarials currently, or within the past 7 days, of the date of Ty21a
41
42 109 administration (ascertained verbally)

44 110 ***Vaccine storage and transport***

46 111 In order to maintain a reliable vaccine cold chain, the vaccines and diluents to be used will be stored
47
48 112 and transported within the recommended temperature range of $+2^{\circ}\text{C}$ to $+8^{\circ}\text{C}$. Care will be taken to
49
50 113 ensure that the vaccines are not frozen. BCG, being sensitive to light, will be kept in the dark
51
52 114 (normally within its secondary packaging) for as long as possible to protect it during storage and
53
54 115 transportation. All vaccines will be kept in appropriate refrigeration equipment with a temperature
55
56 116 monitoring device to ensure temperatures remain between $+2^{\circ}\text{C}$ and $+8^{\circ}\text{C}$. Cold boxes/vaccines
57
58 117 carriers with temperature monitors will be used to transport vaccines and the diluents from the
59
60 118 MRC/UVRI and LSHTM Uganda Research Unit (Entebbe) to Koome island and while transporting
60 119 vaccines to immunisation sessions. Designated staff will be given responsibility for managing the

1
2
3 120 vaccine cold chain. All cold chain equipment including the temperature monitoring devices used for
4
5 121 this project will comply with relevant technical specifications as defined by the EPI standards. Basic
6
7 122 routine maintenance will be regularly carried out on all cold chain equipment.

8 9 123 ***Additional laboratory measurements***

10
11 124 Additional assays will comprise HIV serology, pregnancy testing and full blood counts. HIV testing
12
13 125 and pregnancy testing will be accompanied by appropriate counselling by trained staff.

- 14
15 126 • HIV serology will be done on blood samples using rapid tests and according to prevailing
16
17 127 national algorithms.²⁶ This will be done at baseline.
- 18
19 128 • Pregnancy testing will be done using urine samples and standard operating procedures for
20
21 129 assessment of urine β -human chorionic gonadotropin (β hCG). This will be done at baseline
22
23 130 and before immunisation on each immunisation day.
- 24
25 131 • Full blood counts will be conducted using a haematology analyser. Mild, moderate and
26
27 132 severe anaemia will be defined according to WHO guidelines, by age.²⁷ This will be done at
28
29 133 baseline to test for anaemia as part of the eligibility assessment, and pre-immunisation as
30
31 134 part of the assessment of immunological profile.

32
33 135 Individuals found to be HIV positive or pregnant will be referred to appropriate providers for further
34
35 136 care. Individuals with severe anaemia (haemoglobin <82g/L) will be excluded from the randomised
36
37 137 intervention (since the intervention might be beneficial in management of anaemia). They will be
38
39 138 treated for anaemia.

40 41 139 ***Sample handling and archive***

42
43 140 Blood and other samples will be processed according to local laboratory standard operating
44
45 141 procedures (SOPs). All samples will reach the laboratory in anonymised form.

46
47 142 A sample archive will be developed. Although our current programme of work will address specific
48
49 143 hypotheses regarding pathways of effects of parasites and interventions, the sample archive will
50
51 144 provide a major asset for exploration of new leads arising from this work, or for an alternative,
52
53 145 “systems biology” approach employing (for example) proteomic, genomic, epigenetic and
54
55 146 transcriptomic analyses, and investigating the microbiome and virome. Information provided to
56
57 147 participants, and consent forms, will include considerations of sample storage, and the possibility of
58
59 148 sample analysis in laboratories within and outside Uganda. Participants will be able to decide if they
60
149 will permit such future use of any leftover samples. We plan to store the samples for up to 20 years.
150
151 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 ethical review committees.

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3 152 If they elect not to permit this, all of those leftover samples will be discarded after the completion of
4
5 153 the work included in the current protocol.

6
7 154 ***Operational considerations***

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9 155 *Programme governance*

10
11 156 A Programme Steering Committee has been set up to guide progress across all projects. This
12
13 157 comprises the following:

- 14
15 158 • An independent chair
16
17 159 • Representatives from the Ministry of Health programmes for immunisation and for vector
18
19 160 borne disease control
20
21 161 • Representatives of district authorities (Mukono and Jinja districts)
22
23 162 • Community representatives
24
25 163 • Principal investigator and co-investigators
26
27 164 • Project leader and post-doctoral immunologist
28
29 165 • Trial statistician
30
31 166 • Laboratory manager
32
33 167 • Medical Research Council observer

34
35 168 *Informed consent*

36
37 169 Both written informed assent from the participants and written informed consent from a parent or
38
39 170 guardian will be required for participation, although these may not necessarily be obtained at the
40
41 171 same time. Information will be provided in both English and the appropriate local language. For
42
43 172 individuals who cannot speak the languages used, or who cannot read or write, a witness who can
44
45 173 read the information sheet and translate the information to the participant or parent/guardian will
46
47 174 be used. Two different types of age specific assent forms will be used for the group of participants
48
49 175 aged 9 – 12 years and for the group aged 13 – 17 years. Informed consent by emancipated or
50
51 176 mature minors will be obtained using a designated consent form for these categories of participants.

52
53 177 The aims of the study, all tests, treatments and immunisations to be carried out and potential risks
54
55 178 will be explained. The participant will be given the opportunity to ask about details of the trial, and
56
57 179 will then have time to consider whether or not to participate. If they do decide to participate, they
58
59 180 and their parent/guardian will sign and date two copies of the assent and consent forms, one for
60
181 them to take away and keep, and one to be stored securely by the research team. Separate
182 information and consent forms will be provided (i) for consent for storage of samples for future

183 studies and for anonymous sharing of data from this study and (ii) for possible genetic studies; the
184 information sheet will explain that these data may be used in analyses related to this protocol.

185 *Screening and Eligibility Assessment*

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

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

194 *Enrolment*

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

197 *Discontinuation/withdrawal criteria*

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

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

210 Any participant who becomes pregnant during the trial will be followed up until the end of the
211 pregnancy but no further immunisations will be given unless indicated during pregnancy (as is the
212 case for tetanus toxoid). The trial allocation for this participant will be unblinded and the participant
213 will only be given further treatment if clinically indicated. The babies will also be followed up and

1
2
3 214 examined for any adverse effects. We will not routinely perform venepuncture in a pregnant
4
5 215 participant.

6
7 216 The reason for withdrawal will be recorded in the case report form (CRF). If withdrawal is due to an
8
9 217 AE, appropriate follow-up visits or medical care will be arranged, with the agreement of the
10
11 218 participant, until the AE has resolved, stabilised or a non-trial related causality has been assigned.

12
13 219 If a participant withdraws from the study samples collected before their withdrawal from the trial
14
15 220 will be used/ stored unless the participant specifically requests otherwise.

16 221 *Trial discontinuation*

17
18 222 The trial will be discontinued in the event of new scientific information that renders continuation
19
20 223 futile or unethical, or for any other reason, at the discretion of the Programme Steering Committee.

21 224 *End of study definition*

22
23 225 The trial will be completed when the last participant enrolled into the trial has completed their final
24
25 226 follow up visit.

26 227 *Safety assessments and oversight*

27
28 228 No new investigational drug or product will be used in the proposed trial. However, standard
29
30 229 approaches for monitoring safety and reporting of serious adverse events will be followed.

31 230 *Monitoring*

32
33 231 The trial will be monitored by both internal and external monitors according to a pre-defined
34
35 232 monitoring plan which will include a site initiation visit, monitoring visits at least annually, and a
36
37 233 close-out visit. The monitors will assess patient safety, data integrity, and adherence to the protocol
38
39 234 and to Good Clinical Research Practice procedures.

40 235 ***Considerations regarding standard of care***

41
42 236 *S. mansoni* infection status will be determined retrospectively through assays conducted in bulk on
43
44 237 stored samples (plasma CAA). These results will not, therefore, be useful to determine management
45
46 238 of individual participants.

47
48 239 Participants in the standard treatment arm will receive lower levels of anthelmintic treatment.
49
50 240 However, all trial arms will receive a minimum of well-implemented national standard of care.

51
52 241 Standard of care will comprise annual praziquantel treatment. Our own results from the Lake
53
54 242 Victoria Island Intervention Study on Worms and Allergy-related diseases (LaVIISWA),²⁸ which
55
56 243 compared annual versus quarterly intervention for schistosomiasis at community level over three
57
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3 244 years, showed no advantage of quarterly treatment for morbidity outcomes attributed to
4
5 245 schistosomiasis.
6
7 246 Schistosomiasis can cause anaemia. To manage the expected differential benefits of the
8
9 247 interventions for anaemia, a full blood count will be performed at baseline, as discussed above;
10
11 248 anaemic children will be managed appropriately and severely anaemic children excluded.
12
13 249 Albendazole will be provided twice a year to manage nematode infections (after collection of
14
15 250 primary and secondary endpoint samples).

16 251 ***Procedures to be followed in the event of abnormal findings***

17
18 252 Abnormal clinical findings from medical history, examination or blood tests will be assessed as to
19
20 253 their clinical significance throughout the trials. If an abnormal test result is deemed clinically
21
22 254 significant, it may be repeated. If a test remains clinically significant, the participant will be informed
23
24 255 and appropriate medical care arranged as appropriate and with the permission of the participant.
25
26 256 Specific details regarding findings, discussion with participants and resulting actions will be recorded
27
28 257 in the clinical records. Decisions to exclude the participant from enrolling in the trial or to withdraw
29
30 258 a participant from the trial will be at the discretion of the Investigator.

31 259 ***Data and Safety Monitoring Board (DSMB)***

32
33 260 A data and safety monitoring board (DSMB) has been appointed to provide real-time safety
34
35 261 oversight. The DSMB will be notified within 7 days of the Investigators' being aware of the
36
37 262 occurrence of SAEs. The DSMB may recommend the Investigators to place the trial on hold if
38
39 263 deemed necessary following an intervention-related SAE. The DSMB will be chaired by a clinician
40
41 264 experienced in clinical trials. There will be a minimum of two other appropriately qualified
42
43 265 committee members. In the case of events related to a blinded intervention, the DSMB can request
44
45 266 unblinding. Membership will include a statistician, and at least one Ugandan member. All
46
47 267 correspondence between Investigators and the DSMB will be conveyed by the Principal Investigator
48
49 268 to the trial Sponsor. The Chair of the DSMB will be contacted for advice and independent review by
50
51 269 the Investigator or trial Sponsor in the following situations:

- 50 270
- 51 271 • The occurrence of any SAE
 - 52 272 • Any other situation where the Investigator or trial Sponsor feels independent advice or
53 273 review is important

54
55 273 ***Ethical and regulatory considerations***

56
57 274 *Further information regarding risks*

1
2
3 275 The immunisations to be given have recognised side effects which are usually mild and resolve
4
5 276 spontaneously in a few days to one week. Parenteral vaccines are likely to result in pain and
6
7 277 swelling at the site of injection and mild fever; very occasionally pain and swelling can be severe and
8
9 278 associated with difficulty in moving the shoulder. Sometimes headache and tiredness occur. Rarely
10
11 279 a vaccine may cause a severe allergic reaction. For most vaccines this is estimated at less than one
12
13 280 in a million doses (but 1 in 55,000 for Yellow Fever vaccine).²⁹ Individuals with a history of a
14
15 281 possible allergic reaction to drugs or vaccines, or to vaccine components including eggs or chicken
16
17 282 proteins, will be excluded from the studies. The research team will be trained and prepared to
18
19 283 manage severe allergic reactions.

20
21 284 Adverse reactions to Yellow Fever vaccine include severe nervous system reaction (about 1 person in
22
23 285 125,000) and severe, life-threatening illness with organ failure (about 1 person in 250,000). The
24
25 286 mortality for this severe, life-threatening adverse effect is reported as about 50%.²⁹

26
27 287 BCG immunisation is likely to induce a scar in many cases. This may develop over several weeks,
28
29 288 starting as a small papule at the injection site which may become ulcerated and then heal over a
30
31 289 period of 2 to 5 months; and lymphadenopathy may develop. Occasionally a more severe local
32
33 290 reaction occurs (estimated at 1 per 1,000-10,000 doses): for example, an abscess develops and scars
34
35 291 may develop into keloids. Rarely BCG can cause disseminated disease (1 per 230,000 to 640,000
36
37 292 doses), or disease in sites remote from the immunisation site. Disseminated BCG disease usually
38
39 293 occurs in immunocompromised people: HIV positive people will be excluded from these studies.³⁰
40
41 294 BCG "pre-immunisation" may interfere with the response to the subsequent live vaccines; indeed
42
43 295 our hypothesis, and published results, suggest that it may suppress replication of YF 17D vaccine.³¹
44
45 296 However, this reduced replication has not been shown to correlate with, or result in, reduced levels
46
47 297 of neutralising antibody titres (which are the desired protective outcome).^{13 31}

48
49 298 Oral typhoid vaccine (Ty21a) may occasionally be associated with stomach pain, nausea, vomiting
50
51 299 and (rarely) rash.²⁹

52
53 300 Praziquantel has been in use for about 30 years. It has a well-recognised profile of side effects
54
55 301 including dizziness, nausea, vomiting, abdominal pain, diarrhoea (sometimes with blood) and
56
57 302 urticarial rash. The symptoms are considered to arise largely from the effects of killing worms and to
58
59 303 be more severe in people with heavy infections. Symptoms are better tolerated when the drugs are
60
304 given after food and we will provide treatment after a meal or snack. Simple medications, such as
305 paracetamol and cetirizine, can alleviate symptoms and these will be available on treatment days.

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Reporting checklist for protocol of a clinical trial.

Based on the SPIRIT guidelines.

Instructions to authors

Complete this checklist by entering the page numbers from your manuscript where readers will find each of the items listed below.

Your article may not currently address all the items on the checklist. Please modify your text to include the missing information. If you are certain that an item does not apply, please write "n/a" and provide a short explanation.

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In your methods section, say that you used the SPIRIT reporting guidelines, and cite them as:

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	Reporting Item	Page Number
Administrative information		
Title	#1 Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	1
Trial registration	#2a Trial identifier and registry name. If not yet registered, name of intended registry	2
Trial registration: data set	#2b All items from the World Health Organization Trial Registration Data Set	n/a
Protocol version	#3 Date and version identifier	Information available at ISRCTN60517191
Funding	#4 Sources and types of financial, material, and other support	15
Roles and	#5a Names, affiliations, and roles of protocol	15

responsibilities: contributorship		contributors	
Roles and responsibilities: sponsor contact information	#5b	Name and contact information for the trial sponsor	Information available at ISRCTN60517191
Roles and responsibilities: sponsor and funder	#5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities	16
Roles and responsibilities: committees	#5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)	Information detailed in supplementary information file
Introduction			
Background and rationale	#6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention	4
Background and rationale: choice of comparators	#6b	Explanation for choice of comparators	8
Objectives	#7	Specific objectives or hypotheses	6
Trial design	#8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, non-inferiority, exploratory)	7

**Methods:
Participants,
interventions, and
outcomes**

1	Study setting	#9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained	7
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8	Eligibility criteria	#10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists)	7
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14	Interventions:	#11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	8
15	description			
16				
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19				
20	Interventions:	#11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving / worsening disease)	Detailed in supplementary information file
21	modifications			
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27	Interventions:	#11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return; laboratory tests)	Detailed in supplementary information file
28	adherence			
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32	Interventions:	#11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	Detailed in supplementary information file
33	concomitant care			
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37	Outcomes	#12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended	10
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50	Participant timeline	#13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure)	14
51				
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53				Also detailed in supplementary information file, Table S1
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1	Sample size	#14	Estimated number of participants needed to achieve	11
2			study objectives and how it was determined,	
3			including clinical and statistical assumptions	
4			supporting any sample size calculations	
5				
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7				
8	Recruitment	#15	Strategies for achieving adequate participant	Detailed in
9			enrolment to reach target sample size	supplementary
10				information file
11				
12				

13 **Methods:**

14 **Assignment of** 15 **interventions (for** 16 **controlled trials)**

19				
20	Allocation: sequence	#16a	Method of generating the allocation sequence (eg,	8
21	generation		computer-generated random numbers), and list of	
22			any factors for stratification. To reduce predictability	
23			of a random sequence, details of any planned	
24			restriction (eg, blocking) should be provided in a	
25			separate document that is unavailable to those who	
26			enrol participants or assign interventions	
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31	Allocation	#16b	Mechanism of implementing the allocation sequence	9
32	concealment		(eg, central telephone; sequentially numbered,	
33	mechanism		opaque, sealed envelopes), describing any steps to	
34			conceal the sequence until interventions are assigned	
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38	Allocation:	#16c	Who will generate the allocation sequence, who will	8, 9
39	implementation		enrol participants, and who will assign participants to	
40			interventions	
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43	Blinding (masking)	#17a	Who will be blinded after assignment to	9
44			interventions (eg, trial participants, care providers,	
45			outcome assessors, data analysts), and how	
46				
47				
48				
49	Blinding (masking):	#17b	If blinded, circumstances under which unblinding is	Detailed in
50	emergency		permissible, and procedure for revealing a	supplementary
51	unblinding		participant's allocated intervention during the trial	information file
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54 **Methods: Data** 55 **collection,** 56 **management, and** 57 **analysis**

1	Data collection plan	#18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol	10. More details in supplementary information file
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14	Data collection plan: retention	#18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols	Detailed in supplementary information file
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21	Data management	#19	Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol	13. More information in the statistical analysis plan found at ISRCTN60517191
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31	Statistics: outcomes	#20a	Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol	13. More information in the statistical analysis plan found at ISRCTN60517191
32				
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40	Statistics: additional analyses	#20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	11. More information in the statistical analysis plan found at ISRCTN60517191
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49	Statistics: analysis population and missing data	#20c	Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation)	Information in the statistical analysis plan found at ISRCTN60517191
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Methods: Monitoring

1	Data monitoring:	#21a	Composition of data monitoring committee (DMC);	Detailed in
2	formal committee		summary of its role and reporting structure;	supplementary
3			statement of whether it is independent from the	information file
4			sponsor and competing interests; and reference to	
5			where further details about its charter can be found,	
6			if not in the protocol. Alternatively, an explanation of	
7			why a DMC is not needed	
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12	Data monitoring:	#21b	Description of any interim analyses and stopping	Detailed in
13	interim analysis		guidelines, including who will have access to these	supplementary
14			interim results and make the final decision to	information file
15			terminate the trial	
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19	Harms	#22	Plans for collecting, assessing, reporting, and	Detailed in
20			managing solicited and spontaneously reported	supplementary
21			adverse events and other unintended effects of trial	information file
22			interventions or trial conduct	
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26	Auditing	#23	Frequency and procedures for auditing trial conduct,	Detailed in
27			if any, and whether the process will be independent	supplementary
28			from investigators and the sponsor	information file
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31	Ethics and			
32	dissemination			
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35	Research ethics	#24	Plans for seeking research ethics committee /	12
36	approval		institutional review board (REC / IRB) approval	
37				
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39	Protocol	#25	Plans for communicating important protocol	11
40	amendments		modifications (eg, changes to eligibility criteria,	
41			outcomes, analyses) to relevant parties (eg,	
42			investigators, REC / IRBs, trial participants, trial	
43			registries, journals, regulators)	
44				
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47	Consent or assent	#26a	Who will obtain informed consent or assent from	12.
48			potential trial participants or authorised surrogates,	
49			and how (see Item 32)	Also detailed in
50				supplementary
51				information file
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55	Consent or assent:	#26b	Additional consent provisions for collection and use	Detailed in
56	ancillary studies		of participant data and biological specimens in	supplementary
57			ancillary studies, if applicable	information file
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1	Confidentiality	#27	How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial	Detailed in supplementary information file
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8	Declaration of interests	#28	Financial and other competing interests for principal investigators for the overall trial and each study site	15
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11	Data access	#29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators	n/a
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17	Ancillary and post trial care	#30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation	Detailed in supplementary information file
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22	Dissemination policy: trial results	#31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions	2, 13
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32	Dissemination policy: authorship	#31b	Authorship eligibility guidelines and any intended use of professional writers	n/a
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36	Dissemination policy: reproducible research	#31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	13
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41	Appendices			
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43	Informed consent materials	#32	Model consent form and other related documentation given to participants and authorised surrogates	n/a
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47	Biological specimens	#33	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable	n/a
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BMJ Open

The effect of intensive treatment for schistosomiasis on immune responses to vaccines among rural Ugandan island adolescents: randomised controlled trial protocol A for the 'POPulation differences in VACcine responses' (POPVAC) programme

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Date Submitted by the Author:	05-Oct-2020
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Primary Subject Heading:	Infectious diseases
Secondary Subject Heading:	Epidemiology, Immunology (including allergy), Public health
Keywords:	Infection control < INFECTIOUS DISEASES, PARASITOLOGY, Public health < INFECTIOUS DISEASES, Immunology < TROPICAL MEDICINE, Epidemiology < TROPICAL MEDICINE, Paediatric infectious disease & immunisation < PAEDIATRICS

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2
3 1 **The effect of intensive treatment for schistosomiasis on immune responses to vaccines among**
4 **rural Ugandan island adolescents: randomised controlled trial protocol A for the ‘POPulation**
5 **differences in VACCine responses’ (POPVAC) programme**
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9 4 Gyaviira Nkurunungi^{1,¶,*}, Ludoviko Zirimenya^{1,¶}, Jacent Nassuuna^{1,¶}, Agnes Natukunda^{1,¶}, Prossy N
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1
2
3 **20 Abstract**
4

5 **21 Introduction**
6

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8 Several licensed and investigational vaccines have lower efficacy, and induce impaired immune
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10 responses, in low-income versus high-income countries and in rural, versus urban, settings.
11
12 Understanding these population differences is essential to optimising vaccine effectiveness in the
13
14 tropics. We suggest that repeated exposure to and immunomodulation by chronic helminth
15
16 infections partly explains population differences in vaccine response.
17

16 **27 Methods and analysis**
17

18
19 We have designed an individually randomised, parallel group trial of intensive versus standard
20
21 praziquantel (PZQ) intervention against schistosomiasis, to determine effects on vaccine response
22
23 outcomes among school-going adolescents (9 to 17 years) from rural *Schistosoma mansoni* (*Sm*)-
24
25 endemic Ugandan islands. Vaccines to be studied comprise BCG on day 'zero'; yellow fever, oral
26
27 typhoid and Human Papilloma Virus (HPV) vaccines at week 4; and HPV and Tetanus/diphtheria
28
29 booster vaccine at week 28. The intensive arm will receive PZQ doses three times, each two weeks
30
31 apart, before BCG immunisation, followed by a dose at week 8 and quarterly thereafter. The
32
33 standard arm will receive PZQ at week 8 and week 52. We expect to enrol 480 participants, with 80%
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35 *Sm* infected at the outset.
36

37
38 Primary outcomes are BCG-specific IFN- γ ELISpot responses eight weeks after BCG immunisation and
39
40 for other vaccines, antibody responses to key vaccine antigens at four weeks after immunisation.
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42 Secondary analyses will determine effects of intensive anthelmintic treatment on correlates of
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44 protective immunity, on vaccine response waning, on priming vs boosting immunisations, and on *Sm*
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46 infection status and intensity. Exploratory immunology assays using archived samples will enable
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48 assessment of mechanistic links between helminths and vaccine responses.
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45 **43 Ethics and dissemination**
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48 Ethics approval has been obtained from relevant Ugandan and UK ethics committees. Results will be
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50 shared with Uganda Ministry of Health, relevant district councils, community leaders and study
51
52 participants. Further dissemination will be done through conference proceedings and publications.
53

52 **47 Trial registration**
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54
55 Current Controlled Trials identifier: ISRCTN60517191.
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3 50 **Article summary**
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5 51 *Strengths and limitations of this study*
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- 7 52
- 8 • This will be the first adequately powered intervention study to investigate effects of
9 schistosomiasis treatment on vaccine responses in adolescents.
10
 - 11 54 • Effects on both live-attenuated and inert vaccines will be studied.
12
 - 13 55 • Our strong immunoepidemiological design and nested immunological studies will address
14 56 specific hypotheses regarding pathways of effects.
15
 - 16 57 • The sample archives developed will provide a major asset for exploration of new leads
17 58 arising from this hypothesis-driven work, or for an alternative, “systems biology” approach
18 59 investigating (for example) transcriptome, microbiome and virome.
19
 - 20 60 • Even with intensive anthelmintic intervention, it may be difficult to “successfully” treat
21 61 *Schistosoma* infection in our endemic setting due to re-infections; however, we still expect a
22 62 substantial difference in intensity between the two trial arms.
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28 64 **Word count**

29 65 3250

30 66 **Keywords**

31 67 Vaccine; Schistosomiasis; Praziquantel; Immunization
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68 Introduction

69 Vaccine-specific immune responses are often impaired, and vaccine efficacy and effectiveness lower,
70 in tropical low-income countries (LICs) compared to temperate high-income countries and in rural,
71 compared to urban, LIC settings.¹⁻⁸ This has been recognised for both live vaccines (such as BCG,^{2,3,5,9}
72 polio,¹ yellow fever⁴ vaccines) and non-live vaccines (such as influenza¹⁰ and tetanus¹¹).

73 Investigational malaria⁷ and viral-vectored tuberculosis⁶ and Ebola¹² vaccines are also affected.

74 Previous exposure to the target pathogen (or related organisms) may mask the benefit of the
75 vaccine.^{13,14} However, pre-vaccination exposure does not explain why Ebola trial vaccine-specific

76 responses differ between healthy UK and Senegalese adults,¹² as the target organism is rare.

77 Therefore, environmentally-dependent mechanisms may play an important role.⁵

78 A long-held hypothesis is that parasites, particularly helminths, modulate vaccine responses through

79 profound pre- and post-immunisation bystander effects on immunological activation and

80 regulation.¹⁵⁻¹⁷ Helminths might also impact vaccines responses through interactions with the

81 complex ecosystem of mammalian gut bacteria, fungi, protozoa and viruses (the “trans-kingdom”

82 concept¹⁸ detailed elsewhere in this journal [bmjopen-2020-040425]). Helminth-induced gut mucosa

83 damage, the associated translocation of microbial products into the systemic circulation¹⁹⁻²¹ and

84 systemic immune activation or regulation mediated by microbial products might contribute to

85 modulation of responses to vaccines and other infections.

86 Helminth-mediated modulation of vaccine responses has not been substantiated in human

87 populations. No appropriately powered trials have been conducted to evaluate reversibility of their

88 effects. In animal models, helminths generally impair priming and accelerate waning of vaccine

89 responses, although effects vary with helminth species, vaccine type and the timing of infection and

90 immunisation.²² Most observational studies in humans also suggest suppressed or biased responses

91 during helminth infection, especially during systemic infections, such as schistosomiasis and the

92 filariases. There is modest evidence that treating geohelminths in humans improves responses to

93 BCG^{23,24} or oral cholera vaccine²⁵ and we found that schistosomiasis treatment improved the

94 measles-booster response in pre-school children.²⁶ There is therefore a strong case for a

95 comprehensive assessment of the effects of helminths and their treatment on vaccine responses.

96 The extent to which helminths and related “trans-kingdom” mediators causally and reversibly

97 impact immunological characteristics associated with vaccine responses may best be determined by

98 intervention studies. This trial protocol A of the ‘Population differences in Vaccine responses’

99 programme (POPVAC A; Current Controlled Trials identifier: ISRCTN60517191) has been designed to

100 evaluate the effect of *Schistosoma mansoni* and its treatment on vaccine responses. This study is

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101 one of three parallel trials whose designs and cross-cutting analyses are described separately in this
102 journal (bmjopen-2020-040425, bmjopen-2020-040427 and bmjopen-2020-040430).

For peer review only

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3 103 **Hypothesis**
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5 104 The overarching goal of the POPVAC programme is to understand population differences in vaccine
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7 105 responses in Uganda, in order to identify strategies through which vaccine effectiveness can be
8
9 106 optimised for the low-income, tropical settings where they are especially needed. For this Trial A, we
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11 107 focus on the hypothesis that *Schistosoma mansoni* infection suppresses responses to unrelated
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13 108 vaccines; and that this effect can be reversed, at least in part, by intensive praziquantel treatment
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15 109 intervention.

16 110 **Objective**

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18 111 To determine whether there are reversible effects of chronic *Schistosoma mansoni* infection on
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20 112 vaccine response in adolescents, using an intervention study.
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113 **Methods and analysis**

114 ***Setting and participants***

115 SPIRIT reporting guidelines²⁷ have been used. We will conduct an individually randomised, parallel
116 group trial of intensive versus standard intervention against schistosomiasis (described below) in the
117 *S. mansoni*-endemic Koome islands of Lake Victoria, Mukono district, Uganda.²⁸ We aim to enroll 480
118 participants, randomising 240 to each intervention arm. The study cohort will recruit participants
119 aged 9 to 17 years in primary school years 1 to 6. Adolescents²⁹ in this study setting bear a heavy
120 parasite burden.³⁰ In addition, this age-group is a target group for vaccines against sexually
121 transmitted infections (currently human papilloma virus [HPV] – in future, it is hoped, for vaccines
122 against HIV) and for booster immunisations.

123 ***Recruitment criteria***

124 *Inclusion criteria*

- 125 i. Attending the selected school and planning to continue to attend the school for the duration
126 of the study
- 127 ii. Aged 9 to 17 years and enrolled in primary 1 to 6 (to avoid primary leaving examinations in
128 late year 7, and loss to follow up of children leaving after primary 7)
- 129 iii. Written informed assent by participant and consent by parent or guardian
- 130 iv. Females agree to avoid pregnancy for the duration of the trial
- 131 v. Willing to provide locator information and to be contacted during the course of the trial
- 132 vi. Able and willing (in the investigator's opinion) to comply with all the study requirements

133 *Exclusion criteria*

- 134 i. Clinically significant history of immunodeficiency (including HIV), cancer, cardiovascular
135 disease, gastrointestinal disease, liver disease, renal disease, endocrine disorder and
136 neurological illness
- 137 ii. History of serious psychiatric condition or disorder
- 138 iii. Moderate or severe acute illness characterised by any of the following symptoms: fever,
139 impaired consciousness, convulsions, difficulty in breathing, vomiting; or as otherwise
140 determined by the attending project clinician.
- 141 iv. Concurrent oral or systemic steroid medication or the concurrent use of other
142 immunosuppressive agents within 2 months prior to enrolment
- 143 v. History of allergic reaction to immunisation or any allergy likely to be exacerbated by any
144 component of the study vaccines including egg or chicken proteins

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3 145 vi. History of previous immunisation with Yellow Fever (YF), oral typhoid or Human Papillomavirus
4 146 (HPV) vaccine; previous immunisation with BCG or Tetanus and diphtheria vaccine (Td) at age
5 147 ≥ 5 years
6
7 148 vii. Tendency to develop keloid scars
8
9 149 viii. Haemoglobin less than 82g/L
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11 150 ix. Positive HIV serology
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13 151 x. Positive pregnancy test
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15 152 xi. Female currently lactating, confirmed pregnancy or intention to become pregnant during the
16 153 trial period
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18 154 xii. Use of an investigational medicinal product or non-registered drug, live vaccine, or medical
19 155 device other than the study vaccines for 30 days prior to dosing with the study vaccine, or
20 156 planned use during the study period
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22 157 xiii. Administration of immunoglobulins and/or any blood products within the three months
23 158 preceding the planned trial immunisation date
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27 159 Further information on recruitment criteria can be found in Supplementary information.
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29 160 **Interventions**

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31 161 We will individually randomise participants to intensive or standard praziquantel (PZQ) treatment, in
32 162 a 1:1 ratio. The intensive arm will receive three doses of PZQ (approximately 40mg/kg, assessed by
33 163 height pole³¹) each two weeks apart prior to the first immunisation (the last of these 2-3 weeks before
34 164 immunisation), followed by PZQ at 8 weeks (after primary endpoint sampling) and thereafter quarterly
35 165 PZQ (approximately; timings adjusted to accommodate school terms) during follow up. The standard
36 166 arm will receive their first dose of PZQ at week 8 (after immunisation and after primary endpoint
37 167 sampling) and a second dose at week 52 (to conform to Uganda Ministry of Health policy, which is
38 168 annual treatment) (**Figure 1**). No placebo will be used in this trial because all participants will be
39 169 treated (albeit at different frequencies) and participants are unlikely to seek additional treatments
40 170 outside the trial schedule: praziquantel treatment is not popular because of the recognised (although
41 171 temporary) adverse effects (described in Supplementary information).
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50 172 **Randomisation and allocation to treatment arm**

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52 173 A randomisation code will be generated by an independent statistician using a randomly permuted
53 174 block size (sizes 4, 6, 8 and 10) and used to allocate participants to either receive quarterly PZQ
54 175 (intensive arm) or annual PZQ (standard arm). A set of envelopes will be prepared, labelled
55 176 sequentially with the randomisation numbers and containing a card indicating the corresponding
56 177 allocation (to intensive or standard treatment). The randomisation code will be kept securely by the
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178 trial statistician and made available only to those responsible for providing or preparing the trial
 179 interventions. A second copy will be held by a data manager or statistician not otherwise involved in
 180 the trial at the MRC/UVRI and LSHTM Uganda Research Unit. At enrolment, eligibility criteria will be
 181 checked and eligible participants will be allocated sequentially to the next randomisation number
 182 until the required sample size is achieved. Randomisation implementation will be done by a clinician
 183 using the sequentially numbered opaque sealed envelopes. When the next randomisation number in
 184 the sequence is allocated, the envelope bearing that number will be opened to reveal the allocation.

185 **Blinding**

186 Clinicians and participants will not be blinded to the treatment allocation since they will not
 187 participate in outcome ascertainment; only immunology laboratory staff who are assessing trial
 188 outcomes will be blinded.

189 **Immunisations**

190 We will study a portfolio of licensed vaccines (live and inert, oral and parental, priming and boosting)
 191 expected to be beneficial (in some cases, already given) to adolescents in Uganda. Our schedule
 192 (**Table 1**, supplementary **Table S1**) will comprise three main immunisation days (week 0, week 4 and
 193 week 28). Additional HPV immunisation will be provided for girls aged 14 years or above, and a
 194 second Tetanus/diphtheria boost will be given after completion of the study, to accord with the
 195 national Expanded Programme on Immunisation (EPI) routines, but the response to these will not
 196 specifically be assessed. Further rationale for the selection of vaccines is detailed in the
 197 Supplementary information.

198

Table 1. Immunisation schedule

	Immunisation week 0	Immunisation week 4	[Immunisation week 8]	Immunisation week 28	[Immunisation week 52]
Live vaccines	BCG vaccination / re-vaccination ¹	Yellow fever (YF-17D) Oral typhoid (Ty21a)			
Non-live vaccines		HPV prime ²	HPV boost for girls aged ≥ 14 years ^{3,4}	HPV boost ² and Tetanus/ diphtheria (Td) boost	Tetanus/ diphtheria (Td) boost ^{4,5}
1. Prior BCG status may vary (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) 2. Both girls and boys will receive the HPV vaccine 3. The National EPI programme recommends three doses of HPV vaccine for older girls 4. These doses will be given to comply with guidelines but outcomes specifically relating to these doses will not be assessed 5. Priming by immunisation in infancy is assumed					

199

200 ***Schedule of immunisation and sampling***

201 The schedule of immunisation and sampling is outlined in **Figure 1** and **Table S1**. While optimal
202 timings for outcome measures vary between vaccines, sampling at 8 weeks post BCG and 4 weeks
203 post YF-17D, Ty21a, HPV and Td is proposed for the primary endpoints, targeting the establishment
204 of memory responses and approximate peak of antibody responses. A secondary endpoint at one
205 year will assess waning. All analyses will take baseline measurements into account. Immunisation
206 postponement criteria are detailed in Supplementary information.

207 ***Outcomes***

208 *Primary outcomes*

209 These will be assessed in all participants.

- 210 i. **BCG:** BCG-specific IFN- γ ELISpot response eight weeks post BCG immunisation.
- 211 ii. **YF-17D:** neutralising antibody titres (plaque-reduction neutralisation test) at four weeks post
212 YF immunisation.
- 213 iii. **Ty21a:** *Salmonella typhi* lipopolysaccharide (LPS)-specific immunoglobulin (Ig)G concentration
214 at four weeks post Ty21a immunisation.
- 215 iv. **HPV:** IgG specific for L1-proteins of HPV-16/18 at four weeks post HPV priming immunisation.
- 216 v. **Td:** Tetanus and diphtheria toxoid-specific IgG concentration at four weeks post Td
217 immunisation.

218 *Secondary outcomes*

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

- 222 i. **Protective immunity.** Proportions with protective neutralising antibody (YF); protective IgG
223 levels (TT);³² seroconversion rates (Ty21a) at four weeks post the corresponding
224 immunisation.
- 225 ii. **Response waning.** Primary outcome measures (all vaccines) repeated at week 52, and area-
226 under-the curve (AUC) analyses. Parasitic infection may accelerate,³³ and anti-parasitic
227 interventions delay, waning.
- 228 iii. **Priming versus boosting.** Effects on priming versus boosting will be examined for HPV only,
229 comparing outcomes four weeks after the first, and four weeks after the second vaccine dose.

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3 230 iv. **Current *S. mansoni* infection status and intensity** will be determined by serum/plasma levels
4 231 of circulating anodic antigen (CAA). The method is quantitative, highly specific for *Schistosoma*
5 232 infection, and much more sensitive than the conventional Kato Katz method.³⁴ CAA will be
6 233 assessed retrospectively on stored samples collected at baseline, on immunisation days, and
7 234 on primary and secondary endpoint days.

11
12 235 Furthermore, our sample collection will offer opportunities for an array of exploratory
13 236 immunological evaluations on stored samples, focusing mainly on vaccine antigen specific outcomes.
14 237 Exploratory assays will provide further detail on the role of immunological profiles and trans-
15 238 kingdom effects in mediating helminth modulation of vaccine-specific responses.

19 239 *Additional evaluation of parasite infection exposure*

- 21 240 i. **Prior exposure to schistosomiasis** will be evaluated by ELISA for IgG to schistosome egg
22 241 antigen using stored blood samples collected at baseline.
- 23 242 ii. **The presence of other helminth infections** will be determined retrospectively using stool
24 243 PCR of samples collected at baseline and at weeks 28 and 52.³⁰ In accordance with national
25 244 guidelines, all participants will be treated with albendazole or mebendazole after collection
26 245 of samples for primary endpoints at week 8 and 28, and after collection of samples for
27 246 secondary endpoints at week 52.
- 28 247 iii. **Current malaria infection status and intensity** will be assessed retrospectively by PCR on
29 248 stored samples collected on immunisation days and at week 52. Individuals presenting with
30 249 fever will be investigated using rapid diagnostic tests for malaria and treated based on the
31 250 results and according to prevailing national guidelines.
- 32 251 iv. **Prior malaria exposure** will be evaluated by ELISA for IgG to malaria antigen using stored
33 252 samples collected at baseline.

34 253 *Sample size considerations*

35 254 Based on the literature^{4 35 36} and preliminary data, we anticipate that, following log to base 10
36 255 transformations that will be applied to normalise primary outcome measures, standard deviations
37 256 (SDs) of primary outcome measures will lie between 0.3 and 0.6 on this log scale, and that effective
38 257 treatment may increase responses by approximately 0.2 on the log scale (based on Tweyongyere *et*
39 258 *al.*²⁶). We have therefore powered our study to detect differences of this magnitude (0.2 on the log
40 259 scale) or (in some cases) smaller (**Table 2**). We assume *S. mansoni* prevalence of $\geq 80\%$.

260 Based on these assumptions, we plan to include 480 participants in total (240 quarterly PZQ, 240
 261 annual PZQ); of whom 384 are expected to be *S. mansoni* infected,²⁸ giving 192 participants in each
 262 trial arm who are infected at baseline.

263 **Table 2** shows power estimates, for 5% significance level and assuming 20% loss to follow-up.

264

Table 2. Power estimates (5% significance level)

Standard deviation (log ₁₀)	Difference in mean log ₁₀ transformed outcome, between trial arms						
	0.08	0.10	0.12	0.14	0.16	0.18	0.20
192 intensive PZQ vs 192 standard PZQ (<i>S. mansoni</i> 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%
Cells highlighted in grey correspond to >80% power; differences in mean log ₁₀ transformed outcome of 0.08, 0.10, 0.12, 0.14, 0.16, 0.18 and 0.20 are equivalent to geometric mean ratios for untransformed outcomes of 1.20, 1.26, 1.32, 1.38, 1.45, 1.51 and 1.59, respectively.							

265

266 ***Ethics and dissemination***

267 Ethical approval has been granted from the Research Ethics Committee of the Uganda Virus
 268 Research Institute (UVRI REC, reference: GC/127/19/05/664) and the London School of Hygiene and
 269 Tropical Medicine (LSHTM, reference: 16032), and from the Uganda National Council for Science and
 270 Technology (UNCST, reference: HS2486) and the Uganda National Drug Authority (NDA, reference:
 271 CTA0093). Any protocol amendments will be submitted to ethics committees and regulatory bodies
 272 for approval before implementation.

273 Participants are adolescents and therefore a vulnerable human population. Care will be taken to
 274 provide adequate, age and education-status appropriate information and to ensure that it is
 275 understood; and to emphasise that participation is voluntary. Participants will be enrolled only when
 276 they have given their own assent and when consent has been given by the parent or guardian. No
 277 major risks to the participants are anticipated since all the treatments and vaccines to be given are
 278 licensed and known to be safe. The main risk to participants will be time lost from school work: we
 279 will work with teachers and parents to minimise disruption to classes, and will avoid enrolment of
 280 primary 7 students since these classes are involved in national examinations. Further risks are
 281 discussed in Supplementary information.

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

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3 285 ***Patient and public involvement***
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5 286 Concepts involved in this work have been discussed with colleagues at the Vector Control Division
6
7 287 and Expanded Programme on Immunisation in the Ministry of Health (Uganda), with the Mukono
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9 288 District Council and with community leaders and Village Health Teams from Koome subcounty. We
10
11 289 also have held meetings to explain the proposed work to teachers, parents, participants and village
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13 290 members, and to address their questions about issues such as study length, the study's ethical
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15 291 approval status, why adults were excluded from the study, and to explain to them why boys will also
16
17 292 receive the HPV vaccine. Study findings will be shared with these stakeholders and with participants.

18 293 ***Data management and analysis***

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20 294 Socio-demographic information and clinical and laboratory measurements will be recorded and
21
22 295 managed using Research Electronic Data Capture (REDCap) tools,^{37 38} with paper-based forms as
23
24 296 back-up. All data will be recorded under a unique study ID number. When paper forms must be
25
26 297 used, data will be double entered in a study-specific database, with standard checks for
27
28 298 discrepancies. All data for analysis will be anonymised and stored on a secure and password-
29
30 299 protected server, with access limited to essential research personnel.

31
32 300 Baseline characteristics will be summarised by trial arm, and the effect of intensive (compared to
33
34 301 standard) praziquantel treatment on the outcomes will be analysed. Information on infection status
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36 302 will only be available after randomisation. The primary analysis will be done on individuals identified
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38 303 as infected at baseline (through randomisation, these will be balanced between treatment arms);
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40 304 this will test the hypothesis that treating the infection (and subsequent reinfections) reverses the
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42 305 parasite's effects on vaccine responses. If treating *S. mansoni* reverses adverse parasite effects on
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44 306 vaccine responses, this may be a beneficial public health intervention. However, routine screening
45
46 307 for parasite infection before immunisation would be laborious. Secondary analyses will include all
47
48 308 randomised individuals; this will provide insight into the broader benefit of the interventions as
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50 309 public health measures. The effect of intensive versus standard praziquantel treatment on primary
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52 310 outcomes will be assessed using unpaired t-tests, with results presented as a mean difference in
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54 311 vaccine response measure together with 95% confidence interval and p-value. We anticipate that
55
56 312 outcomes will be positively skewed, and will apply log transformations to normalise distributions
57
58 313 before analysis if required. The detailed analytical plan is available on the online trial registration site
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60 314 (<http://www.isrctn.com/ISRCTN60517191>).

315 Discussion

316 This will be the first adequately powered intervention study to investigate the effect of
317 schistosomiasis treatment on vaccine responses in adolescents. This study will determine whether *S.*
318 *mansoni* infection has a causal, reversible, impact on the response to live or inert vaccines, including
319 effects on vaccine replication, immune response profile, priming, boosting and waning. The results
320 will add to understanding of population differences in vaccine responses and on interventions that
321 may enhance responses. If treating helminths improves vaccine responses in adolescents, combined
322 parasite-control/immunisation programmes offer an attractive, practical public health intervention
323 for schools and communities.

324 There are risks associated with our approach to addressing the trial objective. **First**, there is a risk of
325 failure to clear *S. mansoni* infections, and repeated reinfection during the trial. This issue can be
326 challenging because of incomplete cure or maturation of immature worms after treatment, and
327 lifestyles in endemic communities that result in repeated exposure. To mitigate this, we will
328 administer three PZQ treatments over a six-week period before the first immunisations, and
329 continuing quarterly treatment in the intensive arm. **Second**, there is a risk that *S. mansoni* infection
330 has long-term effects, not removed by treatment, mediated, for example, by epigenetic change.³⁹
331 However, studies show that parasite treatment results in immunological changes,^{40,41} and our data
332 suggest at least partial recovery of the measles vaccine response among young children treated for
333 schistosomiasis.²⁶ By initiating intervention six to eight weeks before the first immunisations, and
334 providing repeated intervention in the intensive arms, we hope to achieve significant resolution of *S.*
335 *mansoni* effects.

336 We are interested in the effects of removing *S. mansoni*. Treating parasites can induce acute
337 immunological change due to release of previously hidden antigens.^{42,43} To minimise such effects,
338 immunisations will be given at least 2 weeks after PZQ (the longest practicable interval; **Figure 1**).

339 Laboratory analyses will also highlight immune parameters and cellular populations that link
340 environmental exposures to vaccine responses. Identifying processes associated with poor or good
341 outcomes will inform strategies in vaccine design (for example, the genetic modification of vaccines,
342 or innovative use of adjuvants, to counter any adverse immunological milieu, currently an area of
343 intense research for cancer vaccines⁴⁴); ultimately supporting the development of effective vaccines
344 tailored to the low-income settings that most need them.

345

346 Study timeline

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3 347 Applications for ethical approval were submitted in May 2018, with approval received in September
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5 348 2018 (Uganda Virus Research Institute Research Ethics Committee), May 2019 (National Drug
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7 349 Authority and Uganda National Council for Science and Technology), June 2019 (London School of
8
9 350 Hygiene and Tropical Medicine). Collaborator/investigator/trial steering committee meetings were
10
11 351 also held during the initial 12-month planning period. The study began recruitment in July 2019.
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13 352 Intervention will be up to 12 months, with completion of the project scheduled for September 2020.
14

15 353

16 354 **Competing interests**

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18 355 Alison Elliott reports a grant from the Medical research Council, UK (POPVAC programme funding).
19
20 356 The rest of the authors declare that they have no conflicts of interest.

21 357 **Author contributions**

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23
24 358 AME conceived the study. AME, GN, ELW, AN, JN, SC, LZ and JK contributed to study design. LZ, GO,
25
26 359 PNK, EN, GK, RA, CN, CO, MN, CZ, SA and FA are site clinicians/nurses/clinical laboratory technicians
27
28 360 providing valuable input on clinical considerations of the intervention. MS, SK and RK are field
29
30 361 workers handling the organisational integration of the intervention. AN, AM and ELW are involved in
31
32 362 organisation of the databases, trial randomization, treatment allocation and drawing up of analytical
33
34 363 plans. GN, LZ, JN, AN, SC, ELW and AME drafted the manuscript. All authors reviewed the
35
36 364 manuscript, contributed to it and approved the final version.

36 365 **Acknowledgements**

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38
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40
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42
43 368 diphtheria vaccines were kind donations from the Serum Institute of India. We thank the Vector
44
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46
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48
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50
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60
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8 384 and is also part of the EDCTP2 programme supported by the European Union.

9
10 385 The study sponsor (London School of Hygiene and Tropical Medicine) and funders had no role in
11 386 study design; collection, management, analysis, and interpretation of data; writing of the protocol;
12 387 and the decision to submit the protocol for publication.

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19 388 **POPVAC trial team**

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21 389 **Principal investigator:** Alison Elliott; **Project leader:** Ludoviko Zirimenya; **laboratory staff:** Gyaviira
22 390 Nkurunungi, Stephen Cose, Rebecca Amongin, Beatrice Nassanga, Jacent Nassuuna, Irene Nambuya,
23 391 Prossy Kabuubi, Emmanuel Niwagaba, Gloria Oduru, Grace Kabami; **statisticians and data**
24 392 **managers:** Emily Webb, Agnes Natukunda, Helen Akurut, Alex Mutebe; **clinicians:** Anne Wajja, Milly
25 393 Namutebi, Christopher Zziwa, Joel Serubanja; **nurses:** Caroline Onen, Esther Nakazibwe, Josephine
26 394 Tumusiime, Caroline Ninsiima, Susan Amongi, Florence Akello; **internal monitor:** Mirriam
27 395 Akello; **field workers:** Robert Kizindo, Moses Sewankambo, Denis Nsubuga, Samuel Kiwanuka, Fred
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15 534 **FIGURE LEGENDS**

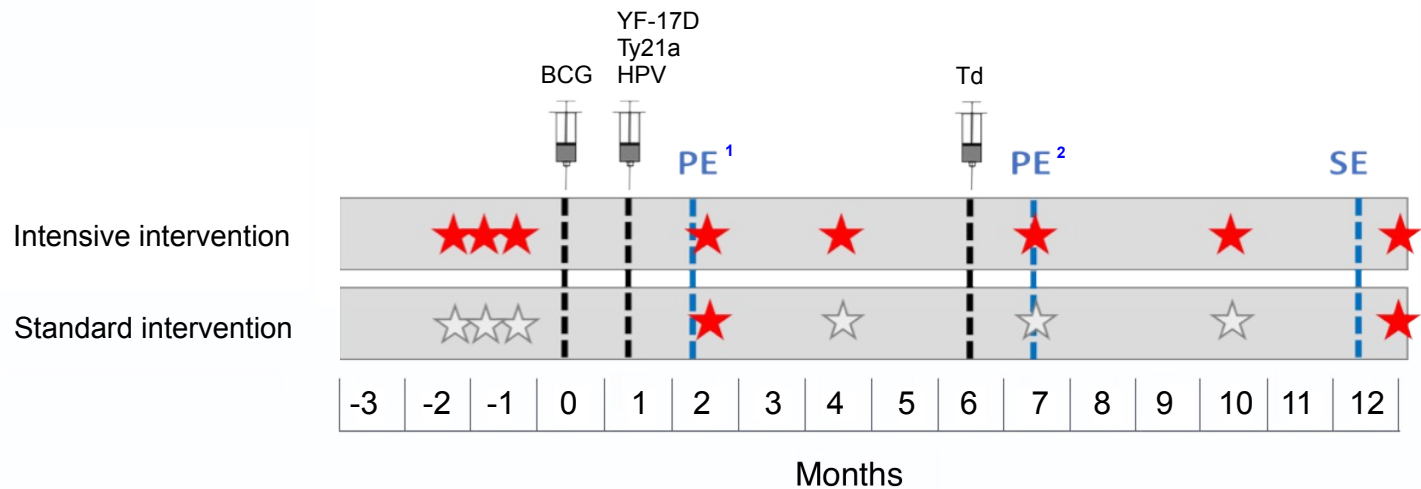
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17 535 **Figure 1. Outline of immunisations and anthelmintic intervention**




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19 536 ¹Primary endpoints will be at eight weeks post BCG and four weeks post Yellow fever (YF-17D), oral
20 537 typhoid (Ty21a), Human Papilloma Virus (HPV) and Tetanus/diphtheria (Td) vaccination.

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22 538 ²Primary endpoint for responses to Td given at 28 weeks.
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 praziquantel;  - - - immunisations; **PE** - - - - primary endpoint; **SE** - - - - secondary endpoint
 standard arm, no praziquantel

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3 **SUPPLEMENTARY INFORMATION**
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8 **The effect of intensive treatment for schistosomiasis on immune responses to vaccines among**
9 **rural Ugandan island adolescents: randomised controlled trial protocol A for the ‘POPulation**
10 **differences in VACCine responses’ (POPVAC) programme**
11

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14 Milly Namutebi¹, Christopher Zziwa¹, Susan Amongi¹, Caroline Ninsiima¹, Caroline Onen¹, Florence
15 Akello¹, Moses Sewankambo¹, Samuel Kiwanuka¹, Robert Kizindo¹, James Kaweesa², Stephen Cose^{1,3},
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Table S1. Schedule of visits and procedures

VISIT NUMBER	1	2 & 3	4	5 ⁹	5.2	6	7	8	9	10	11
WEEKS FROM 1 ST IMMUNISATION	-8 ¹	-6 ¹⁰ , -4, -2	0	4	4 weeks +4 days	8	20	28	32	44	52
	Screening	Treatment (Rx) only	Immunisations	Immunisations		Primary Endpoint (PE), Immunisations	Rx only	Immunisations	PE/Secondary Endpoint (SE) ²	Rx only	SE, Immunisations
RANDOMISED INTENSIVE VS STANDARD PRAZIQUANTEL INTERVENTION											
PZQ intensive arm (x)		x				x ³	x		x ³	x	x ³
PZQ standard arm						x ³					x ³
Albendazole						x ³			x ³		x ³
VACCINES											
BCG			x								
YF-17D				x							
Ty21a				x							
HPV				x		[x] ⁴		x			
Td								x			[x] ⁵
INVESTIGATIONS/PROCEDURES											
Inclusion/exclusion criteria	x										
Informed consent	x										
Questionnaire	x		x	x	x	x		x	x		x
Examination	x		(x)	(x)	(x)	(x)		(x)	(x)		(x)
Urine β-HCG test (female only) 1mL	x		x	x				x			
Urine YF viral load					x						
Stool for PCR and storage	x							x			x
Stool for coproantibody and storage	x					x					
BLOOD SAMPLES											
Malaria PCR (1ml)			x	x				x			x
Serology for HIV, prior malaria and <i>S. mansoni</i> (0.5ml)	x										
Mansonella perstans (1ml)	x										
Serum/plasma CAA (1ml)	x		x	x		x		x	x		x
Hb ⁸ / Full blood count (0.5ml)	x		x	x				x			
Assessments of pre-immunisation responses, and/or vaccine response outcomes and/or exploratory immunology; storage ⁷ (10 – 20 mls)			x	x		x		x	x		x
Blood for gene expression (2mls)			x	x				x			
Blood vol (mL)	4		27	17		10-20		27	10		14
Cumulative blood vol (mL) ⁷	4		31	48		68		95	105		119

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3 **PE:** primary endpoint; **SE:** secondary endpoint; **Rx only:** treatment only

4 Immunisation days highlighted in green, primary end point days in red, days for treatment only in grey

5 (x) performed if clinically indicated

6 1. Screening and enrolment into Project A will take place about 8 weeks before immunisation 0 to allow initiation of the praziquantel intervention.

7 2. Week 32 is primary endpoint for responses to Td given at 28 weeks; secondary endpoint for HPV booster

8 3. Treatments given after sampling when schedules coincide

9 4. Week 8 HPV dose will be given for previously-unvaccinated girls aged ≥ 14 years

10 5. Week 52 Td booster dose will be provided as a service

11 6. Exploratory immunology blood volume will be guided by guidelines from Harvard Mass General, where a maximum of 3ml/kg body weight is taken at any one time point and not more than 3ml/kg is taken over any 8-week period (ref http://www.drgreene.com/21_1616.html.) These guidelines have been followed in a previous study vaccinating adolescents with investigational tuberculosis vaccine MVA85A (in Uganda).¹ The total blood volume planned is 68 ml over the initial intensive sampling period of 8 weeks. Revision of sample volumes based on weight will only be required for participants who weigh less than 22 kg; the average weight of children aged 9 years is expected to be 28kg (with 21kg the 3rd centile) with greater weights for older children.²

12 7. At baseline, it will only be Hb estimation by Haemocue

13 8. Oral typhoid vaccine doses will be administered on three alternate days namely visit 5, 5.1 and 5.2.

14 9. The first PZQ treatment at week -6 will be administered at the end of the screening visit

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23 **Further information on recruitment criteria**

- 24 • Participants who are excluded from the trial because they have been discovered (during
25 screening procedures) to be suffering from a previously undiagnosed condition thought to
26 require further medical attention will be referred appropriately for further investigation and
27 treatment.
- 28 • Participants discovered to have severe anaemia will be excluded from the trial and treated
29 for anaemia
- 30 • Participants discovered to be HIV-positive will be counselled and offered a CD4 T-cell count
31 and referred to a provider of antiretroviral treatment (“Test and Treat” – i.e. initiation of
32 treatment regardless of CD4 count is recommended for these high-risk communities).
- 33 • Participants discovered to be pregnant will be counselled and referred to an antenatal clinic
34 of their choice.

35 This trial proposes to recruit all participants within a short time-frame. It will not, therefore, be
36 possible to reconsider enrolment of potential participants with temporary exclusion criteria after
37 treatment and resolution of the condition.

38 **Further rationale for the selection of vaccines**

39 *Bacillus Calmette–Guérin (BCG)*

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

44 Worldwide, TB is among the top 10 causes of death; Uganda has an estimated incidence of
45 202/100,000 people.³ Infectious, sputum positive, pulmonary TB classically emerges in adolescence,
46 driving the on-going epidemic.⁴ Thus adolescent booster immunisation is a key TB control strategy.⁵
47 However, BCG vaccine response and efficacy are often impaired in tropical and rural settings⁶⁻⁸ and
48 new TB vaccines are similarly affected.⁹ In the past, the WHO has been hesitant to recommend BCG
49 re-vaccination. However, in 2017 WHO’s Strategic Advisory Group of Experts (SAGE) recommended:
50 “Further research is warranted to explore whether certain sub-groups of age, geographic or *M.*
51 *tuberculosis* exposure categories would benefit from re-vaccination.”¹⁰ Recent results suggest that,
52 despite the variability of BCG efficacy between populations, BCG vaccination in adolescence offers
53 benefit in some tropical settings, especially for individuals who are not yet infected with
54 *Mycobacterium tuberculosis*, and may also be cost-effective.^{7,11} Also, BCG vaccine is currently being
55 used among adolescents in South Africa as a comparator in a trial of a novel TB vaccine (trial

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3 56 registration NCT02075203). BCG efficacy in Ugandan adolescents, and differences in BCG vaccine
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5 57 responses between urban and rural Ugandan populations, have not been tested. Information
6
7 58 obtained from this study is expected to further inform the use of BCG in adolescents, and also to
8
9 59 inform the development of new vaccines for tuberculosis.

10 60 *Yellow fever vaccine*

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12 61 Yellow fever vaccine YF-17D is a live replicating parenteral vaccine. The vaccine (Stamaril; Sanofi
13
14 62 Pasteur) is available for purchase in Uganda. Yellow Fever (YF) causes outbreaks in Uganda and the
15
16 63 wider region¹² and YF-17D is a candidate for Uganda's expanded programme on immunisation (EPI).
17
18 64 Lower vaccine replication, lower neutralising antibody induction, and greater waning, are described
19
20 65 in Uganda compared to Switzerland.¹³ YF-17D is a potential vector for novel vaccine constructs,¹⁴
21
22 66 adding relevance to vaccine development.

23 67 *Typhoid vaccine Ty21a*

24
25 68 Typhoid vaccine Ty21a is a live replicating oral vaccine and also a potential vector for new vaccine
26
27 69 constructs.¹⁵ The Ty21a vaccine will be purchased from PaxVax, Redwood City, California.
28
29 70 Substantial, multi-year typhoid outbreaks occur in Uganda and immunisation campaigns have been
30
31 71 advocated as cost effective.¹⁶ Schistosomiasis has been associated with prolonged *S. typhi* infection¹⁷
32
33 72 and impaired antibody responses to killed typhoid vaccines.¹⁸

34
35 73 Ty21a was developed in the 1970s. Although not routinely used in Uganda, it has been (and is
36
37 74 currently) registered in many countries. It was first registered in the United States and United
38
39 75 Kingdom in the 1980s, and is recommended by the WHO for both endemic and epidemic settings.¹⁹
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41 76 It has comparable efficacy to the parenteral Vi polysaccharide typhoid vaccine, good durability and
42
43 77 minimal adverse effects.¹⁹ It is proposed for use in this study to model effects of study exposures
44
45 78 and intervention on the response to a live oral vaccine.

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47 79 The Ty21a vaccine is given as a three-dose regimen on alternate days.

48 80 *Human Papilloma Virus (HPV) vaccine*

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50 81 The Human Papilloma Virus (HPV) vaccine is a protein virus-like particle. The quadrivalent HPV
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52 82 Vaccine Gardasil (Merck) is available for purchase in Uganda and is the vaccine used by the national
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54 83 EPI programme. Studies after three vaccine doses have found somewhat enhanced responses in the
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56 84 presence of malaria, but no effect of helminths.²⁰ No study has previously investigated parasite
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58 85 effects on the priming response, but recent results for tetanus suggest that priming may be more
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60 86 susceptible than boosting to adverse effects.²¹ This will be important if forthcoming trials support
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88 89 single-dose HPV immunisation (NCT02834637). HPV immunisation is being rolled out among girls to

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3 88 prevent cervical neoplasia, the most common cancer among Ugandan women and we will
4
5 89 coordinate provision with the national HPV immunisation programme.²² HPV immunisation is also
6
7 90 beneficial for boys since HPV infection is associated with anogenital warts, anal cancer and
8
9 91 oropharyngeal cancers in both males and females, and with penile cancer in men,²³ and we will
10
11 92 include boys in these studies.

12 93 *Tetanus and diphtheria vaccines*

14 94 Tetanus and diphtheria (Td) vaccines comprise inert toxoids. Schistosomiasis is associated with a Th2
15
16 95 biased response to tetanus toxoid²⁴ and with suppressed antibody responses among those with low
17
18 96 pre-immunisation antibody levels.²¹ Booster immunisation is recommended for young women to
19
20 97 prevent maternal and neonatal tetanus. Recent evidence emphasises the need to protect young
21
22 98 men also.²⁵

23 99 ***Immunisation Postponement Criteria***

25 100 If any one of the following is identified at the time scheduled for immunisation, the participant may
26
27 101 be immunised at a later date, or withdrawn, at the discretion of the Investigator. The participant
28
29 102 must be followed until resolution of the event as with any adverse event:

- 31 103 • Acute disease at the time of immunisation. Acute disease is defined as the presence of a
32
33 104 moderate or severe illness with or without fever. All vaccines can be administered to persons
34
35 105 with a minor illness such as diarrhoea or mild upper respiratory infection with or without low-
36
37 106 grade fever, i.e. temperature of $\leq 37.5^{\circ}\text{C}$ (99.5°F)
- 38 107 • Temperature of $>37.5^{\circ}\text{C}$ (99.5°F) at the time of immunisation
- 40 108 • Taking antibiotics or antimalarials currently, or within the past 7 days, of the date of Ty21a
41
42 109 administration (ascertained verbally)

44 110 ***Vaccine storage and transport***

46 111 In order to maintain a reliable vaccine cold chain, the vaccines and diluents to be used will be stored
47
48 112 and transported within the recommended temperature range of $+2^{\circ}\text{C}$ to $+8^{\circ}\text{C}$. Care will be taken to
49
50 113 ensure that the vaccines are not frozen. BCG, being sensitive to light, will be kept in the dark
51
52 114 (normally within its secondary packaging) for as long as possible to protect it during storage and
53
54 115 transportation. All vaccines will be kept in appropriate refrigeration equipment with a temperature
55
56 116 monitoring device to ensure temperatures remain between $+2^{\circ}\text{C}$ and $+8^{\circ}\text{C}$. Cold boxes/vaccines
57
58 117 carriers with temperature monitors will be used to transport vaccines and the diluents from the
59
60 118 MRC/UVRI and LSHTM Uganda Research Unit (Entebbe) to Koome island and while transporting
60 119 vaccines to immunisation sessions. Designated staff will be given responsibility for managing the

1
2
3 120 vaccine cold chain. All cold chain equipment including the temperature monitoring devices used for
4
5 121 this project will comply with relevant technical specifications as defined by the EPI standards. Basic
6
7 122 routine maintenance will be regularly carried out on all cold chain equipment.

8 9 123 ***Additional laboratory measurements***

10
11 124 Additional assays will comprise HIV serology, pregnancy testing and full blood counts. HIV testing
12
13 125 and pregnancy testing will be accompanied by appropriate counselling by trained staff.

- 14
15 126 • HIV serology will be done on blood samples using rapid tests and according to prevailing
16
17 127 national algorithms.²⁶ This will be done at baseline.
- 18
19 128 • Pregnancy testing will be done using urine samples and standard operating procedures for
20
21 129 assessment of urine β -human chorionic gonadotropin (β hCG). This will be done at baseline
22
23 130 and before immunisation on each immunisation day.
- 24
25 131 • Full blood counts will be conducted using a haematology analyser. Mild, moderate and
26
27 132 severe anaemia will be defined according to WHO guidelines, by age.²⁷ This will be done at
28
29 133 baseline to test for anaemia as part of the eligibility assessment, and pre-immunisation as
30
31 134 part of the assessment of immunological profile.

32
33 135 Individuals found to be HIV positive or pregnant will be referred to appropriate providers for further
34
35 136 care. Individuals with severe anaemia (haemoglobin <82g/L) will be excluded from the randomised
36
37 137 intervention (since the intervention might be beneficial in management of anaemia). They will be
38
39 138 treated for anaemia.

40 41 139 ***Sample handling and archive***

42
43 140 Blood and other samples will be processed according to local laboratory standard operating
44
45 141 procedures (SOPs). All samples will reach the laboratory in anonymised form.

46
47 142 A sample archive will be developed. Although our current programme of work will address specific
48
49 143 hypotheses regarding pathways of effects of parasites and interventions, the sample archive will
50
51 144 provide a major asset for exploration of new leads arising from this work, or for an alternative,
52
53 145 “systems biology” approach employing (for example) proteomic, genomic, epigenetic and
54
55 146 transcriptomic analyses, and investigating the microbiome and virome. Information provided to
56
57 147 participants, and consent forms, will include considerations of sample storage, and the possibility of
58
59 148 sample analysis in laboratories within and outside Uganda. Participants will be able to decide if they
60
149 will permit such future use of any leftover samples. We plan to store the samples for up to 20 years.
150
151 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 ethical review committees.

1
2
3 152 If they elect not to permit this, all of those leftover samples will be discarded after the completion of
4
5 153 the work included in the current protocol.

6
7 154 ***Operational considerations***

8
9 155 *Programme governance*

10
11 156 A Programme Steering Committee has been set up to guide progress across all projects. This
12
13 157 comprises the following:

- 14
15 158 • An independent chair
16
17 159 • Representatives from the Ministry of Health programmes for immunisation and for vector
18
19 160 borne disease control
20
21 161 • Representatives of district authorities (Mukono and Jinja districts)
22
23 162 • Community representatives
24
25 163 • Principal investigator and co-investigators
26
27 164 • Project leader and post-doctoral immunologist
28
29 165 • Trial statistician
30
31 166 • Laboratory manager
32
33 167 • Medical Research Council observer

34
35 168 *Informed consent*

36
37 169 Both written informed assent from the participants and written informed consent from a parent or
38
39 170 guardian will be required for participation, although these may not necessarily be obtained at the
40
41 171 same time. Information will be provided in both English and the appropriate local language. For
42
43 172 individuals who cannot speak the languages used, or who cannot read or write, a witness who can
44
45 173 read the information sheet and translate the information to the participant or parent/guardian will
46
47 174 be used. Two different types of age specific assent forms will be used for the group of participants
48
49 175 aged 9 – 12 years and for the group aged 13 – 17 years. Informed consent by emancipated or mature
50
51 176 minors will be obtained using a designated consent form for these categories of participants.

52
53 177 The aims of the study, all tests, treatments and immunisations to be carried out and potential risks
54
55 178 will be explained. The participant will be given the opportunity to ask about details of the trial, and
56
57 179 will then have time to consider whether or not to participate. If they do decide to participate, they
58
59 180 and their parent/guardian will sign and date two copies of the assent and consent forms, one for
60
181 them to take away and keep, and one to be stored securely by the research team. Separate
182
information and consent forms will be provided (i) for consent for storage of samples for future

1
2
3 183 studies and for anonymous sharing of data from this study and (ii) for possible genetic studies; the
4
5 184 information sheet will explain that these data may be used in analyses related to this protocol.
6

7 185 *Screening and Eligibility Assessment*
8

9 186 Once the informed consent process has been completed, and consent (and assent) given, a baseline
10
11 187 medical history (including concomitant medication) will be collected. Vital signs will be checked and
12
13 188 a physical examination will be performed. Inclusion and exclusion criteria will be checked.

14
15 189 Participants will undergo pre- and post-test counselling for HIV and (for girls) pregnancy testing by a
16
17 190 trained and experienced nurse- or clinician-counsellor. Blood, urine and stool samples will be
18
19 191 obtained, for tests as specified in the schedule of procedures. These tests are to exclude the major,
20
21 192 immunomodulating co-infection, HIV, and conditions that might impact safety (anaemia,
22
23 193 pregnancy).

24 194 *Enrolment*

25
26 195 Participants who consent/assent, complete the screening processes, satisfy all the inclusion criteria
27
28 196 and meet none of the exclusion criteria will be enrolled.

29
30 197 *Discontinuation/withdrawal criteria*

31
32 198 In accordance with the principles of the current revision of the Declaration of Helsinki and any other
33
34 199 applicable regulations, a participant has the right to withdraw from the study at any time and for any
35
36 200 reason, and is not obliged to give his or her reasons for doing so. The Investigator may withdraw the
37
38 201 participant at any time in the interests of the participant's health and well-being. In addition, the
39
40 202 participant may withdraw/be withdrawn for any of the following reasons:

- 41 203
- 42 204 • Ineligibility (either arising during the study or retrospectively, having been overlooked at
43 screening)
 - 44 205 • Administrative decision by the Investigator
 - 45 206 • Significant protocol deviation
 - 46 207 • Participant non-compliance with study requirements
 - 47 208 • An adverse event which requires discontinuation of the study involvement or results in
48 inability to continue to comply with study procedures.
- 49
50
51 209

52
53 210 Any participant who becomes pregnant during the trial will be followed up until the end of the
54
55 211 pregnancy but no further immunisations will be given unless indicated during pregnancy (as is the
56
57 212 case for tetanus toxoid). The trial allocation for this participant will be unblinded and the participant
58
59 213 will only be given further treatment if clinically indicated. The babies will also be followed up and
60

1
2
3 214 examined for any adverse effects. We will not routinely perform venepuncture in a pregnant
4
5 215 participant.

6
7 216 The reason for withdrawal will be recorded in the case report form (CRF). If withdrawal is due to an
8
9 217 AE, appropriate follow-up visits or medical care will be arranged, with the agreement of the
10
11 218 participant, until the AE has resolved, stabilised or a non-trial related causality has been assigned.

12
13 219 If a participant withdraws from the study samples collected before their withdrawal from the trial
14
15 220 will be used/ stored unless the participant specifically requests otherwise.

16 221 *Trial discontinuation*

17
18 222 The trial will be discontinued in the event of new scientific information that renders continuation
19
20 223 futile or unethical, or for any other reason, at the discretion of the Programme Steering Committee.

21
22 224 *End of study definition*

23
24 225 The trial will be completed when the last participant enrolled into the trial has completed their final
25
26 226 follow up visit.

27
28 227 *Safety assessments and oversight*

29
30 228 No new investigational drug or product will be used in the proposed trial. However, standard
31
32 229 approaches for monitoring safety and reporting of serious adverse events will be followed.

33
34 230 *Monitoring*

35
36 231 The trial will be monitored by both internal and external monitors according to a pre-defined
37
38 232 monitoring plan which will include a site initiation visit, monitoring visits at least annually, and a
39
40 233 close-out visit. The monitors will assess patient safety, data integrity, and adherence to the protocol
41
42 234 and to Good Clinical Research Practice procedures.

43
44 235 ***Considerations regarding standard of care***

45
46 236 *S. mansoni* infection status will be determined retrospectively through assays conducted in bulk on
47
48 237 stored samples (plasma CAA). These results will not, therefore, be useful to determine management
49
50 238 of individual participants.

51 239 Participants in the standard treatment arm will receive lower levels of anthelmintic treatment.

52 240 However, all trial arms will receive a minimum of well-implemented national standard of care.

53
54 241 Standard of care will comprise annual praziquantel treatment. Our own results from the Lake

55
56 242 Victoria Island Intervention Study on Worms and Allergy-related diseases (LaVIISWA),²⁸ which

57
58 243 compared annual versus quarterly intervention for schistosomiasis at community level over three
59
60

1
2
3 244 years, showed no advantage of quarterly treatment for morbidity outcomes attributed to
4
5 245 schistosomiasis.
6
7 246 Schistosomiasis can cause anaemia. To manage the expected differential benefits of the
8
9 247 interventions for anaemia, a full blood count will be performed at baseline, as discussed above;
10
11 248 anaemic children will be managed appropriately and severely anaemic children excluded.
12
13 249 Albendazole will be provided twice a year to manage nematode infections (after collection of
14
15 250 primary and secondary endpoint samples).

16 251 ***Procedures to be followed in the event of abnormal findings***

17
18 252 Abnormal clinical findings from medical history, examination or blood tests will be assessed as to
19
20 253 their clinical significance throughout the trials. If an abnormal test result is deemed clinically
21
22 254 significant, it may be repeated. If a test remains clinically significant, the participant will be informed
23
24 255 and appropriate medical care arranged as appropriate and with the permission of the participant.
25
26 256 Specific details regarding findings, discussion with participants and resulting actions will be recorded
27
28 257 in the clinical records. Decisions to exclude the participant from enrolling in the trial or to withdraw
29
30 258 a participant from the trial will be at the discretion of the Investigator.

31 259 ***Data and Safety Monitoring Board (DSMB)***

32
33 260 A data and safety monitoring board (DSMB) has been appointed to provide real-time safety
34
35 261 oversight. The DSMB will be notified within 7 days of the Investigators' being aware of the
36
37 262 occurrence of SAEs. The DSMB may recommend the Investigators to place the trial on hold if
38
39 263 deemed necessary following an intervention-related SAE. The DSMB will be chaired by a clinician
40
41 264 experienced in clinical trials. There will be a minimum of two other appropriately qualified
42
43 265 committee members. In the case of events related to a blinded intervention, the DSMB can request
44
45 266 unblinding. Membership will include a statistician, and at least one Ugandan member. All
46
47 267 correspondence between Investigators and the DSMB will be conveyed by the Principal Investigator
48
49 268 to the trial Sponsor. The Chair of the DSMB will be contacted for advice and independent review by
50
51 269 the Investigator or trial Sponsor in the following situations:

- 52 270 • The occurrence of any SAE
- 53 271 • Any other situation where the Investigator or trial Sponsor feels independent advice or
- 54 272 review is important

55 273 ***Ethical and regulatory considerations***

56
57
58 274 *Further information regarding risks*
59
60

1
2
3 275 The immunisations to be given have recognised side effects which are usually mild and resolve
4
5 276 spontaneously in a few days to one week. Parenteral vaccines are likely to result in pain and
6
7 277 swelling at the site of injection and mild fever; very occasionally pain and swelling can be severe and
8
9 278 associated with difficulty in moving the shoulder. Sometimes headache and tiredness occur. Rarely
10
11 279 a vaccine may cause a severe allergic reaction. For most vaccines this is estimated at less than one
12
13 280 in a million doses (but 1 in 55,000 for Yellow Fever vaccine).²⁹ Individuals with a history of a
14
15 281 possible allergic reaction to drugs or vaccines, or to vaccine components including eggs or chicken
16
17 282 proteins, will be excluded from the studies. The research team will be trained and prepared to
18
19 283 manage severe allergic reactions.

20
21 284 Adverse reactions to Yellow Fever vaccine include severe nervous system reaction (about 1 person in
22
23 285 125,000) and severe, life-threatening illness with organ failure (about 1 person in 250,000). The
24
25 286 mortality for this severe, life-threatening adverse effect is reported as about 50%.²⁹

26
27 287 BCG immunisation is likely to induce a scar in many cases. This may develop over several weeks,
28
29 288 starting as a small papule at the injection site which may become ulcerated and then heal over a
30
31 289 period of 2 to 5 months; and lymphadenopathy may develop. Occasionally a more severe local
32
33 290 reaction occurs (estimated at 1 per 1,000-10,000 doses): for example, an abscess develops and scars
34
35 291 may develop into keloids. Rarely BCG can cause disseminated disease (1 per 230,000 to 640,000
36
37 292 doses), or disease in sites remote from the immunisation site. Disseminated BCG disease usually
38
39 293 occurs in immunocompromised people: HIV positive people will be excluded from these studies.³⁰
40
41 294 BCG "pre-immunisation" may interfere with the response to the subsequent live vaccines; indeed
42
43 295 our hypothesis, and published results, suggest that it may suppress replication of YF 17D vaccine.³¹
44
45 296 However, this reduced replication has not been shown to correlate with, or result in, reduced levels
46
47 297 of neutralising antibody titres (which are the desired protective outcome).^{13 31}

48
49 298 Oral typhoid vaccine (Ty21a) may occasionally be associated with stomach pain, nausea, vomiting
50
51 299 and (rarely) rash.²⁹

52
53 300 Praziquantel has been in use for about 30 years. It has a well-recognised profile of side effects
54
55 301 including dizziness, nausea, vomiting, abdominal pain, diarrhoea (sometimes with blood) and
56
57 302 urticarial rash. The symptoms are considered to arise largely from the effects of killing worms and to
58
59 303 be more severe in people with heavy infections. Symptoms are better tolerated when the drugs are
60
304 given after food and we will provide treatment after a meal or snack. Simple medications, such as
305 paracetamol and cetirizine, can alleviate symptoms and these will be available on treatment days.

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Reporting checklist for protocol of a clinical trial.

Based on the SPIRIT guidelines.

Instructions to authors

Complete this checklist by entering the page numbers from your manuscript where readers will find each of the items listed below.

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	Reporting Item	Page Number
Administrative information		
Title	#1 Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	1
Trial registration	#2a Trial identifier and registry name. If not yet registered, name of intended registry	2
Trial registration: data set	#2b All items from the World Health Organization Trial Registration Data Set	n/a
Protocol version	#3 Date and version identifier	Information available at ISRCTN60517191
Funding	#4 Sources and types of financial, material, and other support	15
Roles and	#5a Names, affiliations, and roles of protocol	15

responsibilities: contributorship		contributors	
Roles and responsibilities: sponsor contact information	#5b	Name and contact information for the trial sponsor	Information available at ISRCTN60517191
Roles and responsibilities: sponsor and funder	#5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities	16
Roles and responsibilities: committees	#5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)	Information detailed in supplementary information file
Introduction			
Background and rationale	#6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention	4
Background and rationale: choice of comparators	#6b	Explanation for choice of comparators	8
Objectives	#7	Specific objectives or hypotheses	6
Trial design	#8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, non-inferiority, exploratory)	7
Methods: Participants, interventions, and outcomes			

1	Study setting	#9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained	7
2				
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8	Eligibility criteria	#10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists)	7
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14	Interventions:	#11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	8
15	description			
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19				
20	Interventions:	#11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving / worsening disease)	Detailed in supplementary information file
21	modifications			
22				
23				
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27	Interventions:	#11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return; laboratory tests)	Detailed in supplementary information file
28	adherence			
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32	Interventions:	#11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	Detailed in supplementary information file
33	concomitant care			
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35				
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37	Outcomes	#12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended	10
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50	Participant timeline	#13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure)	14
51				
52				
53				Also detailed in supplementary information file, Table S1
54				
55				
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1	Sample size	#14	Estimated number of participants needed to achieve	11
2			study objectives and how it was determined,	
3			including clinical and statistical assumptions	
4			supporting any sample size calculations	
5				
6				
7				
8	Recruitment	#15	Strategies for achieving adequate participant	Detailed in
9			enrolment to reach target sample size	supplementary
10				information file
11				
12				
13	Methods:			
14	Assignment of			
15	interventions (for			
16	controlled trials)			
17				
18				
19				
20	Allocation: sequence	#16a	Method of generating the allocation sequence (eg,	8
21	generation		computer-generated random numbers), and list of	
22			any factors for stratification. To reduce predictability	
23			of a random sequence, details of any planned	
24			restriction (eg, blocking) should be provided in a	
25			separate document that is unavailable to those who	
26			enrol participants or assign interventions	
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31	Allocation	#16b	Mechanism of implementing the allocation sequence	9
32	concealment		(eg, central telephone; sequentially numbered,	
33	mechanism		opaque, sealed envelopes), describing any steps to	
34			conceal the sequence until interventions are assigned	
35				
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37				
38	Allocation:	#16c	Who will generate the allocation sequence, who will	8, 9
39	implementation		enrol participants, and who will assign participants to	
40			interventions	
41				
42				
43	Blinding (masking)	#17a	Who will be blinded after assignment to	9
44			interventions (eg, trial participants, care providers,	
45			outcome assessors, data analysts), and how	
46				
47				
48				
49	Blinding (masking):	#17b	If blinded, circumstances under which unblinding is	Detailed in
50	emergency		permissible, and procedure for revealing a	supplementary
51	unblinding		participant's allocated intervention during the trial	information file
52				
53				

**Methods: Data
collection,
management, and
analysis**

1	Data collection plan	#18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol	10. More details in supplementary information file
2				
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14	Data collection plan: retention	#18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols	Detailed in supplementary information file
15				
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21	Data management	#19	Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol	13. More information in the statistical analysis plan found at ISRCTN60517191
22				
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31	Statistics: outcomes	#20a	Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol	13. More information in the statistical analysis plan found at ISRCTN60517191
32				
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39				
40	Statistics: additional analyses	#20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	11. More information in the statistical analysis plan found at ISRCTN60517191
41				
42				
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48				
49	Statistics: analysis population and missing data	#20c	Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation)	Information in the statistical analysis plan found at ISRCTN60517191
50				
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Methods: Monitoring

1	Data monitoring:	#21a	Composition of data monitoring committee (DMC);	Detailed in
2	formal committee		summary of its role and reporting structure;	supplementary
3			statement of whether it is independent from the	information file
4			sponsor and competing interests; and reference to	
5			where further details about its charter can be found,	
6			if not in the protocol. Alternatively, an explanation of	
7			why a DMC is not needed	
8				
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12	Data monitoring:	#21b	Description of any interim analyses and stopping	Detailed in
13	interim analysis		guidelines, including who will have access to these	supplementary
14			interim results and make the final decision to	information file
15			terminate the trial	
16				
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18				
19	Harms	#22	Plans for collecting, assessing, reporting, and	Detailed in
20			managing solicited and spontaneously reported	supplementary
21			adverse events and other unintended effects of trial	information file
22			interventions or trial conduct	
23				
24				
25				
26	Auditing	#23	Frequency and procedures for auditing trial conduct,	Detailed in
27			if any, and whether the process will be independent	supplementary
28			from investigators and the sponsor	information file
29				
30				
31	Ethics and			
32	dissemination			
33				
34				
35	Research ethics	#24	Plans for seeking research ethics committee /	12
36	approval		institutional review board (REC / IRB) approval	
37				
38				
39	Protocol	#25	Plans for communicating important protocol	11
40	amendments		modifications (eg, changes to eligibility criteria,	
41			outcomes, analyses) to relevant parties (eg,	
42			investigators, REC / IRBs, trial participants, trial	
43			registries, journals, regulators)	
44				
45				
46				
47	Consent or assent	#26a	Who will obtain informed consent or assent from	12.
48			potential trial participants or authorised surrogates,	
49			and how (see Item 32)	
50				Also detailed in
51				supplementary
52				information file
53				
54				
55	Consent or assent:	#26b	Additional consent provisions for collection and use	Detailed in
56	ancillary studies		of participant data and biological specimens in	supplementary
57			ancillary studies, if applicable	information file
58				
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1	Confidentiality	#27	How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial	Detailed in supplementary information file
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8	Declaration of interests	#28	Financial and other competing interests for principal investigators for the overall trial and each study site	15
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10				
11	Data access	#29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators	n/a
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17	Ancillary and post trial care	#30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation	Detailed in supplementary information file
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22	Dissemination policy: trial results	#31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions	2, 13
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32	Dissemination policy: authorship	#31b	Authorship eligibility guidelines and any intended use of professional writers	n/a
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36	Dissemination policy: reproducible research	#31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	13
37				
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41	Appendices			
42				
43	Informed consent materials	#32	Model consent form and other related documentation given to participants and authorised surrogates	Supplementary files provided
44				
45				
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47	Biological specimens	#33	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable	n/a
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BMJ Open

The effect of intensive treatment for schistosomiasis on immune responses to vaccines among rural Ugandan island adolescents: randomised controlled trial protocol A for the 'POPulation differences in VACcine responses' (POPVAC) programme

Journal:	<i>BMJ Open</i>
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Article Type:	Protocol
Date Submitted by the Author:	05-Oct-2020
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Primary Subject Heading:	Infectious diseases
Secondary Subject Heading:	Epidemiology, Immunology (including allergy), Public health
Keywords:	Infection control < INFECTIOUS DISEASES, PARASITOLOGY, Public health < INFECTIOUS DISEASES, Immunology < TROPICAL MEDICINE, Epidemiology < TROPICAL MEDICINE, Paediatric infectious disease & immunisation < PAEDIATRICS

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3 1 **The effect of intensive treatment for schistosomiasis on immune responses to vaccines among**
4 **rural Ugandan island adolescents: randomised controlled trial protocol A for the ‘POPulation**
5 **differences in VACCine responses’ (POPVAC) programme**
6
7

8
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12 Akello¹, Moses Sewankambo¹, Samuel Kiwanuka¹, Robert Kizindo¹, James Kaweesa², Stephen Cose^{1,3},
13
14 7 Emily L Webb⁴, Alison M Elliott^{1,3} **for the POPVAC trial team**
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16 8

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2
3 **20 Abstract**
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5 **21 Introduction**
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7
8 Several licensed and investigational vaccines have lower efficacy, and induce impaired immune
9
10 responses, in low-income versus high-income countries and in rural, versus urban, settings.
11
12 Understanding these population differences is essential to optimising vaccine effectiveness in the
13
14 tropics. We suggest that repeated exposure to and immunomodulation by chronic helminth
15
16 infections partly explains population differences in vaccine response.
17

16 **27 Methods and analysis**
17

18
19 We have designed an individually randomised, parallel group trial of intensive versus standard
20
21 praziquantel (PZQ) intervention against schistosomiasis, to determine effects on vaccine response
22
23 outcomes among school-going adolescents (9 to 17 years) from rural *Schistosoma mansoni* (*Sm*)-
24
25 endemic Ugandan islands. Vaccines to be studied comprise BCG on day 'zero'; yellow fever, oral
26
27 typhoid and Human Papilloma Virus (HPV) vaccines at week 4; and HPV and Tetanus/diphtheria
28
29 booster vaccine at week 28. The intensive arm will receive PZQ doses three times, each two weeks
30
31 apart, before BCG immunisation, followed by a dose at week 8 and quarterly thereafter. The
32
33 standard arm will receive PZQ at week 8 and week 52. We expect to enrol 480 participants, with 80%
34
35 *Sm* infected at the outset.
36

37
38 Primary outcomes are BCG-specific IFN- γ ELISpot responses eight weeks after BCG immunisation and
39
40 for other vaccines, antibody responses to key vaccine antigens at four weeks after immunisation.
41
42 Secondary analyses will determine effects of intensive anthelmintic treatment on correlates of
43
44 protective immunity, on vaccine response waning, on priming vs boosting immunisations, and on *Sm*
45
46 infection status and intensity. Exploratory immunology assays using archived samples will enable
47
48 assessment of mechanistic links between helminths and vaccine responses.
49

45 **43 Ethics and dissemination**
46

47
48 Ethics approval has been obtained from relevant Ugandan and UK ethics committees. Results will be
49
50 shared with Uganda Ministry of Health, relevant district councils, community leaders and study
51
52 participants. Further dissemination will be done through conference proceedings and publications.
53

52 **47 Trial registration**
53

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55 Current Controlled Trials identifier: ISRCTN60517191.
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3 50 **Article summary**
4

5 51 *Strengths and limitations of this study*
6

- 7 52
- 8 • This will be the first adequately powered intervention study to investigate effects of
9 schistosomiasis treatment on vaccine responses in adolescents.
10
 - 11 54 • Effects on both live-attenuated and inert vaccines will be studied.
12
 - 13 55 • Our strong immunoepidemiological design and nested immunological studies will address
14 specific hypotheses regarding pathways of effects.
15
 - 16 57 • The sample archives developed will provide a major asset for exploration of new leads
17 arising from this hypothesis-driven work, or for an alternative, “systems biology” approach
18 58 investigating (for example) transcriptome, microbiome and virome.
19 59
 - 20 60 • Even with intensive anthelmintic intervention, it may be difficult to “successfully” treat
21 *Schistosoma* infection in our endemic setting due to re-infections; however, we still expect a
22 substantial difference in intensity between the two trial arms.
23 61
24 62
25 63

26
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28 64 **Word count**
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30 65 3250
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32 66 **Keywords**
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34 67 Vaccine; Schistosomiasis; Praziquantel; Immunization
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68 Introduction

69 Vaccine-specific immune responses are often impaired, and vaccine efficacy and effectiveness lower,
70 in tropical low-income countries (LICs) compared to temperate high-income countries and in rural,
71 compared to urban, LIC settings.¹⁻⁸ This has been recognised for both live vaccines (such as BCG,^{2,3,5,9}
72 polio,¹ yellow fever⁴ vaccines) and non-live vaccines (such as influenza¹⁰ and tetanus¹¹).

73 Investigational malaria⁷ and viral-vectored tuberculosis⁶ and Ebola¹² vaccines are also affected.

74 Previous exposure to the target pathogen (or related organisms) may mask the benefit of the
75 vaccine.^{13,14} However, pre-vaccination exposure does not explain why Ebola trial vaccine-specific

76 responses differ between healthy UK and Senegalese adults,¹² as the target organism is rare.

77 Therefore, environmentally-dependent mechanisms may play an important role.⁵

78 A long-held hypothesis is that parasites, particularly helminths, modulate vaccine responses through

79 profound pre- and post-immunisation bystander effects on immunological activation and

80 regulation.¹⁵⁻¹⁷ Helminths might also impact vaccines responses through interactions with the

81 complex ecosystem of mammalian gut bacteria, fungi, protozoa and viruses (the “trans-kingdom”

82 concept¹⁸ detailed elsewhere in this journal [bmjopen-2020-040425]). Helminth-induced gut mucosa

83 damage, the associated translocation of microbial products into the systemic circulation¹⁹⁻²¹ and

84 systemic immune activation or regulation mediated by microbial products might contribute to

85 modulation of responses to vaccines and other infections.

86 Helminth-mediated modulation of vaccine responses has not been substantiated in human

87 populations. No appropriately powered trials have been conducted to evaluate reversibility of their

88 effects. In animal models, helminths generally impair priming and accelerate waning of vaccine

89 responses, although effects vary with helminth species, vaccine type and the timing of infection and

90 immunisation.²² Most observational studies in humans also suggest suppressed or biased responses

91 during helminth infection, especially during systemic infections, such as schistosomiasis and the

92 filariases. There is modest evidence that treating geohelminths in humans improves responses to

93 BCG^{23,24} or oral cholera vaccine²⁵ and we found that schistosomiasis treatment improved the

94 measles-booster response in pre-school children.²⁶ There is therefore a strong case for a

95 comprehensive assessment of the effects of helminths and their treatment on vaccine responses.

96 The extent to which helminths and related “trans-kingdom” mediators causally and reversibly

97 impact immunological characteristics associated with vaccine responses may best be determined by

98 intervention studies. This trial protocol A of the ‘Population differences in Vaccine responses’

99 programme (POPVAC A; Current Controlled Trials identifier: ISRCTN60517191) has been designed to

100 evaluate the effect of *Schistosoma mansoni* and its treatment on vaccine responses. This study is

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101 one of three parallel trials whose designs and cross-cutting analyses are described separately in this
102 journal (bmjopen-2020-040425, bmjopen-2020-040427 and bmjopen-2020-040430).

For peer review only

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2
3 103 **Hypothesis**
4

5 104 The overarching goal of the POPVAC programme is to understand population differences in vaccine
6
7 105 responses in Uganda, in order to identify strategies through which vaccine effectiveness can be
8
9 106 optimised for the low-income, tropical settings where they are especially needed. For this Trial A, we
10
11 107 focus on the hypothesis that *Schistosoma mansoni* infection suppresses responses to unrelated
12
13 108 vaccines; and that this effect can be reversed, at least in part, by intensive praziquantel treatment
14
15 109 intervention.

16 110 **Objective**

17
18 111 To determine whether there are reversible effects of chronic *Schistosoma mansoni* infection on
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20 112 vaccine response in adolescents, using an intervention study.
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113 **Methods and analysis**

114 ***Setting and participants***

115 SPIRIT reporting guidelines²⁷ have been used. We will conduct an individually randomised, parallel
116 group trial of intensive versus standard intervention against schistosomiasis (described below) in the
117 *S. mansoni*-endemic Koome islands of Lake Victoria, Mukono district, Uganda.²⁸ We aim to enroll 480
118 participants, randomising 240 to each intervention arm. The study cohort will recruit participants
119 aged 9 to 17 years in primary school years 1 to 6. Adolescents²⁹ in this study setting bear a heavy
120 parasite burden.³⁰ In addition, this age-group is a target group for vaccines against sexually
121 transmitted infections (currently human papilloma virus [HPV] – in future, it is hoped, for vaccines
122 against HIV) and for booster immunisations.

123 ***Recruitment criteria***

124 *Inclusion criteria*

- 125 i. Attending the selected school and planning to continue to attend the school for the duration
126 of the study
- 127 ii. Aged 9 to 17 years and enrolled in primary 1 to 6 (to avoid primary leaving examinations in
128 late year 7, and loss to follow up of children leaving after primary 7)
- 129 iii. Written informed assent by participant and consent by parent or guardian
- 130 iv. Females agree to avoid pregnancy for the duration of the trial
- 131 v. Willing to provide locator information and to be contacted during the course of the trial
- 132 vi. Able and willing (in the investigator's opinion) to comply with all the study requirements

133 *Exclusion criteria*

- 134 i. Clinically significant history of immunodeficiency (including HIV), cancer, cardiovascular
135 disease, gastrointestinal disease, liver disease, renal disease, endocrine disorder and
136 neurological illness
- 137 ii. History of serious psychiatric condition or disorder
- 138 iii. Moderate or severe acute illness characterised by any of the following symptoms: fever,
139 impaired consciousness, convulsions, difficulty in breathing, vomiting; or as otherwise
140 determined by the attending project clinician.
- 141 iv. Concurrent oral or systemic steroid medication or the concurrent use of other
142 immunosuppressive agents within 2 months prior to enrolment
- 143 v. History of allergic reaction to immunisation or any allergy likely to be exacerbated by any
144 component of the study vaccines including egg or chicken proteins

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2
3 145 vi. History of previous immunisation with Yellow Fever (YF), oral typhoid or Human Papillomavirus
4 146 (HPV) vaccine; previous immunisation with BCG or Tetanus and diphtheria vaccine (Td) at age
5 147 ≥ 5 years
6
7
8 148 vii. Tendency to develop keloid scars
9
10 149 viii. Haemoglobin less than 82g/L
11
12 150 ix. Positive HIV serology
13
14 151 x. Positive pregnancy test
15 152 xi. Female currently lactating, confirmed pregnancy or intention to become pregnant during the
16 153 trial period
17
18 154 xii. Use of an investigational medicinal product or non-registered drug, live vaccine, or medical
19 155 device other than the study vaccines for 30 days prior to dosing with the study vaccine, or
20 156 planned use during the study period
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22 157 xiii. Administration of immunoglobulins and/or any blood products within the three months
23 158 preceding the planned trial immunisation date
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27 159 Further information on recruitment criteria can be found in Supplementary information.
28

29 160 **Interventions**

30
31 161 We will individually randomise participants to intensive or standard praziquantel (PZQ) treatment, in
32 162 a 1:1 ratio. The intensive arm will receive three doses of PZQ (approximately 40mg/kg, assessed by
33 163 height pole³¹) each two weeks apart prior to the first immunisation (the last of these 2-3 weeks before
34 164 immunisation), followed by PZQ at 8 weeks (after primary endpoint sampling) and thereafter quarterly
35 165 PZQ (approximately; timings adjusted to accommodate school terms) during follow up. The standard
36 166 arm will receive their first dose of PZQ at week 8 (after immunisation and after primary endpoint
37 167 sampling) and a second dose at week 52 (to conform to Uganda Ministry of Health policy, which is
38 168 annual treatment) (**Figure 1**). No placebo will be used in this trial because all participants will be
39 169 treated (albeit at different frequencies) and participants are unlikely to seek additional treatments
40 170 outside the trial schedule: praziquantel treatment is not popular because of the recognised (although
41 171 temporary) adverse effects (described in Supplementary information).
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50 172 **Randomisation and allocation to treatment arm**

51
52 173 A randomisation code will be generated by an independent statistician using a randomly permuted
53 174 block size (sizes 4, 6, 8 and 10) and used to allocate participants to either receive quarterly PZQ
54 175 (intensive arm) or annual PZQ (standard arm). A set of envelopes will be prepared, labelled
55 176 sequentially with the randomisation numbers and containing a card indicating the corresponding
56 177 allocation (to intensive or standard treatment). The randomisation code will be kept securely by the
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178 trial statistician and made available only to those responsible for providing or preparing the trial
 179 interventions. A second copy will be held by a data manager or statistician not otherwise involved in
 180 the trial at the MRC/UVRI and LSHTM Uganda Research Unit. At enrolment, eligibility criteria will be
 181 checked and eligible participants will be allocated sequentially to the next randomisation number
 182 until the required sample size is achieved. Randomisation implementation will be done by a clinician
 183 using the sequentially numbered opaque sealed envelopes. When the next randomisation number in
 184 the sequence is allocated, the envelope bearing that number will be opened to reveal the allocation.

185 **Blinding**

186 Clinicians and participants will not be blinded to the treatment allocation since they will not
 187 participate in outcome ascertainment; only immunology laboratory staff who are assessing trial
 188 outcomes will be blinded.

189 **Immunisations**

190 We will study a portfolio of licensed vaccines (live and inert, oral and parental, priming and boosting)
 191 expected to be beneficial (in some cases, already given) to adolescents in Uganda. Our schedule
 192 (**Table 1**, supplementary **Table S1**) will comprise three main immunisation days (week 0, week 4 and
 193 week 28). Additional HPV immunisation will be provided for girls aged 14 years or above, and a
 194 second Tetanus/diphtheria boost will be given after completion of the study, to accord with the
 195 national Expanded Programme on Immunisation (EPI) routines, but the response to these will not
 196 specifically be assessed. Further rationale for the selection of vaccines is detailed in the
 197 Supplementary information.

198

Table 1. Immunisation schedule

	Immunisation week 0	Immunisation week 4	[Immunisation week 8]	Immunisation week 28	[Immunisation week 52]
Live vaccines	BCG vaccination / re-vaccination ¹	Yellow fever (YF-17D) Oral typhoid (Ty21a)			
Non-live vaccines		HPV prime ²	HPV boost for girls aged ≥ 14 years ^{3,4}	HPV boost ² and Tetanus/ diphtheria (Td) boost	Tetanus/ diphtheria (Td) boost ^{4,5}
1. Prior BCG status may vary (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) 2. Both girls and boys will receive the HPV vaccine 3. The National EPI programme recommends three doses of HPV vaccine for older girls 4. These doses will be given to comply with guidelines but outcomes specifically relating to these doses will not be assessed 5. Priming by immunisation in infancy is assumed					

199

200 ***Schedule of immunisation and sampling***

201 The schedule of immunisation and sampling is outlined in **Figure 1** and **Table S1**. While optimal
202 timings for outcome measures vary between vaccines, sampling at 8 weeks post BCG and 4 weeks
203 post YF-17D, Ty21a, HPV and Td is proposed for the primary endpoints, targeting the establishment
204 of memory responses and approximate peak of antibody responses. A secondary endpoint at one
205 year will assess waning. All analyses will take baseline measurements into account. Immunisation
206 postponement criteria are detailed in Supplementary information.

207 ***Outcomes***

208 *Primary outcomes*

209 These will be assessed in all participants.

- 210 i. **BCG:** BCG-specific IFN- γ ELISpot response eight weeks post BCG immunisation.
- 211 ii. **YF-17D:** neutralising antibody titres (plaque-reduction neutralisation test) at four weeks post
212 YF immunisation.
- 213 iii. **Ty21a:** *Salmonella typhi* lipopolysaccharide (LPS)-specific immunoglobulin (Ig)G concentration
214 at four weeks post Ty21a immunisation.
- 215 iv. **HPV:** IgG specific for L1-proteins of HPV-16/18 at four weeks post HPV priming immunisation.
- 216 v. **Td:** Tetanus and diphtheria toxoid-specific IgG concentration at four weeks post Td
217 immunisation.

218 *Secondary outcomes*

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

- 222 i. **Protective immunity.** Proportions with protective neutralising antibody (YF); protective IgG
223 levels (TT);³² seroconversion rates (Ty21a) at four weeks post the corresponding
224 immunisation.
- 225 ii. **Response waning.** Primary outcome measures (all vaccines) repeated at week 52, and area-
226 under-the curve (AUC) analyses. Parasitic infection may accelerate,³³ and anti-parasitic
227 interventions delay, waning.
- 228 iii. **Priming versus boosting.** Effects on priming versus boosting will be examined for HPV only,
229 comparing outcomes four weeks after the first, and four weeks after the second vaccine dose.

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3 230 iv. **Current *S. mansoni* infection status and intensity** will be determined by serum/plasma levels
4 231 of circulating anodic antigen (CAA). The method is quantitative, highly specific for *Schistosoma*
5 232 infection, and much more sensitive than the conventional Kato Katz method.³⁴ CAA will be
6 233 assessed retrospectively on stored samples collected at baseline, on immunisation days, and
7 234 on primary and secondary endpoint days.

11
12 235 Furthermore, our sample collection will offer opportunities for an array of exploratory
13 236 immunological evaluations on stored samples, focusing mainly on vaccine antigen specific outcomes.
14 237 Exploratory assays will provide further detail on the role of immunological profiles and trans-
15 238 kingdom effects in mediating helminth modulation of vaccine-specific responses.

19 239 *Additional evaluation of parasite infection exposure*

- 21 240 i. **Prior exposure to schistosomiasis** will be evaluated by ELISA for IgG to schistosome egg
22 241 antigen using stored blood samples collected at baseline.
- 23 242 ii. **The presence of other helminth infections** will be determined retrospectively using stool
24 243 PCR of samples collected at baseline and at weeks 28 and 52.³⁰ In accordance with national
25 244 guidelines, all participants will be treated with albendazole or mebendazole after collection
26 245 of samples for primary endpoints at week 8 and 28, and after collection of samples for
27 246 secondary endpoints at week 52.
- 28 247 iii. **Current malaria infection status and intensity** will be assessed retrospectively by PCR on
29 248 stored samples collected on immunisation days and at week 52. Individuals presenting with
30 249 fever will be investigated using rapid diagnostic tests for malaria and treated based on the
31 250 results and according to prevailing national guidelines.
- 32 251 iv. **Prior malaria exposure** will be evaluated by ELISA for IgG to malaria antigen using stored
33 252 samples collected at baseline.

34 253 *Sample size considerations*

35 254 Based on the literature^{4 35 36} and preliminary data, we anticipate that, following log to base 10
36 255 transformations that will be applied to normalise primary outcome measures, standard deviations
37 256 (SDs) of primary outcome measures will lie between 0.3 and 0.6 on this log scale, and that effective
38 257 treatment may increase responses by approximately 0.2 on the log scale (based on Tweyongyere *et*
39 258 *al.*²⁶). We have therefore powered our study to detect differences of this magnitude (0.2 on the log
40 259 scale) or (in some cases) smaller (**Table 2**). We assume *S. mansoni* prevalence of $\geq 80\%$.

260 Based on these assumptions, we plan to include 480 participants in total (240 quarterly PZQ, 240
 261 annual PZQ); of whom 384 are expected to be *S. mansoni* infected,²⁸ giving 192 participants in each
 262 trial arm who are infected at baseline.

263 **Table 2** shows power estimates, for 5% significance level and assuming 20% loss to follow-up.

264

Table 2. Power estimates (5% significance level)

Standard deviation (log ₁₀)	Difference in mean log ₁₀ transformed outcome, between trial arms						
	0.08	0.10	0.12	0.14	0.16	0.18	0.20
192 intensive PZQ vs 192 standard PZQ (<i>S. mansoni</i> 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%
Cells highlighted in grey correspond to >80% power; differences in mean log ₁₀ transformed outcome of 0.08, 0.10, 0.12, 0.14, 0.16, 0.18 and 0.20 are equivalent to geometric mean ratios for untransformed outcomes of 1.20, 1.26, 1.32, 1.38, 1.45, 1.51 and 1.59, respectively.							

265

266 ***Ethics and dissemination***

267 Ethical approval has been granted from the Research Ethics Committee of the Uganda Virus
 268 Research Institute (UVRI REC, reference: GC/127/19/05/664) and the London School of Hygiene and
 269 Tropical Medicine (LSHTM, reference: 16032), and from the Uganda National Council for Science and
 270 Technology (UNCST, reference: HS2486) and the Uganda National Drug Authority (NDA, reference:
 271 CTA0093). Any protocol amendments will be submitted to ethics committees and regulatory bodies
 272 for approval before implementation.

273 Participants are adolescents and therefore a vulnerable human population. Care will be taken to
 274 provide adequate, age and education-status appropriate information and to ensure that it is
 275 understood; and to emphasise that participation is voluntary. Participants will be enrolled only when
 276 they have given their own assent and when consent has been given by the parent or guardian. No
 277 major risks to the participants are anticipated since all the treatments and vaccines to be given are
 278 licensed and known to be safe. The main risk to participants will be time lost from school work: we
 279 will work with teachers and parents to minimise disruption to classes, and will avoid enrolment of
 280 primary 7 students since these classes are involved in national examinations. Further risks are
 281 discussed in Supplementary information.

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

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3 285 ***Patient and public involvement***
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5 286 Concepts involved in this work have been discussed with colleagues at the Vector Control Division
6
7 287 and Expanded Programme on Immunisation in the Ministry of Health (Uganda), with the Mukono
8
9 288 District Council and with community leaders and Village Health Teams from Koome subcounty. We
10
11 289 also have held meetings to explain the proposed work to teachers, parents, participants and village
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13 290 members, and to address their questions about issues such as study length, the study's ethical
14
15 291 approval status, why adults were excluded from the study, and to explain to them why boys will also
16
17 292 receive the HPV vaccine. Study findings will be shared with these stakeholders and with participants.

18 293 ***Data management and analysis***
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20 294 Socio-demographic information and clinical and laboratory measurements will be recorded and
21
22 295 managed using Research Electronic Data Capture (REDCap) tools,^{37 38} with paper-based forms as
23
24 296 back-up. All data will be recorded under a unique study ID number. When paper forms must be
25
26 297 used, data will be double entered in a study-specific database, with standard checks for
27
28 298 discrepancies. All data for analysis will be anonymised and stored on a secure and password-
29
30 299 protected server, with access limited to essential research personnel.

31 300 Baseline characteristics will be summarised by trial arm, and the effect of intensive (compared to
32
33 301 standard) praziquantel treatment on the outcomes will be analysed. Information on infection status
34
35 302 will only be available after randomisation. The primary analysis will be done on individuals identified
36
37 303 as infected at baseline (through randomisation, these will be balanced between treatment arms);
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39 304 this will test the hypothesis that treating the infection (and subsequent reinfections) reverses the
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41 305 parasite's effects on vaccine responses. If treating *S. mansoni* reverses adverse parasite effects on
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43 306 vaccine responses, this may be a beneficial public health intervention. However, routine screening
44
45 307 for parasite infection before immunisation would be laborious. Secondary analyses will include all
46
47 308 randomised individuals; this will provide insight into the broader benefit of the interventions as
48
49 309 public health measures. The effect of intensive versus standard praziquantel treatment on primary
50
51 310 outcomes will be assessed using unpaired t-tests, with results presented as a mean difference in
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53 311 vaccine response measure together with 95% confidence interval and p-value. We anticipate that
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55 312 outcomes will be positively skewed, and will apply log transformations to normalise distributions
56
57 313 before analysis if required. The detailed analytical plan is available on the online trial registration site
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59 314 (<http://www.isrctn.com/ISRCTN60517191>).
60

315 Discussion

316 This will be the first adequately powered intervention study to investigate the effect of
317 schistosomiasis treatment on vaccine responses in adolescents. This study will determine whether *S.*
318 *mansoni* infection has a causal, reversible, impact on the response to live or inert vaccines, including
319 effects on vaccine replication, immune response profile, priming, boosting and waning. The results
320 will add to understanding of population differences in vaccine responses and on interventions that
321 may enhance responses. If treating helminths improves vaccine responses in adolescents, combined
322 parasite-control/immunisation programmes offer an attractive, practical public health intervention
323 for schools and communities.

324 There are risks associated with our approach to addressing the trial objective. **First**, there is a risk of
325 failure to clear *S. mansoni* infections, and repeated reinfection during the trial. This issue can be
326 challenging because of incomplete cure or maturation of immature worms after treatment, and
327 lifestyles in endemic communities that result in repeated exposure. To mitigate this, we will
328 administer three PZQ treatments over a six-week period before the first immunisations, and
329 continuing quarterly treatment in the intensive arm. **Second**, there is a risk that *S. mansoni* infection
330 has long-term effects, not removed by treatment, mediated, for example, by epigenetic change.³⁹
331 However, studies show that parasite treatment results in immunological changes,^{40,41} and our data
332 suggest at least partial recovery of the measles vaccine response among young children treated for
333 schistosomiasis.²⁶ By initiating intervention six to eight weeks before the first immunisations, and
334 providing repeated intervention in the intensive arms, we hope to achieve significant resolution of *S.*
335 *mansoni* effects.

336 We are interested in the effects of removing *S. mansoni*. Treating parasites can induce acute
337 immunological change due to release of previously hidden antigens.^{42,43} To minimise such effects,
338 immunisations will be given at least 2 weeks after PZQ (the longest practicable interval; **Figure 1**).

339 Laboratory analyses will also highlight immune parameters and cellular populations that link
340 environmental exposures to vaccine responses. Identifying processes associated with poor or good
341 outcomes will inform strategies in vaccine design (for example, the genetic modification of vaccines,
342 or innovative use of adjuvants, to counter any adverse immunological milieu, currently an area of
343 intense research for cancer vaccines⁴⁴); ultimately supporting the development of effective vaccines
344 tailored to the low-income settings that most need them.

345

346 Study timeline

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3 347 Applications for ethical approval were submitted in May 2018, with approval received in September
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5 348 2018 (Uganda Virus Research Institute Research Ethics Committee), May 2019 (National Drug
6
7 349 Authority and Uganda National Council for Science and Technology), June 2019 (London School of
8
9 350 Hygiene and Tropical Medicine). Collaborator/investigator/trial steering committee meetings were
10
11 351 also held during the initial 12-month planning period. The study began recruitment in July 2019.
12
13 352 Intervention will be up to 12 months, with completion of the project scheduled for September 2020.
14

15 353

16 354 **Competing interests**

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18 355 Alison Elliott reports a grant from the Medical research Council, UK (POPVAC programme funding).
19
20 356 The rest of the authors declare that they have no conflicts of interest.

21 357 **Author contributions**

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23
24 358 AME conceived the study. AME, GN, ELW, AN, JN, SC, LZ and JK contributed to study design. LZ, GO,
25
26 359 PNK, EN, GK, RA, CN, CO, MN, CZ, SA and FA are site clinicians/nurses/clinical laboratory technicians
27
28 360 providing valuable input on clinical considerations of the intervention. MS, SK and RK are field
29
30 361 workers handling the organisational integration of the intervention. AN, AM and ELW are involved in
31
32 362 organisation of the databases, trial randomization, treatment allocation and drawing up of analytical
33
34 363 plans. GN, LZ, JN, AN, SC, ELW and AME drafted the manuscript. All authors reviewed the
35
36 364 manuscript, contributed to it and approved the final version.

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38
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42
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48
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12
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15
16 386 study design; collection, management, analysis, and interpretation of data; writing of the protocol;
17
18 387 and the decision to submit the protocol for publication.

19 388 **POPVAC trial team**

20
21 389 **Principal investigator:** Alison Elliott; **Project leader:** Ludoviko Zirimenya; **laboratory staff:** Gyaviira
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24
25 391 Prossy Kabuubi, Emmanuel Niwagaba, Gloria Oduru, Grace Kabami; **statisticians and data**
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27 392 **managers:** Emily Webb, Agnes Natukunda, Helen Akurut, Alex Mutebe; **clinicians:** Anne Wajja, Milly
28
29 393 Namutebi, Christopher Zziwa, Joel Serubanja; **nurses:** Caroline Onen, Esther Nakazibwe, Josephine
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31 394 Tumusiime, Caroline Ninsiima, Susan Amongi, Florence Akello; **internal monitor:** Mirriam
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33 395 Akello; **field workers:** Robert Kizindo, Moses Sewankambo, Denis Nsubuga, Samuel Kiwanuka, Fred
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35 396 Kiwudhu; **boatman:** David Abiriga; **administrative management:** Moses Kizza, Samsi Nansukusa;
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37 397 **internal and external collaborators:** Pontiano Kaleebu, Hermelijn Smits, Maria Yazdanbakhsh,
38
39 398 Govert van Dam, Paul Corstjens, Sarah Staedke, Henry Luzze, James Kaweesa, Edridah Tukahebwa,
40
41 399 Elly Tumushabe, Moses Muwanga

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15 534 **FIGURE LEGENDS**

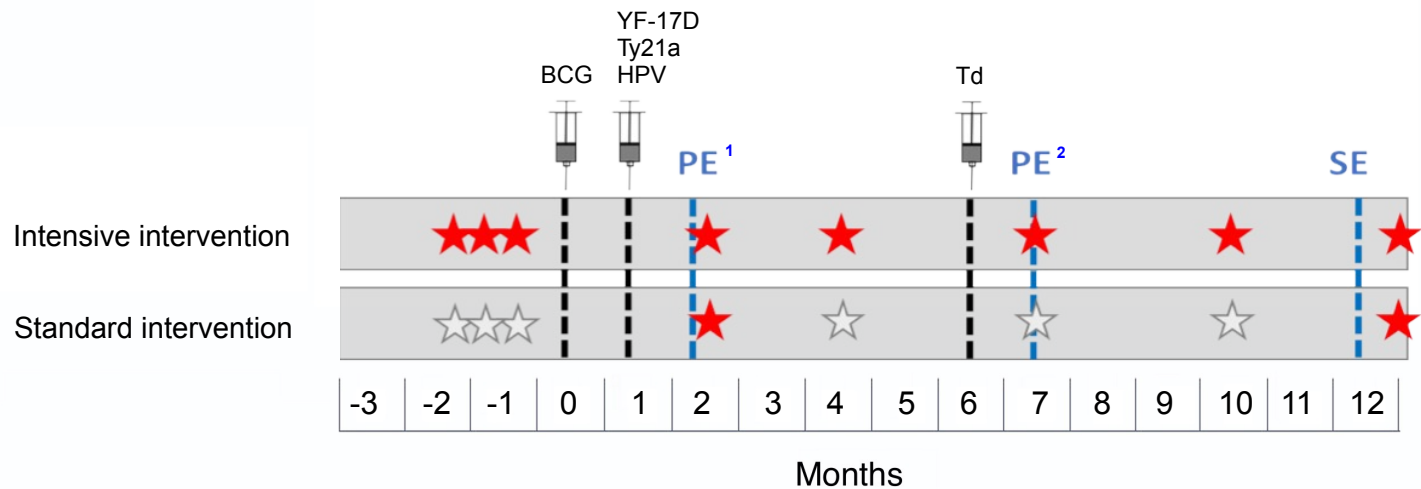
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17 535 **Figure 1. Outline of immunisations and anthelmintic intervention**






18
19 536 ¹Primary endpoints will be at eight weeks post BCG and four weeks post Yellow fever (YF-17D), oral
20 537 typhoid (Ty21a), Human Papilloma Virus (HPV) and Tetanus/diphtheria (Td) vaccination.

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22 538 ²Primary endpoint for responses to Td given at 28 weeks.
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 praziquantel;
  - - - immunisations;
 PE  primary endpoint;
 SE  secondary endpoint
 standard arm, no praziquantel

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3 **SUPPLEMENTARY INFORMATION**
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8 **The effect of intensive treatment for schistosomiasis on immune responses to vaccines among**
9 **rural Ugandan island adolescents: randomised controlled trial protocol A for the ‘POPulation**
10 **differences in VACCine responses’ (POPVAC) programme**
11

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14 Milly Namutebi¹, Christopher Zziwa¹, Susan Amongi¹, Caroline Ninsiima¹, Caroline Onen¹, Florence
15 Akello¹, Moses Sewankambo¹, Samuel Kiwanuka¹, Robert Kizindo¹, James Kaweesa², Stephen Cose^{1,3},
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Table S1. Schedule of visits and procedures

VISIT NUMBER	1	2 & 3	4	5 ⁹	5.2	6	7	8	9	10	11
WEEKS FROM 1 ST IMMUNISATION	-8 ¹	-6 ¹⁰ , -4, -2	0	4	4 weeks +4 days	8	20	28	32	44	52
	Screening	Treatment (Rx) only	Immunisations	Immunisations		Primary Endpoint (PE), Immunisations	Rx only	Immunisations	PE/Secondary Endpoint (SE) ²	Rx only	SE, Immunisations
RANDOMISED INTENSIVE VS STANDARD PRAZIQUANTEL INTERVENTION											
PZQ intensive arm (x)		x				x ³	x		x ³	x	x ³
PZQ standard arm						x ³					x ³
Albendazole						x ³			x ³		x ³
VACCINES											
BCG			x								
YF-17D				x							
Ty21a				x							
HPV				x		[x] ⁴		x			
Td								x			[x] ⁵
INVESTIGATIONS/PROCEDURES											
Inclusion/exclusion criteria	x										
Informed consent	x										
Questionnaire	x		x	x	x	x		x	x		x
Examination	x		(x)	(x)	(x)	(x)		(x)	(x)		(x)
Urine β-HCG test (female only) 1mL	x		x	x				x			
Urine YF viral load					x						
Stool for PCR and storage	x							x			x
Stool for coproantibody and storage	x					x					
BLOOD SAMPLES											
Malaria PCR (1ml)			x	x				x			x
Serology for HIV, prior malaria and <i>S. mansoni</i> (0.5ml)	x										
Mansonella perstans (1ml)	x										
Serum/plasma CAA (1ml)	x		x	x		x		x	x		x
Hb ⁸ / Full blood count (0.5ml)	x		x	x				x			
Assessments of pre-immunisation responses, and/or vaccine response outcomes and/or exploratory immunology; storage ⁷ (10 – 20 mls)			x	x		x		x	x		x
Blood for gene expression (2mls)			x	x				x			
Blood vol (mL)	4		27	17		10-20		27	10		14
Cumulative blood vol (mL) ⁷	4		31	48		68		95	105		119

1
2
3 **PE:** primary endpoint; **SE:** secondary endpoint; **Rx only:** treatment only

4 Immunisation days highlighted in green, primary end point days in red, days for treatment only in grey

5 (x) performed if clinically indicated

6 1. Screening and enrolment into Project A will take place about 8 weeks before immunisation 0 to allow initiation of the praziquantel intervention.

7 2. Week 32 is primary endpoint for responses to Td given at 28 weeks; secondary endpoint for HPV booster

8 3. Treatments given after sampling when schedules coincide

9 4. Week 8 HPV dose will be given for previously-unvaccinated girls aged ≥ 14 years

10 5. Week 52 Td booster dose will be provided as a service

11 6. Exploratory immunology blood volume will be guided by guidelines from Harvard Mass General, where a maximum of 3ml/kg body weight is taken at any one time point and not more than 3ml/kg is taken over any 8-week period (ref http://www.drgreene.com/21_1616.html.) These guidelines have been followed in a previous study vaccinating adolescents with investigational tuberculosis vaccine MVA85A (in Uganda).¹ The total blood volume planned is 68 ml over the initial intensive sampling period of 8 weeks. Revision of sample volumes based on weight will only be required for participants who weigh less than 22 kg; the average weight of children aged 9 years is expected to be 28kg (with 21kg the 3rd centile) with greater weights for older children.²

13 7. At baseline, it will only be Hb estimation by Haemocue

14 8. Oral typhoid vaccine doses will be administered on three alternate days namely visit 5, 5.1 and 5.2.

15 9. The first PZQ treatment at week -6 will be administered at the end of the screening visit

16 22

23 **Further information on recruitment criteria**

- 24 • Participants who are excluded from the trial because they have been discovered (during
25 screening procedures) to be suffering from a previously undiagnosed condition thought to
26 require further medical attention will be referred appropriately for further investigation and
27 treatment.
- 28 • Participants discovered to have severe anaemia will be excluded from the trial and treated
29 for anaemia
- 30 • Participants discovered to be HIV-positive will be counselled and offered a CD4 T-cell count
31 and referred to a provider of antiretroviral treatment (“Test and Treat” – i.e. initiation of
32 treatment regardless of CD4 count is recommended for these high-risk communities).
- 33 • Participants discovered to be pregnant will be counselled and referred to an antenatal clinic
34 of their choice.

35 This trial proposes to recruit all participants within a short time-frame. It will not, therefore, be
36 possible to reconsider enrolment of potential participants with temporary exclusion criteria after
37 treatment and resolution of the condition.

38 **Further rationale for the selection of vaccines**

39 *Bacillus Calmette–Guérin (BCG)*

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

44 Worldwide, TB is among the top 10 causes of death; Uganda has an estimated incidence of
45 202/100,000 people.³ Infectious, sputum positive, pulmonary TB classically emerges in adolescence,
46 driving the on-going epidemic.⁴ Thus adolescent booster immunisation is a key TB control strategy.⁵
47 However, BCG vaccine response and efficacy are often impaired in tropical and rural settings⁶⁻⁸ and
48 new TB vaccines are similarly affected.⁹ In the past, the WHO has been hesitant to recommend BCG
49 re-vaccination. However, in 2017 WHO’s Strategic Advisory Group of Experts (SAGE) recommended:
50 “Further research is warranted to explore whether certain sub-groups of age, geographic or *M.*
51 *tuberculosis* exposure categories would benefit from re-vaccination.”¹⁰ Recent results suggest that,
52 despite the variability of BCG efficacy between populations, BCG vaccination in adolescence offers
53 benefit in some tropical settings, especially for individuals who are not yet infected with
54 *Mycobacterium tuberculosis*, and may also be cost-effective.^{7,11} Also, BCG vaccine is currently being
55 used among adolescents in South Africa as a comparator in a trial of a novel TB vaccine (trial

1
2
3 56 registration NCT02075203). BCG efficacy in Ugandan adolescents, and differences in BCG vaccine
4
5 57 responses between urban and rural Ugandan populations, have not been tested. Information
6
7 58 obtained from this study is expected to further inform the use of BCG in adolescents, and also to
8
9 59 inform the development of new vaccines for tuberculosis.

10 60 *Yellow fever vaccine*

11
12 61 Yellow fever vaccine YF-17D is a live replicating parenteral vaccine. The vaccine (Stamaril; Sanofi
13
14 62 Pasteur) is available for purchase in Uganda. Yellow Fever (YF) causes outbreaks in Uganda and the
15
16 63 wider region¹² and YF-17D is a candidate for Uganda's expanded programme on immunisation (EPI).
17
18 64 Lower vaccine replication, lower neutralising antibody induction, and greater waning, are described
19
20 65 in Uganda compared to Switzerland.¹³ YF-17D is a potential vector for novel vaccine constructs,¹⁴
21
22 66 adding relevance to vaccine development.

23 67 *Typhoid vaccine Ty21a*

24
25 68 Typhoid vaccine Ty21a is a live replicating oral vaccine and also a potential vector for new vaccine
26
27 69 constructs.¹⁵ The Ty21a vaccine will be purchased from PaxVax, Redwood City, California.
28
29 70 Substantial, multi-year typhoid outbreaks occur in Uganda and immunisation campaigns have been
30
31 71 advocated as cost effective.¹⁶ Schistosomiasis has been associated with prolonged *S. typhi* infection¹⁷
32
33 72 and impaired antibody responses to killed typhoid vaccines.¹⁸

34
35 73 Ty21a was developed in the 1970s. Although not routinely used in Uganda, it has been (and is
36
37 74 currently) registered in many countries. It was first registered in the United States and United
38
39 75 Kingdom in the 1980s, and is recommended by the WHO for both endemic and epidemic settings.¹⁹
40
41 76 It has comparable efficacy to the parenteral Vi polysaccharide typhoid vaccine, good durability and
42
43 77 minimal adverse effects.¹⁹ It is proposed for use in this study to model effects of study exposures
44
45 78 and intervention on the response to a live oral vaccine.

46
47 79 The Ty21a vaccine is given as a three-dose regimen on alternate days.

48 80 *Human Papilloma Virus (HPV) vaccine*

49
50 81 The Human Papilloma Virus (HPV) vaccine is a protein virus-like particle. The quadrivalent HPV
51
52 82 Vaccine Gardasil (Merck) is available for purchase in Uganda and is the vaccine used by the national
53
54 83 EPI programme. Studies after three vaccine doses have found somewhat enhanced responses in the
55
56 84 presence of malaria, but no effect of helminths.²⁰ No study has previously investigated parasite
57
58 85 effects on the priming response, but recent results for tetanus suggest that priming may be more
59
60 86 susceptible than boosting to adverse effects.²¹ This will be important if forthcoming trials support
87
88 89 single-dose HPV immunisation (NCT02834637). HPV immunisation is being rolled out among girls to

1
2
3 88 prevent cervical neoplasia, the most common cancer among Ugandan women and we will
4
5 89 coordinate provision with the national HPV immunisation programme.²² HPV immunisation is also
6
7 90 beneficial for boys since HPV infection is associated with anogenital warts, anal cancer and
8
9 91 oropharyngeal cancers in both males and females, and with penile cancer in men,²³ and we will
10
11 92 include boys in these studies.

12 93 *Tetanus and diphtheria vaccines*

14 94 Tetanus and diphtheria (Td) vaccines comprise inert toxoids. Schistosomiasis is associated with a Th2
15
16 95 biased response to tetanus toxoid²⁴ and with suppressed antibody responses among those with low
17
18 96 pre-immunisation antibody levels.²¹ Booster immunisation is recommended for young women to
19
20 97 prevent maternal and neonatal tetanus. Recent evidence emphasises the need to protect young
21
22 98 men also.²⁵

23 99 ***Immunisation Postponement Criteria***

25 100 If any one of the following is identified at the time scheduled for immunisation, the participant may
26
27 101 be immunised at a later date, or withdrawn, at the discretion of the Investigator. The participant
28
29 102 must be followed until resolution of the event as with any adverse event:

- 31 103 • Acute disease at the time of immunisation. Acute disease is defined as the presence of a
32
33 104 moderate or severe illness with or without fever. All vaccines can be administered to persons
34
35 105 with a minor illness such as diarrhoea or mild upper respiratory infection with or without low-
36
37 106 grade fever, i.e. temperature of $\leq 37.5^{\circ}\text{C}$ (99.5°F)
- 38 107 • Temperature of $>37.5^{\circ}\text{C}$ (99.5°F) at the time of immunisation
- 40 108 • Taking antibiotics or antimalarials currently, or within the past 7 days, of the date of Ty21a
41
42 109 administration (ascertained verbally)

44 110 ***Vaccine storage and transport***

46 111 In order to maintain a reliable vaccine cold chain, the vaccines and diluents to be used will be stored
47
48 112 and transported within the recommended temperature range of $+2^{\circ}\text{C}$ to $+8^{\circ}\text{C}$. Care will be taken to
49
50 113 ensure that the vaccines are not frozen. BCG, being sensitive to light, will be kept in the dark
51
52 114 (normally within its secondary packaging) for as long as possible to protect it during storage and
53
54 115 transportation. All vaccines will be kept in appropriate refrigeration equipment with a temperature
55
56 116 monitoring device to ensure temperatures remain between $+2^{\circ}\text{C}$ and $+8^{\circ}\text{C}$. Cold boxes/vaccines
57
58 117 carriers with temperature monitors will be used to transport vaccines and the diluents from the
59
60 118 MRC/UVRI and LSHTM Uganda Research Unit (Entebbe) to Koome island and while transporting
60 119 vaccines to immunisation sessions. Designated staff will be given responsibility for managing the

1
2
3 120 vaccine cold chain. All cold chain equipment including the temperature monitoring devices used for
4
5 121 this project will comply with relevant technical specifications as defined by the EPI standards. Basic
6
7 122 routine maintenance will be regularly carried out on all cold chain equipment.

8 9 123 ***Additional laboratory measurements***

10
11 124 Additional assays will comprise HIV serology, pregnancy testing and full blood counts. HIV testing
12
13 125 and pregnancy testing will be accompanied by appropriate counselling by trained staff.

- 14
15 126 • HIV serology will be done on blood samples using rapid tests and according to prevailing
16
17 127 national algorithms.²⁶ This will be done at baseline.
- 18
19 128 • Pregnancy testing will be done using urine samples and standard operating procedures for
20
21 129 assessment of urine β -human chorionic gonadotropin (β hCG). This will be done at baseline
22
23 130 and before immunisation on each immunisation day.
- 24
25 131 • Full blood counts will be conducted using a haematology analyser. Mild, moderate and
26
27 132 severe anaemia will be defined according to WHO guidelines, by age.²⁷ This will be done at
28
29 133 baseline to test for anaemia as part of the eligibility assessment, and pre-immunisation as
30
31 134 part of the assessment of immunological profile.

32
33 135 Individuals found to be HIV positive or pregnant will be referred to appropriate providers for further
34
35 136 care. Individuals with severe anaemia (haemoglobin <82g/L) will be excluded from the randomised
36
37 137 intervention (since the intervention might be beneficial in management of anaemia). They will be
38
39 138 treated for anaemia.

40 41 139 ***Sample handling and archive***

42
43 140 Blood and other samples will be processed according to local laboratory standard operating
44
45 141 procedures (SOPs). All samples will reach the laboratory in anonymised form.

46
47 142 A sample archive will be developed. Although our current programme of work will address specific
48
49 143 hypotheses regarding pathways of effects of parasites and interventions, the sample archive will
50
51 144 provide a major asset for exploration of new leads arising from this work, or for an alternative,
52
53 145 “systems biology” approach employing (for example) proteomic, genomic, epigenetic and
54
55 146 transcriptomic analyses, and investigating the microbiome and virome. Information provided to
56
57 147 participants, and consent forms, will include considerations of sample storage, and the possibility of
58
59 148 sample analysis in laboratories within and outside Uganda. Participants will be able to decide if they
60
61 149 will permit such future use of any leftover samples. We plan to store the samples for up to 20 years.
62
63 150 If further storage is needed after that time, permission will be requested from the Uganda Virus
64
65 151 Research Institute and London School of Hygiene and Tropical Medicine ethical review committees.

1
2
3 152 If they elect not to permit this, all of those leftover samples will be discarded after the completion of
4
5 153 the work included in the current protocol.

6
7 154 ***Operational considerations***

8
9 155 *Programme governance*

10
11 156 A Programme Steering Committee has been set up to guide progress across all projects. This
12
13 157 comprises the following:

- 14
15 158 • An independent chair
16
17 159 • Representatives from the Ministry of Health programmes for immunisation and for vector
18
19 160 borne disease control
20
21 161 • Representatives of district authorities (Mukono and Jinja districts)
22
23 162 • Community representatives
24
25 163 • Principal investigator and co-investigators
26
27 164 • Project leader and post-doctoral immunologist
28
29 165 • Trial statistician
30
31 166 • Laboratory manager
32
33 167 • Medical Research Council observer

34
35 168 *Informed consent*

36
37 169 Both written informed assent from the participants and written informed consent from a parent or
38
39 170 guardian will be required for participation, although these may not necessarily be obtained at the
40
41 171 same time. Information will be provided in both English and the appropriate local language. For
42
43 172 individuals who cannot speak the languages used, or who cannot read or write, a witness who can
44
45 173 read the information sheet and translate the information to the participant or parent/guardian will
46
47 174 be used. Two different types of age specific assent forms will be used for the group of participants
48
49 175 aged 9 – 12 years and for the group aged 13 – 17 years. Informed consent by emancipated or mature
50
51 176 minors will be obtained using a designated consent form for these categories of participants.

52
53 177 The aims of the study, all tests, treatments and immunisations to be carried out and potential risks
54
55 178 will be explained. The participant will be given the opportunity to ask about details of the trial, and
56
57 179 will then have time to consider whether or not to participate. If they do decide to participate, they
58
59 180 and their parent/guardian will sign and date two copies of the assent and consent forms, one for
60
181 them to take away and keep, and one to be stored securely by the research team. Separate
182
information and consent forms will be provided (i) for consent for storage of samples for future

1
2
3 183 studies and for anonymous sharing of data from this study and (ii) for possible genetic studies; the
4
5 184 information sheet will explain that these data may be used in analyses related to this protocol.
6

7 185 *Screening and Eligibility Assessment*
8

9 186 Once the informed consent process has been completed, and consent (and assent) given, a baseline
10
11 187 medical history (including concomitant medication) will be collected. Vital signs will be checked and
12
13 188 a physical examination will be performed. Inclusion and exclusion criteria will be checked.

14
15 189 Participants will undergo pre- and post-test counselling for HIV and (for girls) pregnancy testing by a
16
17 190 trained and experienced nurse- or clinician-counsellor. Blood, urine and stool samples will be
18
19 191 obtained, for tests as specified in the schedule of procedures. These tests are to exclude the major,
20
21 192 immunomodulating co-infection, HIV, and conditions that might impact safety (anaemia,
22
23 193 pregnancy).

24 194 *Enrolment*

25
26 195 Participants who consent/assent, complete the screening processes, satisfy all the inclusion criteria
27
28 196 and meet none of the exclusion criteria will be enrolled.

29
30 197 *Discontinuation/withdrawal criteria*

31
32 198 In accordance with the principles of the current revision of the Declaration of Helsinki and any other
33
34 199 applicable regulations, a participant has the right to withdraw from the study at any time and for any
35
36 200 reason, and is not obliged to give his or her reasons for doing so. The Investigator may withdraw the
37
38 201 participant at any time in the interests of the participant's health and well-being. In addition, the
39
40 202 participant may withdraw/be withdrawn for any of the following reasons:

- 41 203
- 42 • Ineligibility (either arising during the study or retrospectively, having been overlooked at
43 screening)
 - 44 • Administrative decision by the Investigator
 - 45 • Significant protocol deviation
 - 46 • Participant non-compliance with study requirements
 - 47 • An adverse event which requires discontinuation of the study involvement or results in
48 inability to continue to comply with study procedures.
- 49 208
50
51 209

52
53 210 Any participant who becomes pregnant during the trial will be followed up until the end of the
54
55 211 pregnancy but no further immunisations will be given unless indicated during pregnancy (as is the
56
57 212 case for tetanus toxoid). The trial allocation for this participant will be unblinded and the participant
58
59 213 will only be given further treatment if clinically indicated. The babies will also be followed up and
60

1
2
3 214 examined for any adverse effects. We will not routinely perform venepuncture in a pregnant
4
5 215 participant.

6
7 216 The reason for withdrawal will be recorded in the case report form (CRF). If withdrawal is due to an
8
9 217 AE, appropriate follow-up visits or medical care will be arranged, with the agreement of the
10
11 218 participant, until the AE has resolved, stabilised or a non-trial related causality has been assigned.

12
13 219 If a participant withdraws from the study samples collected before their withdrawal from the trial
14
15 220 will be used/ stored unless the participant specifically requests otherwise.

16 221 *Trial discontinuation*

17
18 222 The trial will be discontinued in the event of new scientific information that renders continuation
19
20 223 futile or unethical, or for any other reason, at the discretion of the Programme Steering Committee.

21
22 224 *End of study definition*

23
24 225 The trial will be completed when the last participant enrolled into the trial has completed their final
25
26 226 follow up visit.

27
28 227 *Safety assessments and oversight*

29
30 228 No new investigational drug or product will be used in the proposed trial. However, standard
31
32 229 approaches for monitoring safety and reporting of serious adverse events will be followed.

33
34 230 *Monitoring*

35
36 231 The trial will be monitored by both internal and external monitors according to a pre-defined
37
38 232 monitoring plan which will include a site initiation visit, monitoring visits at least annually, and a
39
40 233 close-out visit. The monitors will assess patient safety, data integrity, and adherence to the protocol
41
42 234 and to Good Clinical Research Practice procedures.

43
44 235 ***Considerations regarding standard of care***

45
46 236 *S. mansoni* infection status will be determined retrospectively through assays conducted in bulk on
47
48 237 stored samples (plasma CAA). These results will not, therefore, be useful to determine management
49
50 238 of individual participants.

51 239 Participants in the standard treatment arm will receive lower levels of anthelmintic treatment.

52 240 However, all trial arms will receive a minimum of well-implemented national standard of care.

53
54 241 Standard of care will comprise annual praziquantel treatment. Our own results from the Lake

55
56 242 Victoria Island Intervention Study on Worms and Allergy-related diseases (LaVIISWA),²⁸ which

57
58 243 compared annual versus quarterly intervention for schistosomiasis at community level over three
59
60

1
2
3 244 years, showed no advantage of quarterly treatment for morbidity outcomes attributed to
4
5 245 schistosomiasis.
6
7 246 Schistosomiasis can cause anaemia. To manage the expected differential benefits of the
8
9 247 interventions for anaemia, a full blood count will be performed at baseline, as discussed above;
10
11 248 anaemic children will be managed appropriately and severely anaemic children excluded.
12
13 249 Albendazole will be provided twice a year to manage nematode infections (after collection of
14
15 250 primary and secondary endpoint samples).

16 251 ***Procedures to be followed in the event of abnormal findings***

17
18 252 Abnormal clinical findings from medical history, examination or blood tests will be assessed as to
19
20 253 their clinical significance throughout the trials. If an abnormal test result is deemed clinically
21
22 254 significant, it may be repeated. If a test remains clinically significant, the participant will be informed
23
24 255 and appropriate medical care arranged as appropriate and with the permission of the participant.
25
26 256 Specific details regarding findings, discussion with participants and resulting actions will be recorded
27
28 257 in the clinical records. Decisions to exclude the participant from enrolling in the trial or to withdraw
29
30 258 a participant from the trial will be at the discretion of the Investigator.

31 259 ***Data and Safety Monitoring Board (DSMB)***

32
33 260 A data and safety monitoring board (DSMB) has been appointed to provide real-time safety
34
35 261 oversight. The DSMB will be notified within 7 days of the Investigators' being aware of the
36
37 262 occurrence of SAEs. The DSMB may recommend the Investigators to place the trial on hold if
38
39 263 deemed necessary following an intervention-related SAE. The DSMB will be chaired by a clinician
40
41 264 experienced in clinical trials. There will be a minimum of two other appropriately qualified
42
43 265 committee members. In the case of events related to a blinded intervention, the DSMB can request
44
45 266 unblinding. Membership will include a statistician, and at least one Ugandan member. All
46
47 267 correspondence between Investigators and the DSMB will be conveyed by the Principal Investigator
48
49 268 to the trial Sponsor. The Chair of the DSMB will be contacted for advice and independent review by
50
51 269 the Investigator or trial Sponsor in the following situations:

- 52 270 • The occurrence of any SAE
- 53 271 • Any other situation where the Investigator or trial Sponsor feels independent advice or
- 54 272 review is important

55 273 ***Ethical and regulatory considerations***

56
57
58 274 *Further information regarding risks*
59
60

1
2
3 275 The immunisations to be given have recognised side effects which are usually mild and resolve
4
5 276 spontaneously in a few days to one week. Parenteral vaccines are likely to result in pain and
6
7 277 swelling at the site of injection and mild fever; very occasionally pain and swelling can be severe and
8
9 278 associated with difficulty in moving the shoulder. Sometimes headache and tiredness occur. Rarely
10
11 279 a vaccine may cause a severe allergic reaction. For most vaccines this is estimated at less than one
12
13 280 in a million doses (but 1 in 55,000 for Yellow Fever vaccine).²⁹ Individuals with a history of a
14
15 281 possible allergic reaction to drugs or vaccines, or to vaccine components including eggs or chicken
16
17 282 proteins, will be excluded from the studies. The research team will be trained and prepared to
18
19 283 manage severe allergic reactions.

20
21 284 Adverse reactions to Yellow Fever vaccine include severe nervous system reaction (about 1 person in
22
23 285 125,000) and severe, life-threatening illness with organ failure (about 1 person in 250,000). The
24
25 286 mortality for this severe, life-threatening adverse effect is reported as about 50%.²⁹

26
27 287 BCG immunisation is likely to induce a scar in many cases. This may develop over several weeks,
28
29 288 starting as a small papule at the injection site which may become ulcerated and then heal over a
30
31 289 period of 2 to 5 months; and lymphadenopathy may develop. Occasionally a more severe local
32
33 290 reaction occurs (estimated at 1 per 1,000-10,000 doses): for example, an abscess develops and scars
34
35 291 may develop into keloids. Rarely BCG can cause disseminated disease (1 per 230,000 to 640,000
36
37 292 doses), or disease in sites remote from the immunisation site. Disseminated BCG disease usually
38
39 293 occurs in immunocompromised people: HIV positive people will be excluded from these studies.³⁰
40
41 294 BCG "pre-immunisation" may interfere with the response to the subsequent live vaccines; indeed
42
43 295 our hypothesis, and published results, suggest that it may suppress replication of YF 17D vaccine.³¹
44
45 296 However, this reduced replication has not been shown to correlate with, or result in, reduced levels
46
47 297 of neutralising antibody titres (which are the desired protective outcome).^{13 31}

48
49 298 Oral typhoid vaccine (Ty21a) may occasionally be associated with stomach pain, nausea, vomiting
50
51 299 and (rarely) rash.²⁹

52
53 300 Praziquantel has been in use for about 30 years. It has a well-recognised profile of side effects
54
55 301 including dizziness, nausea, vomiting, abdominal pain, diarrhoea (sometimes with blood) and
56
57 302 urticarial rash. The symptoms are considered to arise largely from the effects of killing worms and to
58
59 303 be more severe in people with heavy infections. Symptoms are better tolerated when the drugs are
60
304 given after food and we will provide treatment after a meal or snack. Simple medications, such as
305 paracetamol and cetirizine, can alleviate symptoms and these will be available on treatment days.

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Reporting checklist for protocol of a clinical trial.

Based on the SPIRIT guidelines.

Instructions to authors

Complete this checklist by entering the page numbers from your manuscript where readers will find each of the items listed below.

Your article may not currently address all the items on the checklist. Please modify your text to include the missing information. If you are certain that an item does not apply, please write "n/a" and provide a short explanation.

Upload your completed checklist as an extra file when you submit to a journal.

In your methods section, say that you used the SPIRIT reporting guidelines, and cite them as:

Chan A-W, Tetzlaff JM, Altman DG, Laupacis A, Gøtzsche PC, Krleža-Jerić K, Hróbjartsson A, Mann H, Dickersin K, Berlin J, Doré C, Parulekar W, Summerskill W, Groves T, Schulz K, Sox H, Rockhold FW, Rennie D, Moher D. SPIRIT 2013 Statement: Defining standard protocol items for clinical trials. *Ann Intern Med.* 2013;158(3):200-207

	Reporting Item	Page Number
Administrative information		
Title	#1 Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	1
Trial registration	#2a Trial identifier and registry name. If not yet registered, name of intended registry	2
Trial registration: data set	#2b All items from the World Health Organization Trial Registration Data Set	n/a
Protocol version	#3 Date and version identifier	Information available at ISRCTN60517191
Funding	#4 Sources and types of financial, material, and other support	15
Roles and	#5a Names, affiliations, and roles of protocol	15

responsibilities: contributorship		contributors	
Roles and responsibilities: sponsor contact information	#5b	Name and contact information for the trial sponsor	Information available at ISRCTN60517191
Roles and responsibilities: sponsor and funder	#5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities	16
Roles and responsibilities: committees	#5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)	Information detailed in supplementary information file
Introduction			
Background and rationale	#6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention	4
Background and rationale: choice of comparators	#6b	Explanation for choice of comparators	8
Objectives	#7	Specific objectives or hypotheses	6
Trial design	#8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, non-inferiority, exploratory)	7
Methods: Participants, interventions, and outcomes			

1	Study setting	#9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained	7
2				
3				
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6				
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8	Eligibility criteria	#10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists)	7
9				
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14	Interventions:	#11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	8
15	description			
16				
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19				
20	Interventions:	#11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving / worsening disease)	Detailed in supplementary information file
21	modifications			
22				
23				
24				
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27	Interventions:	#11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return; laboratory tests)	Detailed in supplementary information file
28	adherence			
29				
30				
31				
32	Interventions:	#11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	Detailed in supplementary information file
33	concomitant care			
34				
35				
36				
37	Outcomes	#12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended	10
38				
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50	Participant timeline	#13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure)	14
51				
52				
53				Also detailed in supplementary information file, Table S1
54				
55				
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1	Sample size	#14	Estimated number of participants needed to achieve	11
2			study objectives and how it was determined,	
3			including clinical and statistical assumptions	
4			supporting any sample size calculations	
5				
6				
7				
8	Recruitment	#15	Strategies for achieving adequate participant	Detailed in
9			enrolment to reach target sample size	supplementary
10				information file
11				
12				
13	Methods:			
14	Assignment of			
15	interventions (for			
16	controlled trials)			
17				
18				
19				
20	Allocation: sequence	#16a	Method of generating the allocation sequence (eg,	8
21	generation		computer-generated random numbers), and list of	
22			any factors for stratification. To reduce predictability	
23			of a random sequence, details of any planned	
24			restriction (eg, blocking) should be provided in a	
25			separate document that is unavailable to those who	
26			enrol participants or assign interventions	
27				
28				
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31	Allocation	#16b	Mechanism of implementing the allocation sequence	9
32	concealment		(eg, central telephone; sequentially numbered,	
33	mechanism		opaque, sealed envelopes), describing any steps to	
34			conceal the sequence until interventions are assigned	
35				
36				
37				
38	Allocation:	#16c	Who will generate the allocation sequence, who will	8, 9
39	implementation		enrol participants, and who will assign participants to	
40			interventions	
41				
42				
43	Blinding (masking)	#17a	Who will be blinded after assignment to	9
44			interventions (eg, trial participants, care providers,	
45			outcome assessors, data analysts), and how	
46				
47				
48				
49	Blinding (masking):	#17b	If blinded, circumstances under which unblinding is	Detailed in
50	emergency		permissible, and procedure for revealing a	supplementary
51	unblinding		participant's allocated intervention during the trial	information file
52				
53				

**Methods: Data
collection,
management, and
analysis**

1	Data collection plan	#18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol	10. More details in supplementary information file
2				
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14	Data collection plan: retention	#18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols	Detailed in supplementary information file
15				
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21	Data management	#19	Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol	13. More information in the statistical analysis plan found at ISRCTN60517191
22				
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31	Statistics: outcomes	#20a	Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol	13. More information in the statistical analysis plan found at ISRCTN60517191
32				
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40	Statistics: additional analyses	#20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	11. More information in the statistical analysis plan found at ISRCTN60517191
41				
42				
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48				
49	Statistics: analysis population and missing data	#20c	Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation)	Information in the statistical analysis plan found at ISRCTN60517191
50				
51				
52				
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Methods: Monitoring

1	Data monitoring:	#21a	Composition of data monitoring committee (DMC);	Detailed in
2	formal committee		summary of its role and reporting structure;	supplementary
3			statement of whether it is independent from the	information file
4			sponsor and competing interests; and reference to	
5			where further details about its charter can be found,	
6			if not in the protocol. Alternatively, an explanation of	
7			why a DMC is not needed	
8				
9				
10				
11				
12	Data monitoring:	#21b	Description of any interim analyses and stopping	Detailed in
13	interim analysis		guidelines, including who will have access to these	supplementary
14			interim results and make the final decision to	information file
15			terminate the trial	
16				
17				
18				
19	Harms	#22	Plans for collecting, assessing, reporting, and	Detailed in
20			managing solicited and spontaneously reported	supplementary
21			adverse events and other unintended effects of trial	information file
22			interventions or trial conduct	
23				
24				
25				
26	Auditing	#23	Frequency and procedures for auditing trial conduct,	Detailed in
27			if any, and whether the process will be independent	supplementary
28			from investigators and the sponsor	information file
29				
30				
31	Ethics and			
32	dissemination			
33				
34				
35	Research ethics	#24	Plans for seeking research ethics committee /	12
36	approval		institutional review board (REC / IRB) approval	
37				
38				
39	Protocol	#25	Plans for communicating important protocol	11
40	amendments		modifications (eg, changes to eligibility criteria,	
41			outcomes, analyses) to relevant parties (eg,	
42			investigators, REC / IRBs, trial participants, trial	
43			registries, journals, regulators)	
44				
45				
46				
47	Consent or assent	#26a	Who will obtain informed consent or assent from	12.
48			potential trial participants or authorised surrogates,	
49			and how (see Item 32)	
50				Also detailed in
51				supplementary
52				information file
53				
54				
55	Consent or assent:	#26b	Additional consent provisions for collection and use	Detailed in
56	ancillary studies		of participant data and biological specimens in	supplementary
57			ancillary studies, if applicable	information file
58				
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1	Confidentiality	#27	How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial	Detailed in supplementary information file
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8	Declaration of interests	#28	Financial and other competing interests for principal investigators for the overall trial and each study site	15
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11	Data access	#29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators	n/a
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17	Ancillary and post trial care	#30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation	Detailed in supplementary information file
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21				
22	Dissemination policy: trial results	#31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions	2, 13
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32	Dissemination policy: authorship	#31b	Authorship eligibility guidelines and any intended use of professional writers	n/a
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36	Dissemination policy: reproducible research	#31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	13
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41	Appendices			
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43	Informed consent materials	#32	Model consent form and other related documentation given to participants and authorised surrogates	Supplementary files provided
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47	Biological specimens	#33	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable	n/a
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BMJ Open

The effect of intensive treatment for schistosomiasis on immune responses to vaccines among rural Ugandan island adolescents: randomised controlled trial protocol A for the 'POPulation differences in VACcine responses' (POPVAC) programme

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Primary Subject Heading:	Infectious diseases
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3 1 **The effect of intensive treatment for schistosomiasis on immune responses to vaccines among**
4 **rural Ugandan island adolescents: randomised controlled trial protocol A for the ‘POPulation**
5 **differences in VACCine responses’ (POPVAC) programme**
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1
2
3 **20 Abstract**
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5 **21 Introduction**
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7
8 Several licensed and investigational vaccines have lower efficacy, and induce impaired immune
9
10 responses, in low-income versus high-income countries and in rural, versus urban, settings.
11
12 Understanding these population differences is essential to optimising vaccine effectiveness in the
13
14 tropics. We suggest that repeated exposure to and immunomodulation by chronic helminth
15
16 infections partly explains population differences in vaccine response.
17

18 **27 Methods and analysis**
19

20
21 We have designed an individually randomised, parallel group trial of intensive versus standard
22
23 praziquantel (PZQ) intervention against schistosomiasis, to determine effects on vaccine response
24
25 outcomes among school-going adolescents (9 to 17 years) from rural *Schistosoma mansoni* (*Sm*)-
26
27 endemic Ugandan islands. Vaccines to be studied comprise BCG on day 'zero'; yellow fever, oral
28
29 typhoid and Human Papilloma Virus (HPV) vaccines at week 4; and HPV and Tetanus/diphtheria
30
31 booster vaccine at week 28. The intensive arm will receive PZQ doses three times, each two weeks
32
33 apart, before BCG immunisation, followed by a dose at week 8 and quarterly thereafter. The
34
35 standard arm will receive PZQ at week 8 and week 52. We expect to enrol 480 participants, with 80%
36
37 *Sm* infected at the outset.

38
39 Primary outcomes are BCG-specific IFN- γ ELISpot responses eight weeks after BCG immunisation and
40
41 for other vaccines, antibody responses to key vaccine antigens at four weeks after immunisation.
42
43 Secondary analyses will determine effects of intensive anthelmintic treatment on correlates of
44
45 protective immunity, on vaccine response waning, on priming vs boosting immunisations, and on *Sm*
46
47 infection status and intensity. Exploratory immunology assays using archived samples will enable
48
49 assessment of mechanistic links between helminths and vaccine responses.
50

51 **43 Ethics and dissemination**
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54 Ethics approval has been obtained from relevant Ugandan and UK ethics committees. Results will be
55
56 shared with Uganda Ministry of Health, relevant district councils, community leaders and study
57
58 participants. Further dissemination will be done through conference proceedings and publications.
59

60 **47 Trial registration**

48 Current Controlled Trials identifier: ISRCTN60517191.
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3 50 **Article summary**
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5 51 *Strengths and limitations of this study*
6

- 7 52
- 8 • This will be the first adequately powered intervention study to investigate effects of
9 schistosomiasis treatment on vaccine responses in adolescents.
10
 - 11 54 • Effects on both live-attenuated and inert vaccines will be studied.
12
 - 13 55 • Our strong immunoepidemiological design and nested immunological studies will address
14 56 specific hypotheses regarding pathways of effects.
15
 - 16 57 • The sample archives developed will provide a major asset for exploration of new leads
17 58 arising from this hypothesis-driven work, or for an alternative, “systems biology” approach
19 59 investigating (for example) transcriptome, microbiome and virome.
20
 - 21 60 • Even with intensive anthelmintic intervention, it may be difficult to “successfully” treat
22 61 *Schistosoma* infection in our endemic setting due to re-infections; however, we still expect a
24 62 substantial difference in intensity between the two trial arms.
25
- 26
27 63

28 64 **Word count**
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30 65 3250
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32 66 **Keywords**
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34 67 Vaccine; Schistosomiasis; Praziquantel; Immunization
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68 Introduction

69 Vaccine-specific immune responses are often impaired, and vaccine efficacy and effectiveness lower,
70 in tropical low-income countries (LICs) compared to temperate high-income countries and in rural,
71 compared to urban, LIC settings.¹⁻⁸ This has been recognised for both live vaccines (such as BCG,^{2,3,5,9}
72 polio,¹ yellow fever⁴ vaccines) and non-live vaccines (such as influenza¹⁰ and tetanus¹¹).

73 Investigational malaria⁷ and viral-vectored tuberculosis⁶ and Ebola¹² vaccines are also affected.

74 Previous exposure to the target pathogen (or related organisms) may mask the benefit of the
75 vaccine.^{13,14} However, pre-vaccination exposure does not explain why Ebola trial vaccine-specific

76 responses differ between healthy UK and Senegalese adults,¹² as the target organism is rare.

77 Therefore, environmentally-dependent mechanisms may play an important role.⁵

78 A long-held hypothesis is that parasites, particularly helminths, modulate vaccine responses through

79 profound pre- and post-immunisation bystander effects on immunological activation and

80 regulation.¹⁵⁻¹⁷ Helminths might also impact vaccines responses through interactions with the

81 complex ecosystem of mammalian gut bacteria, fungi, protozoa and viruses (the “trans-kingdom”

82 concept¹⁸ detailed elsewhere in this journal [bmjopen-2020-040425]). Helminth-induced gut mucosa

83 damage, the associated translocation of microbial products into the systemic circulation¹⁹⁻²¹ and

84 systemic immune activation or regulation mediated by microbial products might contribute to

85 modulation of responses to vaccines and other infections.

86 Helminth-mediated modulation of vaccine responses has not been substantiated in human

87 populations. No appropriately powered trials have been conducted to evaluate reversibility of their

88 effects. In animal models, helminths generally impair priming and accelerate waning of vaccine

89 responses, although effects vary with helminth species, vaccine type and the timing of infection and

90 immunisation.²² Most observational studies in humans also suggest suppressed or biased responses

91 during helminth infection, especially during systemic infections, such as schistosomiasis and the

92 filariases. There is modest evidence that treating geohelminths in humans improves responses to

93 BCG^{23,24} or oral cholera vaccine²⁵ and we found that schistosomiasis treatment improved the

94 measles-booster response in pre-school children.²⁶ There is therefore a strong case for a

95 comprehensive assessment of the effects of helminths and their treatment on vaccine responses.

96 The extent to which helminths and related “trans-kingdom” mediators causally and reversibly

97 impact immunological characteristics associated with vaccine responses may best be determined by

98 intervention studies. This trial protocol A of the ‘Population differences in Vaccine responses’

99 programme (POPVAC A; Current Controlled Trials identifier: ISRCTN60517191) has been designed to

100 evaluate the effect of *Schistosoma mansoni* and its treatment on vaccine responses. This study is

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101 one of three parallel trials whose designs and cross-cutting analyses are described separately in this
102 journal (bmjopen-2020-040425, bmjopen-2020-040427 and bmjopen-2020-040430).

For peer review only

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3 103 **Hypothesis**
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5 104 The overarching goal of the POPVAC programme is to understand population differences in vaccine
6
7 105 responses in Uganda, in order to identify strategies through which vaccine effectiveness can be
8
9 106 optimised for the low-income, tropical settings where they are especially needed. For this Trial A, we
10
11 107 focus on the hypothesis that *Schistosoma mansoni* infection suppresses responses to unrelated
12
13 108 vaccines; and that this effect can be reversed, at least in part, by intensive praziquantel treatment
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15 109 intervention.

16 110 **Objective**

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18 111 To determine whether there are reversible effects of chronic *Schistosoma mansoni* infection on
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20 112 vaccine response in adolescents, using an intervention study.
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113 **Methods and analysis**

114 ***Setting and participants***

115 SPIRIT reporting guidelines²⁷ have been used. We will conduct an individually randomised, parallel
116 group trial of intensive versus standard intervention against schistosomiasis (described below) in the
117 *S. mansoni*-endemic Koome islands of Lake Victoria, Mukono district, Uganda.²⁸ We aim to enroll 480
118 participants, randomising 240 to each intervention arm. The study cohort will recruit participants
119 aged 9 to 17 years in primary school years 1 to 6. Adolescents²⁹ in this study setting bear a heavy
120 parasite burden.³⁰ In addition, this age-group is a target group for vaccines against sexually
121 transmitted infections (currently human papilloma virus [HPV] – in future, it is hoped, for vaccines
122 against HIV) and for booster immunisations.

123 ***Recruitment criteria***

124 *Inclusion criteria*

- 125 i. Attending the selected school and planning to continue to attend the school for the duration
126 of the study
- 127 ii. Aged 9 to 17 years and enrolled in primary 1 to 6 (to avoid primary leaving examinations in
128 late year 7, and loss to follow up of children leaving after primary 7)
- 129 iii. Written informed assent by participant and consent by parent or guardian
- 130 iv. Females agree to avoid pregnancy for the duration of the trial
- 131 v. Willing to provide locator information and to be contacted during the course of the trial
- 132 vi. Able and willing (in the investigator's opinion) to comply with all the study requirements

133 *Exclusion criteria*

- 134 i. Clinically significant history of immunodeficiency (including HIV), cancer, cardiovascular
135 disease, gastrointestinal disease, liver disease, renal disease, endocrine disorder and
136 neurological illness
- 137 ii. History of serious psychiatric condition or disorder
- 138 iii. Moderate or severe acute illness characterised by any of the following symptoms: fever,
139 impaired consciousness, convulsions, difficulty in breathing, vomiting; or as otherwise
140 determined by the attending project clinician.
- 141 iv. Concurrent oral or systemic steroid medication or the concurrent use of other
142 immunosuppressive agents within 2 months prior to enrolment
- 143 v. History of allergic reaction to immunisation or any allergy likely to be exacerbated by any
144 component of the study vaccines including egg or chicken proteins

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3 145 vi. History of previous immunisation with Yellow Fever (YF), oral typhoid or Human Papillomavirus
4 146 (HPV) vaccine; previous immunisation with BCG or Tetanus and diphtheria vaccine (Td) at age
5 147 ≥ 5 years
6
7 148 vii. Tendency to develop keloid scars
8
9 149 viii. Haemoglobin less than 82g/L
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11 150 ix. Positive HIV serology
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13 151 x. Positive pregnancy test
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15 152 xi. Female currently lactating, confirmed pregnancy or intention to become pregnant during the
16 153 trial period
17
18 154 xii. Use of an investigational medicinal product or non-registered drug, live vaccine, or medical
19 155 device other than the study vaccines for 30 days prior to dosing with the study vaccine, or
20 156 planned use during the study period
21
22 157 xiii. Administration of immunoglobulins and/or any blood products within the three months
23 158 preceding the planned trial immunisation date
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27 159 Further information on recruitment criteria can be found in Supplementary information.
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29 160 **Interventions**

30
31 161 We will individually randomise participants to intensive or standard praziquantel (PZQ) treatment, in
32 162 a 1:1 ratio. The intensive arm will receive three doses of PZQ (approximately 40mg/kg, assessed by
33 163 height pole³¹) each two weeks apart prior to the first immunisation (the last of these 2-3 weeks before
34 164 immunisation), followed by PZQ at 8 weeks (after primary endpoint sampling) and thereafter quarterly
35 165 PZQ (approximately; timings adjusted to accommodate school terms) during follow up. The standard
36 166 arm will receive their first dose of PZQ at week 8 (after immunisation and after primary endpoint
37 167 sampling) and a second dose at week 52 (to conform to Uganda Ministry of Health policy, which is
38 168 annual treatment) (**Figure 1**). No placebo will be used in this trial because all participants will be
39 169 treated (albeit at different frequencies) and participants are unlikely to seek additional treatments
40 170 outside the trial schedule: praziquantel treatment is not popular because of the recognised (although
41 171 temporary) adverse effects (described in Supplementary information).
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50 172 **Randomisation and allocation to treatment arm**

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52 173 A randomisation code will be generated by an independent statistician using a randomly permuted
53 174 block size (sizes 4, 6, 8 and 10) and used to allocate participants to either receive quarterly PZQ
54 175 (intensive arm) or annual PZQ (standard arm). A set of envelopes will be prepared, labelled
55 176 sequentially with the randomisation numbers and containing a card indicating the corresponding
56 177 allocation (to intensive or standard treatment). The randomisation code will be kept securely by the
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178 trial statistician and made available only to those responsible for providing or preparing the trial
 179 interventions. A second copy will be held by a data manager or statistician not otherwise involved in
 180 the trial at the MRC/UVRI and LSHTM Uganda Research Unit. At enrolment, eligibility criteria will be
 181 checked and eligible participants will be allocated sequentially to the next randomisation number
 182 until the required sample size is achieved. Randomisation implementation will be done by a clinician
 183 using the sequentially numbered opaque sealed envelopes. When the next randomisation number in
 184 the sequence is allocated, the envelope bearing that number will be opened to reveal the allocation.

185 **Blinding**

186 Clinicians and participants will not be blinded to the treatment allocation since they will not
 187 participate in outcome ascertainment; only immunology laboratory staff who are assessing trial
 188 outcomes will be blinded.

189 **Immunisations**

190 We will study a portfolio of licensed vaccines (live and inert, oral and parental, priming and boosting)
 191 expected to be beneficial (in some cases, already given) to adolescents in Uganda. Our schedule
 192 (**Table 1**, supplementary **Table S1**) will comprise three main immunisation days (week 0, week 4 and
 193 week 28). Additional HPV immunisation will be provided for girls aged 14 years or above, and a
 194 second Tetanus/diphtheria boost will be given after completion of the study, to accord with the
 195 national Expanded Programme on Immunisation (EPI) routines, but the response to these will not
 196 specifically be assessed. Further rationale for the selection of vaccines is detailed in the
 197 Supplementary information.

198

Table 1. Immunisation schedule

	Immunisation week 0	Immunisation week 4	[Immunisation week 8]	Immunisation week 28	[Immunisation week 52]
Live vaccines	BCG vaccination / re-vaccination ¹	Yellow fever (YF-17D) Oral typhoid (Ty21a)			
Non-live vaccines		HPV prime ²	HPV boost for girls aged ≥ 14 years ^{3,4}	HPV boost ² and Tetanus/ diphtheria (Td) boost	Tetanus/ diphtheria (Td) boost ^{4,5}
1. Prior BCG status may vary (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) 2. Both girls and boys will receive the HPV vaccine 3. The National EPI programme recommends three doses of HPV vaccine for older girls 4. These doses will be given to comply with guidelines but outcomes specifically relating to these doses will not be assessed 5. Priming by immunisation in infancy is assumed					

199

200 ***Schedule of immunisation and sampling***

201 The schedule of immunisation and sampling is outlined in **Figure 1** and **Table S1**. Pre-immunisation
202 vaccine responses will be assessed in baseline samples. While optimal timings for outcome measures
203 vary between vaccines, sampling at 8 weeks post BCG and 4 weeks post YF-17D, Ty21a, HPV and Td
204 is proposed for the primary endpoints, targeting the establishment of memory responses and
205 approximate peak of antibody responses. A secondary endpoint at one year will assess waning.
206 Immunisation postponement criteria are detailed in Supplementary information.

207 ***Outcomes***

208 *Primary outcomes*

209 These will be assessed in all participants.

- 210 i. **BCG:** BCG-specific IFN- γ ELISpot response eight weeks post BCG immunisation.
- 211 ii. **YF-17D:** neutralising antibody titres (plaque-reduction neutralisation test) at four weeks post
212 YF immunisation.
- 213 iii. **Ty21a:** *Salmonella typhi* lipopolysaccharide (LPS)-specific immunoglobulin (Ig)G concentration
214 at four weeks post Ty21a immunisation.
- 215 iv. **HPV:** IgG specific for L1-proteins of HPV-16/18 at four weeks post HPV priming immunisation.
- 216 v. **Td:** Tetanus and diphtheria toxoid-specific IgG concentration at four weeks post Td
217 immunisation.

218 *Secondary outcomes*

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

- 222 i. **Protective immunity.** Proportions with protective neutralising antibody (YF); protective IgG
223 levels (TT);³² seroconversion rates (Ty21a) at four weeks post the corresponding
224 immunisation.
- 225 ii. **Response waning.** Primary outcome measures (all vaccines) repeated at week 52, and area-
226 under-the curve (AUC) analyses. Parasitic infection may accelerate,³³ and anti-parasitic
227 interventions delay, waning.
- 228 iii. **Priming versus boosting.** Effects on priming versus boosting will be examined for HPV only,
229 comparing outcomes four weeks after the first, and four weeks after the second vaccine dose.

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2
3 230 iv. **Current *S. mansoni* infection status and intensity** will be determined by serum/plasma levels
4 231 of circulating anodic antigen (CAA). The method is quantitative, highly specific for *Schistosoma*
5 232 infection, and much more sensitive than the conventional Kato Katz method.³⁴ CAA will be
6 233 assessed retrospectively on stored samples collected at baseline, on immunisation days, and
7 234 on primary and secondary endpoint days.

11
12 235 Furthermore, our sample collection will offer opportunities for an array of exploratory
13 236 immunological evaluations on stored samples, focusing mainly on vaccine antigen specific outcomes.
14 237 Exploratory assays will provide further detail on the role of immunological profiles and trans-
15 238 kingdom effects in mediating helminth modulation of vaccine-specific responses.

19 239 ***Evaluation of parasite infection exposure***

20 240 The following measures will also be assessed in all participants, and will be used to describe the
21 241 general infection-exposure experience of the study participants.

- 22 242 i. **Prior exposure to schistosomiasis** will be evaluated by ELISA for IgG to schistosome egg
23 243 antigen using stored blood samples collected at baseline.
- 24 244 ii. **The presence of other helminth infections** will be determined retrospectively using stool
25 245 PCR of samples collected at baseline and at weeks 28 and 52.³⁰ In accordance with national
26 246 guidelines, all participants will be treated with albendazole or mebendazole after collection
27 247 of samples for primary endpoints at week 8 and 28, and after collection of samples for
28 248 secondary endpoints at week 52.
- 29 249 iii. **Current malaria infection status and intensity** will be assessed retrospectively by PCR on
30 250 stored samples collected on immunisation days and at week 52. Individuals presenting with
31 251 fever will be investigated using rapid diagnostic tests for malaria and treated based on the
32 252 results and according to prevailing national guidelines.
- 33 253 iv. **Prior malaria exposure** will be evaluated by ELISA for IgG to malaria antigen using stored
34 254 samples collected at baseline.

47 255 ***Sample size considerations***

48 256 Based on the literature^{4 35 36} and preliminary data, we anticipate that, following log to base 10
49 257 transformations that will be applied to normalise primary outcome measures, standard deviations
50 258 (SDs) of primary outcome measures will lie between 0.3 and 0.6 on this log scale, and that effective
51 259 treatment may increase responses by approximately 0.2 on the log scale (based on Tweyongyere *et*
52 260 *al.*²⁶). We have therefore powered our study to detect differences of this magnitude (0.2 on the log
53 261 scale) or (in some cases) smaller (**Table 2**). We assume *S. mansoni* prevalence of $\geq 80\%$.

262 Based on these assumptions and a two independent samples t-test, we plan to include 480
 263 participants in total (240 quarterly PZQ, 240 annual PZQ); of whom 384 are expected to be *S.*
 264 *mansoni* infected,²⁸ giving 192 participants in each trial arm who are infected at baseline.

265 **Table 2** shows power estimates, for 5% type-1 error rate and assuming 20% loss to follow-up.

266

Table 2. Power estimates (5% type-1 error rate)

Standard deviation (log ₁₀)	Difference in mean log ₁₀ transformed outcome, between trial arms						
	0.08	0.10	0.12	0.14	0.16	0.18	0.20
192 intensive PZQ vs 192 standard PZQ (<i>S. mansoni</i> 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%
Cells highlighted in grey correspond to >80% power; differences in mean log ₁₀ transformed outcome of 0.08, 0.10, 0.12, 0.14, 0.16, 0.18 and 0.20 are equivalent to geometric mean ratios for untransformed outcomes of 1.20, 1.26, 1.32, 1.38, 1.45, 1.51 and 1.59, respectively.							

267

268 ***Ethics and dissemination***

269 Ethical approval has been granted from the Research Ethics Committee of the Uganda Virus
 270 Research Institute (UVRI REC, reference: GC/127/19/05/664) and the London School of Hygiene and
 271 Tropical Medicine (LSHTM, reference: 16032), and from the Uganda National Council for Science and
 272 Technology (UNCST, reference: HS2486) and the Uganda National Drug Authority (NDA, reference:
 273 CTA0093). Any protocol amendments will be submitted to ethics committees and regulatory bodies
 274 for approval before implementation.

275 Participants are adolescents and therefore a vulnerable human population. Care will be taken to
 276 provide adequate, age and education-status appropriate information and to ensure that it is
 277 understood; and to emphasise that participation is voluntary. Participants will be enrolled only when
 278 they have given their own assent and when consent has been given by the parent or guardian.
 279 Model consent and assent forms are shown in Supplementary file 2. No major risks to the
 280 participants are anticipated since all the treatments and vaccines to be given are licensed and known
 281 to be safe. The main risk to participants will be time lost from school work: we will work with
 282 teachers and parents to minimise disruption to classes, and will avoid enrolment of primary 7
 283 students since these classes are involved in national examinations. Further risks are discussed in
 284 Supplementary information.

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3 285 Study findings will be published through open access peer-reviewed journals, presentations at local,
4
5 286 national and international conferences and to the local community through community meetings.
6
7 287 Anonymised participant level datasets generated will be available upon request.

8 9 288 ***Patient and public involvement***

10
11 289 Concepts involved in this work have been discussed with colleagues at the Vector Control Division
12
13 290 and Expanded Programme on Immunisation in the Ministry of Health (Uganda), with the Mukono
14
15 291 District Council and with community leaders and Village Health Teams from Koome subcounty. We
16
17 292 also have held meetings to explain the proposed work to teachers, parents, participants and village
18
19 293 members, and to address their questions about issues such as study length, the study's ethical
20
21 294 approval status, why adults were excluded from the study, and to explain to them why boys will also
22
23 295 receive the HPV vaccine. Study findings will be shared with these stakeholders and with participants.

24 25 296 ***Data management and analysis***

26
27 297 Socio-demographic information and clinical and laboratory measurements will be recorded and
28
29 298 managed using Research Electronic Data Capture (REDCap) tools,^{37 38} with paper-based forms as
30
31 299 back-up. All data will be recorded under a unique study ID number. When paper forms must be
32
33 300 used, data will be double entered in a study-specific database, with standard checks for
34
35 301 discrepancies. All data for analysis will be anonymised and stored on a secure and password-
36
37 302 protected server, with access limited to essential research personnel.

38
39 303 Baseline characteristics including age, sex, school, location of birth, prior vaccination status,
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41 304 helminth infection and prior exposure status and malaria infection and prior exposure status will be
42
43 305 summarised by trial arm. The effect of intensive (compared to standard) praziquantel treatment on
44
45 306 the outcomes will be analysed. Information on infection status will only be available after
46
47 307 randomisation. The primary analysis will be done on individuals identified as infected at baseline
48
49 308 (through randomisation, these will be balanced between treatment arms); this will test the
50
51 309 hypothesis that treating the infection (and subsequent reinfections) reverses the parasite's effects
52
53 310 on vaccine responses. If treating *S. mansoni* reverses adverse parasite effects on vaccine responses,
54
55 311 this may be a beneficial public health intervention. However, routine screening for parasite infection
56
57 312 before immunisation would be laborious. Secondary analyses will include all randomised individuals;
58
59 313 this will provide insight into the broader benefit of the interventions as public health measures. The
60
314 effect of intensive versus standard praziquantel treatment on primary outcomes will be assessed
315 using unpaired t-tests, with results presented as a mean difference in vaccine response measure
316 together with 95% confidence interval and p-value. For all outcomes, we will investigate adjusting
317 for corresponding baseline vaccine responses as this may improve the precision of effect estimates;

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3 318 this will be done using multivariable regression. We anticipate that outcomes will be positively
4
5 319 skewed, and will apply log transformations to normalise distributions before analysis if required. The
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7 320 detailed analytical plan is available on the online trial registration site
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9 321 (<http://www.isrctn.com/ISRCTN60517191>).

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For peer review only

322 Discussion

323 This will be the first adequately powered intervention study to investigate the effect of
324 schistosomiasis treatment on vaccine responses in adolescents. This study will determine whether *S.*
325 *mansoni* infection has a causal, reversible, impact on the response to live or inert vaccines, including
326 effects on vaccine replication, immune response profile, priming, boosting and waning. The results
327 will add to understanding of population differences in vaccine responses and on interventions that
328 may enhance responses. If treating helminths improves vaccine responses in adolescents, combined
329 parasite-control/immunisation programmes offer an attractive, practical public health intervention
330 for schools and communities.

331 There are risks associated with our approach to addressing the trial objective. **First**, there is a risk of
332 failure to clear *S. mansoni* infections, and repeated reinfection during the trial. This issue can be
333 challenging because of incomplete cure or maturation of immature worms after treatment, and
334 lifestyles in endemic communities that result in repeated exposure. To mitigate this, we will
335 administer three PZQ treatments over a six-week period before the first immunisations, and
336 continuing quarterly treatment in the intensive arm. **Second**, there is a risk that *S. mansoni* infection
337 has long-term effects, not removed by treatment, mediated, for example, by epigenetic change.³⁹
338 However, studies show that parasite treatment results in immunological changes,^{40,41} and our data
339 suggest at least partial recovery of the measles vaccine response among young children treated for
340 schistosomiasis.²⁶ By initiating intervention six to eight weeks before the first immunisations, and
341 providing repeated intervention in the intensive arms, we hope to achieve significant resolution of *S.*
342 *mansoni* effects.

343 We are interested in the effects of removing *S. mansoni*. Treating parasites can induce acute
344 immunological change due to release of previously hidden antigens.^{42,43} To minimise such effects,
345 immunisations will be given at least 2 weeks after PZQ (the longest practicable interval; **Figure 1**).

346 Laboratory analyses will also highlight immune parameters and cellular populations that link
347 environmental exposures to vaccine responses. Identifying processes associated with poor or good
348 outcomes will inform strategies in vaccine design (for example, the genetic modification of vaccines,
349 or innovative use of adjuvants, to counter any adverse immunological milieu, currently an area of
350 intense research for cancer vaccines⁴⁴); ultimately supporting the development of effective vaccines
351 tailored to the low-income settings that most need them.

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353 Study timeline

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3 354 Applications for ethical approval were submitted in May 2018, with approval received in September
4 355 2018 (Uganda Virus Research Institute Research Ethics Committee), May 2019 (National Drug
5 356 Authority and Uganda National Council for Science and Technology), June 2019 (London School of
6 357 Hygiene and Tropical Medicine). Collaborator/investigator/trial steering committee meetings were
7
8 358 also held during the initial 12-month planning period. The study began recruitment in July 2019.
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10 359 Intervention will be up to 12 months, with completion of the project scheduled for September 2020.
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14 360

16 361 **Competing interests**

17
18 362 Alison Elliott reports a grant from the Medical research Council, UK (POPVAC programme funding).
19 363 The rest of the authors declare that they have no conflicts of interest.
20
21

22 364 **Author contributions**

23
24 365 AME conceived the study. AME, GN, ELW, AN, JN, SC, LZ and JK contributed to study design. LZ, GO,
25 366 PNK, EN, GK, RA, CN, CO, MN, CZ, SA and FA are site clinicians/nurses/clinical laboratory technicians
26 367 providing valuable input on clinical considerations of the intervention. MS, SK and RK are field
27 368 workers handling the organisational integration of the intervention. AN, AM and ELW are involved in
28 369 organisation of the databases, trial randomization, treatment allocation and drawing up of analytical
29 370 plans. GN, LZ, JN, AN, SC, ELW and AME drafted the manuscript. All authors reviewed the
30 371 manuscript, contributed to it and approved the final version.
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37
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43 378 Richard Hayes) and the Data and Safety Monitoring Board (Dr David Meya, Prof Andrew Prendergast
44 379 and Dr Elizabeth George).
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49

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10
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12
13 391 and is also part of the EDCTP2 programme supported by the European Union.

14 392 The study sponsor (London School of Hygiene and Tropical Medicine) and funders had no role in
15
16 393 study design; collection, management, analysis, and interpretation of data; writing of the protocol;
17
18 394 and the decision to submit the protocol for publication.

19 395 **POPVAC trial team**

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21 396 **Principal investigator:** Alison Elliott; **Project leader:** Ludoviko Zirimenya; **laboratory staff:** Gyaviira
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23 397 Nkurunungi, Stephen Cose, Rebecca Amongin, Beatrice Nassanga, Jacent Nassuuna, Irene Nambuya,
24
25 398 Prossy Kabuubi, Emmanuel Niwagaba, Gloria Oduru, Grace Kabami; **statisticians and data**
26
27 399 **managers:** Emily Webb, Agnes Natukunda, Helen Akurut, Alex Mutebe; **clinicians:** Anne Wajja, Milly
28
29 400 Namutebi, Christopher Zziwa, Joel Serubanja; **nurses:** Caroline Onen, Esther Nakazibwe, Josephine
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31 401 Tumusiime, Caroline Ninsiima, Susan Amongi, Florence Akello; **internal monitor:** Mirriam
32
33 402 Akello; **field workers:** Robert Kizindo, Moses Sewankambo, Denis Nsubuga, Samuel Kiwanuka, Fred
34
35 403 Kiwudhu; **boatman:** David Abiriga; **administrative management:** Moses Kizza, Samsi Nansukusa;
36
37 404 **internal and external collaborators:** Pontiano Kaleebu, Hermelijn Smits, Maria Yazdanbakhsh,
38
39 405 Govert van Dam, Paul Corstjens, Sarah Staedke, Henry Luzze, James Kaweesa, Edridah Tukahebwa,
40
41 406 Elly Tumushabe, Moses Muwanga

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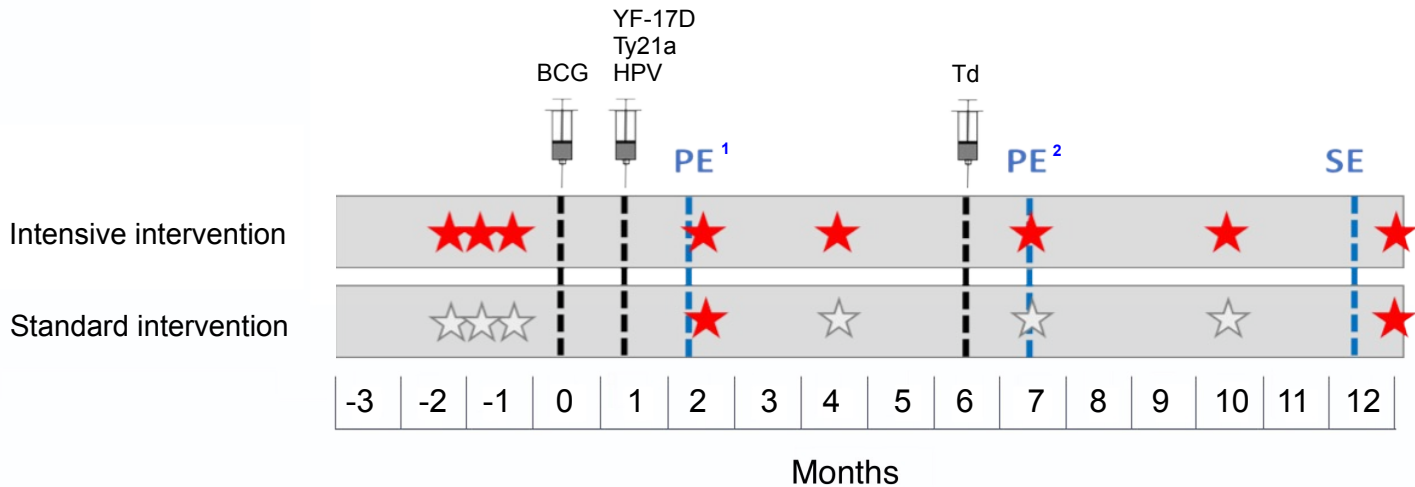
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15 541 **FIGURE LEGENDS**

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17 542 **Figure 1. Outline of immunisations and anthelmintic intervention**

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19 543 ¹Primary endpoints will be at eight weeks post BCG and four weeks post Yellow fever (YF-17D), oral
20 544 typhoid (Ty21a), Human Papilloma Virus (HPV) and Tetanus/diphtheria (Td) vaccination.

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22 545 ²Primary endpoint for responses to Td given at 28 weeks.
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★ praziquantel; - - - immunisations; PE - - - primary endpoint; SE - - - secondary endpoint
★ standard arm, no praziquantel

1
2
3 1 **SUPPLEMENTARY INFORMATION**
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5 2

6
7 3 **The effect of intensive treatment for schistosomiasis on immune responses to vaccines among**
8 **rural Ugandan island adolescents: randomised controlled trial protocol A for the ‘POPulation**
9 **differences in VACCine responses’ (POPVAC) programme**
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Table S1. Schedule of visits and procedures

VISIT NUMBER	1	2 & 3	4	5 ⁹	5.2	6	7	8	9	10	11
WEEKS FROM 1 ST IMMUNISATION	-8 ¹	-6 ¹⁰ , -4, -2	0	4	4 weeks +4 days	8	20	28	32	44	52
	Screening	Treatment (Rx) only	Immunisations	Immunisations		Primary Endpoint (PE), Immunisations	Rx only	Immunisations	PE/Secondary Endpoint (SE) ²	Rx only	SE, Immunisations
RANDOMISED INTENSIVE VS STANDARD PRAZIQUANTEL INTERVENTION											
PZQ intensive arm (x)		x				x ³	x		x ³	x	x ³
PZQ standard arm						x ³					x ³
Albendazole						x ³			x ³		x ³
VACCINES											
BCG			x								
YF-17D				x							
Ty21a				x							
HPV				x		[x] ⁴		x			
Td								x			[x] ⁵
INVESTIGATIONS/PROCEDURES											
Inclusion/exclusion criteria	x										
Informed consent	x										
Questionnaire	x		x	x	x	x		x	x		x
Examination	x		(x)	(x)	(x)	(x)		(x)	(x)		(x)
Urine β-HCG test (female only) 1mL	x		x	x				x			
Urine YF viral load					x						
Stool for PCR and storage	x							x			x
Stool for coproantibody and storage	x					x					
BLOOD SAMPLES											
Malaria PCR (1ml)			x	x				x			x
Serology for HIV, prior malaria and <i>S. mansoni</i> (0.5ml)	x										
Mansonella perstans (1ml)	x										
Serum/plasma CAA (1ml)	x		x	x		x		x	x		x
Hb ⁸ / Full blood count (0.5ml)	x		x	x				x			
Assessments of pre-immunisation responses, and/or vaccine response outcomes and/or exploratory immunology; storage ⁷ (10 – 20 mls)			x	x		x		x	x		x
Blood for gene expression (2mls)			x	x				x			
Blood vol (mL)	4		27	17		10-20		27	10		14
Cumulative blood vol (mL) ⁷	4		31	48		68		95	105		119

PE: primary endpoint; **SE:** secondary endpoint; **Rx only:** treatment only

Immunisation days highlighted in green, primary end point days in red, days for treatment only in grey

(x) performed if clinically indicated

1. Screening and enrolment into Project A will take place about 8 weeks before immunisation 0 to allow initiation of the praziquantel intervention.
2. Week 32 is primary endpoint for responses to Td given at 28 weeks; secondary endpoint for HPV booster
3. Treatments given after sampling when schedules coincide
4. Week 8 HPV dose will be given for previously-unvaccinated girls aged ≥ 14 years
5. Week 52 Td booster dose will be provided as a service
6. Exploratory immunology blood volume will be guided by guidelines from Harvard Mass General, where a maximum of 3ml/kg body weight is taken at any one time point and not more than 3ml/kg is taken over any 8-week period (ref http://www.drgreene.com/21_1616.html.) These guidelines have been followed in a previous study vaccinating adolescents with investigational tuberculosis vaccine MVA85A (in Uganda).¹ The total blood volume planned is 68 ml over the initial intensive sampling period of 8 weeks. Revision of sample volumes based on weight will only be required for participants who weigh less than 22 kg; the average weight of children aged 9 years is expected to be 28kg (with 21kg the 3rd centile) with greater weights for older children.²
7. At baseline, it will only be Hb estimation by Haemocue
8. Oral typhoid vaccine doses will be administered on three alternate days namely visit 5, 5.1 and 5.2.
9. The first PZQ treatment at week -6 will be administered at the end of the screening visit

22

23 **Further information on recruitment criteria**

- 24 • Participants who are excluded from the trial because they have been discovered (during
25 screening procedures) to be suffering from a previously undiagnosed condition thought to
26 require further medical attention will be referred appropriately for further investigation and
27 treatment.
- 28 • Participants discovered to have severe anaemia will be excluded from the trial and treated
29 for anaemia
- 30 • Participants discovered to be HIV-positive will be counselled and offered a CD4 T-cell count
31 and referred to a provider of antiretroviral treatment (“Test and Treat” – i.e. initiation of
32 treatment regardless of CD4 count is recommended for these high-risk communities).
- 33 • Participants discovered to be pregnant will be counselled and referred to an antenatal clinic
34 of their choice.

35 This trial proposes to recruit all participants within a short time-frame. It will not, therefore, be
36 possible to reconsider enrolment of potential participants with temporary exclusion criteria after
37 treatment and resolution of the condition.

38 **Further rationale for the selection of vaccines**

39 *Bacillus Calmette–Guérin (BCG)*

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

44 Worldwide, TB is among the top 10 causes of death; Uganda has an estimated incidence of
45 202/100,000 people.³ Infectious, sputum positive, pulmonary TB classically emerges in adolescence,
46 driving the on-going epidemic.⁴ Thus adolescent booster immunisation is a key TB control strategy.⁵
47 However, BCG vaccine response and efficacy are often impaired in tropical and rural settings⁶⁻⁸ and
48 new TB vaccines are similarly affected.⁹ In the past, the WHO has been hesitant to recommend BCG
49 re-vaccination. However, in 2017 WHO’s Strategic Advisory Group of Experts (SAGE) recommended:
50 “Further research is warranted to explore whether certain sub-groups of age, geographic or *M.*
51 *tuberculosis* exposure categories would benefit from re-vaccination.”¹⁰ Recent results suggest that,
52 despite the variability of BCG efficacy between populations, BCG vaccination in adolescence offers
53 benefit in some tropical settings, especially for individuals who are not yet infected with
54 *Mycobacterium tuberculosis*, and may also be cost-effective.^{7,11} Also, BCG vaccine is currently being
55 used among adolescents in South Africa as a comparator in a trial of a novel TB vaccine (trial

1
2
3 56 registration NCT02075203). BCG efficacy in Ugandan adolescents, and differences in BCG vaccine
4
5 57 responses between urban and rural Ugandan populations, have not been tested. Information
6
7 58 obtained from this study is expected to further inform the use of BCG in adolescents, and also to
8
9 59 inform the development of new vaccines for tuberculosis.

10 60 *Yellow fever vaccine*

11
12 61 Yellow fever vaccine YF-17D is a live replicating parenteral vaccine. The vaccine (Stamaril; Sanofi
13
14 62 Pasteur) is available for purchase in Uganda. Yellow Fever (YF) causes outbreaks in Uganda and the
15
16 63 wider region¹² and YF-17D is a candidate for Uganda's expanded programme on immunisation (EPI).
17
18 64 Lower vaccine replication, lower neutralising antibody induction, and greater waning, are described
19
20 65 in Uganda compared to Switzerland.¹³ YF-17D is a potential vector for novel vaccine constructs,¹⁴
21
22 66 adding relevance to vaccine development.

23 67 *Typhoid vaccine Ty21a*

24
25 68 Typhoid vaccine Ty21a is a live replicating oral vaccine and also a potential vector for new vaccine
26
27 69 constructs.¹⁵ The Ty21a vaccine will be purchased from PaxVax, Redwood City, California.
28
29 70 Substantial, multi-year typhoid outbreaks occur in Uganda and immunisation campaigns have been
30
31 71 advocated as cost effective.¹⁶ Schistosomiasis has been associated with prolonged *S. typhi* infection¹⁷
32
33 72 and impaired antibody responses to killed typhoid vaccines.¹⁸

34
35 73 Ty21a was developed in the 1970s. Although not routinely used in Uganda, it has been (and is
36
37 74 currently) registered in many countries. It was first registered in the United States and United
38
39 75 Kingdom in the 1980s, and is recommended by the WHO for both endemic and epidemic settings.¹⁹
40
41 76 It has comparable efficacy to the parenteral Vi polysaccharide typhoid vaccine, good durability and
42
43 77 minimal adverse effects.¹⁹ It is proposed for use in this study to model effects of study exposures
44
45 78 and intervention on the response to a live oral vaccine.

46
47 79 The Ty21a vaccine is given as a three-dose regimen on alternate days.

48 80 *Human Papilloma Virus (HPV) vaccine*

49
50 81 The Human Papilloma Virus (HPV) vaccine is a protein virus-like particle. The quadrivalent HPV
51
52 82 Vaccine Gardasil (Merck) is available for purchase in Uganda and is the vaccine used by the national
53
54 83 EPI programme. Studies after three vaccine doses have found somewhat enhanced responses in the
55
56 84 presence of malaria, but no effect of helminths.²⁰ No study has previously investigated parasite
57
58 85 effects on the priming response, but recent results for tetanus suggest that priming may be more
59
60 86 susceptible than boosting to adverse effects.²¹ This will be important if forthcoming trials support
87
88 89 single-dose HPV immunisation (NCT02834637). HPV immunisation is being rolled out among girls to

1
2
3 88 prevent cervical neoplasia, the most common cancer among Ugandan women and we will
4
5 89 coordinate provision with the national HPV immunisation programme.²² HPV immunisation is also
6
7 90 beneficial for boys since HPV infection is associated with anogenital warts, anal cancer and
8
9 91 oropharyngeal cancers in both males and females, and with penile cancer in men,²³ and we will
10
11 92 include boys in these studies.

12 93 *Tetanus and diphtheria vaccines*

14 94 Tetanus and diphtheria (Td) vaccines comprise inert toxoids. Schistosomiasis is associated with a Th2
15
16 95 biased response to tetanus toxoid²⁴ and with suppressed antibody responses among those with low
17
18 96 pre-immunisation antibody levels.²¹ Booster immunisation is recommended for young women to
19
20 97 prevent maternal and neonatal tetanus. Recent evidence emphasises the need to protect young
21
22 98 men also.²⁵

23 99 ***Immunisation Postponement Criteria***

25 100 If any one of the following is identified at the time scheduled for immunisation, the participant may
26
27 101 be immunised at a later date, or withdrawn, at the discretion of the Investigator. The participant
28
29 102 must be followed until resolution of the event as with any adverse event:

- 31 103 • Acute disease at the time of immunisation. Acute disease is defined as the presence of a
32
33 104 moderate or severe illness with or without fever. All vaccines can be administered to persons
34
35 105 with a minor illness such as diarrhoea or mild upper respiratory infection with or without low-
36
37 106 grade fever, i.e. temperature of $\leq 37.5^{\circ}\text{C}$ (99.5°F)
- 38 107 • Temperature of $>37.5^{\circ}\text{C}$ (99.5°F) at the time of immunisation
- 40 108 • Taking antibiotics or antimalarials currently, or within the past 7 days, of the date of Ty21a
41
42 109 administration (ascertained verbally)

44 110 ***Vaccine storage and transport***

46 111 In order to maintain a reliable vaccine cold chain, the vaccines and diluents to be used will be stored
47
48 112 and transported within the recommended temperature range of $+2^{\circ}\text{C}$ to $+8^{\circ}\text{C}$. Care will be taken to
49
50 113 ensure that the vaccines are not frozen. BCG, being sensitive to light, will be kept in the dark
51
52 114 (normally within its secondary packaging) for as long as possible to protect it during storage and
53
54 115 transportation. All vaccines will be kept in appropriate refrigeration equipment with a temperature
55
56 116 monitoring device to ensure temperatures remain between $+2^{\circ}\text{C}$ and $+8^{\circ}\text{C}$. Cold boxes/vaccines
57
58 117 carriers with temperature monitors will be used to transport vaccines and the diluents from the
59
60 118 MRC/UVRI and LSHTM Uganda Research Unit (Entebbe) to Koome island and while transporting
60 119 vaccines to immunisation sessions. Designated staff will be given responsibility for managing the

1
2
3 120 vaccine cold chain. All cold chain equipment including the temperature monitoring devices used for
4
5 121 this project will comply with relevant technical specifications as defined by the EPI standards. Basic
6
7 122 routine maintenance will be regularly carried out on all cold chain equipment.

8 9 123 ***Additional laboratory measurements***

10
11 124 Additional assays will comprise HIV serology, pregnancy testing and full blood counts. HIV testing
12
13 125 and pregnancy testing will be accompanied by appropriate counselling by trained staff.

- 14
15 126 • HIV serology will be done on blood samples using rapid tests and according to prevailing
16
17 127 national algorithms.²⁶ This will be done at baseline.
- 18
19 128 • Pregnancy testing will be done using urine samples and standard operating procedures for
20
21 129 assessment of urine β -human chorionic gonadotropin (β hCG). This will be done at baseline
22
23 130 and before immunisation on each immunisation day.
- 24
25 131 • Full blood counts will be conducted using a haematology analyser. Mild, moderate and
26
27 132 severe anaemia will be defined according to WHO guidelines, by age.²⁷ This will be done at
28
29 133 baseline to test for anaemia as part of the eligibility assessment, and pre-immunisation as
30
31 134 part of the assessment of immunological profile.

32
33 135 Individuals found to be HIV positive or pregnant will be referred to appropriate providers for further
34
35 136 care. Individuals with severe anaemia (haemoglobin $<82\text{g/L}$) will be excluded from the randomised
36
37 137 intervention (since the intervention might be beneficial in management of anaemia). They will be
38
39 138 treated for anaemia.

40 41 139 ***Sample handling and archive***

42
43 140 Blood and other samples will be processed according to local laboratory standard operating
44
45 141 procedures (SOPs). All samples will reach the laboratory in anonymised form.

46
47 142 A sample archive will be developed. Although our current programme of work will address specific
48
49 143 hypotheses regarding pathways of effects of parasites and interventions, the sample archive will
50
51 144 provide a major asset for exploration of new leads arising from this work, or for an alternative,
52
53 145 “systems biology” approach employing (for example) proteomic, genomic, epigenetic and
54
55 146 transcriptomic analyses, and investigating the microbiome and virome. Information provided to
56
57 147 participants, and consent forms, will include considerations of sample storage, and the possibility of
58
59 148 sample analysis in laboratories within and outside Uganda. Participants will be able to decide if they
60
149 will permit such future use of any leftover samples. We plan to store the samples for up to 20 years.
150
151 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 ethical review committees.

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2
3 152 If they elect not to permit this, all of those leftover samples will be discarded after the completion of
4
5 153 the work included in the current protocol.

6
7 154 ***Operational considerations***

8
9 155 *Programme governance*

10
11 156 A Programme Steering Committee has been set up to guide progress across all projects. This
12
13 157 comprises the following:

- 14
15 158 • An independent chair
16
17 159 • Representatives from the Ministry of Health programmes for immunisation and for vector
18
19 160 borne disease control
20
21 161 • Representatives of district authorities (Mukono and Jinja districts)
22
23 162 • Community representatives
24
25 163 • Principal investigator and co-investigators
26
27 164 • Project leader and post-doctoral immunologist
28
29 165 • Trial statistician
30
31 166 • Laboratory manager
32
33 167 • Medical Research Council observer

34
35 168 *Informed consent*

36
37 169 Both written informed assent from the participants and written informed consent from a parent or
38
39 170 guardian will be required for participation, although these may not necessarily be obtained at the
40
41 171 same time. Information will be provided in both English and the appropriate local language. For
42
43 172 individuals who cannot speak the languages used, or who cannot read or write, a witness who can
44
45 173 read the information sheet and translate the information to the participant or parent/guardian will
46
47 174 be used. Two different types of age specific assent forms will be used for the group of participants
48
49 175 aged 9 – 12 years and for the group aged 13 – 17 years. Informed consent by emancipated or mature
50
51 176 minors will be obtained using a designated consent form for these categories of participants.

52
53 177 The aims of the study, all tests, treatments and immunisations to be carried out and potential risks
54
55 178 will be explained. The participant will be given the opportunity to ask about details of the trial, and
56
57 179 will then have time to consider whether or not to participate. If they do decide to participate, they
58
59 180 and their parent/guardian will sign and date two copies of the assent and consent forms, one for
60
181 them to take away and keep, and one to be stored securely by the research team. Separate
182 information and consent forms will be provided (i) for consent for storage of samples for future

183 studies and for anonymous sharing of data from this study and (ii) for possible genetic studies; the
184 information sheet will explain that these data may be used in analyses related to this protocol.

185 *Screening and Eligibility Assessment*

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

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

194 *Enrolment*

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

197 *Discontinuation/withdrawal criteria*

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

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

210 Any participant who becomes pregnant during the trial will be followed up until the end of the
211 pregnancy but no further immunisations will be given unless indicated during pregnancy (as is the
212 case for tetanus toxoid). The trial allocation for this participant will be unblinded and the participant
213 will only be given further treatment if clinically indicated. The babies will also be followed up and

1
2
3 214 examined for any adverse effects. We will not routinely perform venepuncture in a pregnant
4
5 215 participant.

6
7 216 The reason for withdrawal will be recorded in the case report form (CRF). If withdrawal is due to an
8
9 217 AE, appropriate follow-up visits or medical care will be arranged, with the agreement of the
10
11 218 participant, until the AE has resolved, stabilised or a non-trial related causality has been assigned.

12
13 219 If a participant withdraws from the study samples collected before their withdrawal from the trial
14
15 220 will be used/ stored unless the participant specifically requests otherwise.

16 221 *Trial discontinuation*

17
18 222 The trial will be discontinued in the event of new scientific information that renders continuation
19
20 223 futile or unethical, or for any other reason, at the discretion of the Programme Steering Committee.

21
22 224 *End of study definition*

23
24 225 The trial will be completed when the last participant enrolled into the trial has completed their final
25
26 226 follow up visit.

27
28 227 *Safety assessments and oversight*

29
30 228 No new investigational drug or product will be used in the proposed trial. However, standard
31
32 229 approaches for monitoring safety and reporting of serious adverse events will be followed.

33
34 230 *Monitoring*

35
36 231 The trial will be monitored by both internal and external monitors according to a pre-defined
37
38 232 monitoring plan which will include a site initiation visit, monitoring visits at least annually, and a
39
40 233 close-out visit. The monitors will assess patient safety, data integrity, and adherence to the protocol
41
42 234 and to Good Clinical Research Practice procedures.

43
44 235 ***Considerations regarding standard of care***

45
46 236 *S. mansoni* infection status will be determined retrospectively through assays conducted in bulk on
47
48 237 stored samples (plasma CAA). These results will not, therefore, be useful to determine management
49
50 238 of individual participants.

51 239 Participants in the standard treatment arm will receive lower levels of anthelmintic treatment.

52
53 240 However, all trial arms will receive a minimum of well-implemented national standard of care.

54
55 241 Standard of care will comprise annual praziquantel treatment. Our own results from the Lake

56
57 242 Victoria Island Intervention Study on Worms and Allergy-related diseases (LaVIISWA),²⁸ which

58
59 243 compared annual versus quarterly intervention for schistosomiasis at community level over three
60

1
2
3 244 years, showed no advantage of quarterly treatment for morbidity outcomes attributed to
4
5 245 schistosomiasis.

6
7 246 Schistosomiasis can cause anaemia. To manage the expected differential benefits of the
8
9 247 interventions for anaemia, a full blood count will be performed at baseline, as discussed above;
10
11 248 anaemic children will be managed appropriately and severely anaemic children excluded.
12
13 249 Albendazole will be provided twice a year to manage nematode infections (after collection of
14
15 250 primary and secondary endpoint samples).

16 251 ***Procedures to be followed in the event of abnormal findings***

17
18 252 Abnormal clinical findings from medical history, examination or blood tests will be assessed as to
19
20 253 their clinical significance throughout the trials. If an abnormal test result is deemed clinically
21
22 254 significant, it may be repeated. If a test remains clinically significant, the participant will be informed
23
24 255 and appropriate medical care arranged as appropriate and with the permission of the participant.
25
26 256 Specific details regarding findings, discussion with participants and resulting actions will be recorded
27
28 257 in the clinical records. Decisions to exclude the participant from enrolling in the trial or to withdraw
29
30 258 a participant from the trial will be at the discretion of the Investigator.

31 259 ***Data and Safety Monitoring Board (DSMB)***

32
33 260 A data and safety monitoring board (DSMB) has been appointed to provide real-time safety
34
35 261 oversight. The DSMB will be notified within 7 days of the Investigators' being aware of the
36
37 262 occurrence of SAEs. The DSMB may recommend the Investigators to place the trial on hold if
38
39 263 deemed necessary following an intervention-related SAE. The DSMB will be chaired by a clinician
40
41 264 experienced in clinical trials. There will be a minimum of two other appropriately qualified
42
43 265 committee members. In the case of events related to a blinded intervention, the DSMB can request
44
45 266 unblinding. Membership will include a statistician, and at least one Ugandan member. All
46
47 267 correspondence between Investigators and the DSMB will be conveyed by the Principal Investigator
48
49 268 to the trial Sponsor. The Chair of the DSMB will be contacted for advice and independent review by
50
51 269 the Investigator or trial Sponsor in the following situations:

- 52
53
54
55
56
57
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59
60
- 270 • The occurrence of any SAE
 - 271 • Any other situation where the Investigator or trial Sponsor feels independent advice or
272 review is important

273 ***Ethical and regulatory considerations***

274 *Further information regarding risks*

1
2
3 275 The immunisations to be given have recognised side effects which are usually mild and resolve
4
5 276 spontaneously in a few days to one week. Parenteral vaccines are likely to result in pain and
6
7 277 swelling at the site of injection and mild fever; very occasionally pain and swelling can be severe and
8
9 278 associated with difficulty in moving the shoulder. Sometimes headache and tiredness occur. Rarely
10
11 279 a vaccine may cause a severe allergic reaction. For most vaccines this is estimated at less than one
12
13 280 in a million doses (but 1 in 55,000 for Yellow Fever vaccine).²⁹ Individuals with a history of a
14
15 281 possible allergic reaction to drugs or vaccines, or to vaccine components including eggs or chicken
16
17 282 proteins, will be excluded from the studies. The research team will be trained and prepared to
18
19 283 manage severe allergic reactions.

20
21 284 Adverse reactions to Yellow Fever vaccine include severe nervous system reaction (about 1 person in
22
23 285 125,000) and severe, life-threatening illness with organ failure (about 1 person in 250,000). The
24
25 286 mortality for this severe, life-threatening adverse effect is reported as about 50%.²⁹

26
27 287 BCG immunisation is likely to induce a scar in many cases. This may develop over several weeks,
28
29 288 starting as a small papule at the injection site which may become ulcerated and then heal over a
30
31 289 period of 2 to 5 months; and lymphadenopathy may develop. Occasionally a more severe local
32
33 290 reaction occurs (estimated at 1 per 1,000-10,000 doses): for example, an abscess develops and scars
34
35 291 may develop into keloids. Rarely BCG can cause disseminated disease (1 per 230,000 to 640,000
36
37 292 doses), or disease in sites remote from the immunisation site. Disseminated BCG disease usually
38
39 293 occurs in immunocompromised people: HIV positive people will be excluded from these studies.³⁰
40
41 294 BCG "pre-immunisation" may interfere with the response to the subsequent live vaccines; indeed
42
43 295 our hypothesis, and published results, suggest that it may suppress replication of YF 17D vaccine.³¹
44
45 296 However, this reduced replication has not been shown to correlate with, or result in, reduced levels
46
47 297 of neutralising antibody titres (which are the desired protective outcome).^{13 31}

48
49 298 Oral typhoid vaccine (Ty21a) may occasionally be associated with stomach pain, nausea, vomiting
50
51 299 and (rarely) rash.²⁹

52
53 300 Praziquantel has been in use for about 30 years. It has a well-recognised profile of side effects
54
55 301 including dizziness, nausea, vomiting, abdominal pain, diarrhoea (sometimes with blood) and
56
57 302 urticarial rash. The symptoms are considered to arise largely from the effects of killing worms and to
58
59 303 be more severe in people with heavy infections. Symptoms are better tolerated when the drugs are
60
304 given after food and we will provide treatment after a meal or snack. Simple medications, such as
305 paracetamol and cetirizine, can alleviate symptoms and these will be available on treatment days.

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MRC/UVRI and LSHTM Uganda Research Unit



Population differences in vaccine responses: the effect of intensive treatment for schistosomiasis on response to vaccines among island adolescents

Short Title: POPVAC Project A

Information Sheet for participants 9 – 12 years

1. Why are we meeting with you?

We are inviting you to take part in a research which is being done by a team from Uganda Virus Research Institute (UVRI), working together with colleagues from the London School of Hygiene and Tropical Medicine, UK. We are meeting you because you are aged between 9 and 12 years and study in a primary school on Koome island. After we tell you about it, we will ask if you'd like to be in this study or not. Only If you agree to take part, will you sign the assent form to show us that you are happy to do so.

The people leading this research study are Professor Alison Elliott and Dr Ludoviko Zirimenya.

2. Why are we doing this research?

We want to **find out whether treating Bilharzia much more frequently (a total of 7 times in a year) can get rid of it completely and whether this will improve vaccine responses.**

In the whole study, there will be about 480 children from schools of Koome islands.

3. What is going to happen in this research study?

Only if you agree, a full health check-up that will involve taking stool, urine and blood samples will be done. If everything is okay, the following will be done:

You will be put into one of the two **Bilharzia treatment** groups by chance

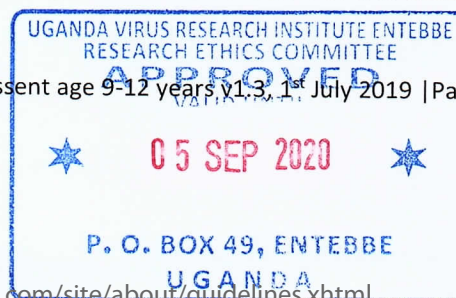
- One group will be treated for Bilharzia using praziquantel (the recommended medicine for Bilharzia) seven times in the year, and an eighth time after the end of the study.
- The other group will be treated for Bilharzia using praziquantel once in the year, and a second time after the end of the study.

You will be immunised with **five (5) vaccines.** These are:

- BCG vaccine. This is intended to protect against tuberculosis.
- Yellow fever vaccine. This protects against Yellow Fever an infection carried by mosquitoes.
- HPV vaccine. Human Papilloma Virus (HPV) causes growths in private parts and cancer in girls and boys.
- Tetanus and diphtheria vaccines. Tetanus and diphtheria are bacterial infections that cause very serious disease.
- Typhoid vaccine. Typhoid is a serious form of fever which is spread through contaminated food.

These immunisations will be given on different times during the entire duration of the study.

POPVAC Project A, assent age 9-12 years v1.3, 1st July 2019 | Page 1 of 5





Most of the vaccines will be injected into your upper arm (either right or left side). The typhoid vaccine is an oral vaccine: it is in the form of capsules which are swallowed. You will be given one capsule per day for three alternating days.

You will be asked to give **blood samples**

We will ask you to give blood samples before and after you are given the vaccines. The amount of blood that we will take at each visit is completely safe and will vary between 4 and 27 millilitres (1 teaspoon and 5 and a half teaspoons) at the different visits.

You will be asked to give **stool and urine samples**

At some visits we will ask you for additional stool and urine samples for testing to check whether the worm infections have responded to treatment or (for girls) whether you have become pregnant.

Some of you will be asked to take part in a special **sugar test** of absorption from the intestines

We think that worm infections may make our intestines a little bit leaky, and this might explain some of their effects. We can test this using a special sugar drink. We will ask about 200 people to do this additional test. If you are asked to do this, it will be done about 8 weeks after your first vaccine is given. You will be asked not to eat overnight or in the morning of the test day. Then you will be given a special drink that contains sugars early in the morning. Afterwards you will also be asked to drink a litre of water (equal to two Rwenzori bottles) over the following few hours. All the urine that you pass during the next five hours will be collected. The volume (amount) of urine will be measured and the amount of sugar in it will be tested, to find out how much of the sugar your body has absorbed.

4. What will the blood, stool and urine samples be used for?

The samples will be used to test for infections, including HIV, malaria and worms, to test for anaemia (the strength of your blood) and to test for pregnancy (among girls). The blood samples will also be used to test how your body responds to vaccines. For some participants, the sugar test will be used to test absorption from the intestines.

5. Will taking part in this study harm me?

We do not expect this to harm you, though you will experience the following:

- You will need to take time off classes during each visit by the study team.
- Taking blood samples is not expected to cause any problem for you, apart from the discomfort or pain, bruising or bleeding, redness at the place where the needle goes into the skin, but this will go away in a few days.
- During the sugar test, you will be asked not to eat overnight and during the morning of the test so you may get hungry (although water and a snack will be provided). This may make you feel a little sick and stools may become a bit loose.
- If you are a girl, even if you know you could not be pregnant, we will do a pregnancy test. We have to be quite sure. You must as well promise that you can avoid getting pregnant while taking part in this study.

POPVAC Project A - assent age 9-12 years v1.3, 1st July 2019 | Page 2 of 5



MRC/UVRI and LSHTM Uganda Research Unit



- Treatment of Bilharzia with praziquantel may make you feel dizzy or sick, give you abdominal pain or diarrhoea, or occasionally cause an itchy rash. The research team will have medicines available to help you if you have a strong reaction to the treatment.
- All the study vaccines are known to be safe. However, even approved vaccines may very occasionally cause a serious reaction, such as an allergic reaction. The research doctors and nurses will be available to help if this happens.

6. Will the study help me?

The treatment for Bilharzia is likely to be good for you, whichever group you are put in. The vaccines are likely to help you by protecting you from infectious diseases. The research results may help all people in the world, because in the end we may get a better understanding whether treating Bilharzia much more frequently (a total of 7 times in a year) can get rid of it completely and whether this will improve vaccine responses.

7. What is the cost of taking part in the trial?

There is no cost to participate in this trial. You will receive a soft drink and the gift of a pen or other simple school material on days when blood samples are drawn, and the gift of a T-shirt at the end of the study when everything is done, in appreciation of your contribution to the work.

8. What happens if I refuse to take part?

It is very important for you to know that you do not have to take part in the research, the choice is yours. No-one will be upset if you decide not to take part. If you agree to take part and later decide that you do not want to take part anymore, that is also okay.

9. What happens if something goes wrong?

The researchers will make every effort to ensure your safety and well-being. MRC/UVRI and LSHTM has a specialist insurance policy in place which would help to pay for treatment if any harm came to you as a result of taking part in the research.

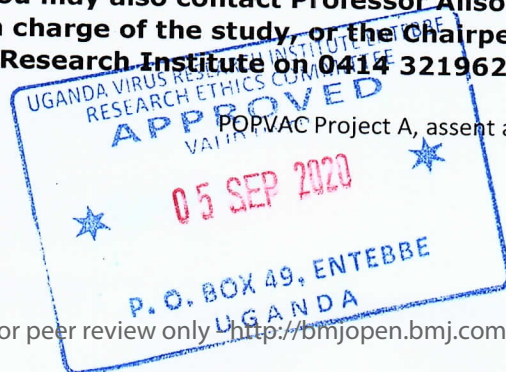
10. Who will have access to your information and samples from this research?

Only research staff trained to keep the information confidential will have access to the records. Your name will be removed from the records and samples, so no-one will be able to find out information about you from our records.

11. Who has reviewed the trial?

This trial has been reviewed by the Uganda National Council for Science and Technology, The Uganda National Drug Authority, the Uganda Virus Research Institute Research and Ethics Committee the ethics committee of the London School of Hygiene and Tropical Medicine in the United Kingdom.

You can find out more about the study at any time by asking one of the members of the research team. You may also contact Professor Alison Elliott (telephone: 0417 704000) who is in charge of the study, or the Chairperson of the Ethics Committee for the Uganda Virus Research Institute on 0414 321962.



POPVAC Project A, assent age 9-12 years v1.3, 1st July 2019 | Page 3 of 5

PVA ID: |A|_|_|_|_|

Witness:

I have read the participant information sheet and the assent statement above

to:..... (PRINT NAME OF PARTICIPANT) in a language which he/she understands. I believe that he/she gives assent to take part in the study.

.....
Witness name

.....
Signature

.....
Date

Witness required only for those using a thumb print instead of the final signature, or unable to read the information and consent form, or if the person taking consent does not speak the participant's language. The witness must not be a member of the research staff or a study participant. The witness must be present for the whole consent process.

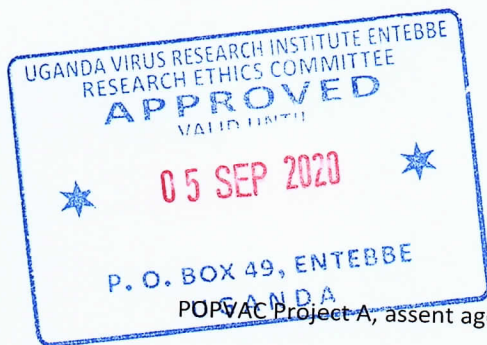
Person taking the assent:

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Researcher name

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Signature

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Date

Comment:



POPVAC Project A, assent age 9-12 years v1.3, 1st July 2019 | Page 5 of 5

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Population differences in vaccine responses: the effect of intensive treatment for schistosomiasis on response to vaccines among island adolescents

Short Title: POPVAC Project A

Information Sheet for participants aged 13 to 17 years

We are inviting you to join in some research which is being done by a team from Uganda Virus Research Institute (UVRI), working together with colleagues from the London School of Hygiene and Tropical Medicine, UK. This form will tell you about the research. If you have any questions, please ask the person who is telling you about this research. Then you can decide whether you want to take part or not. There is no need to take part unless you really wish to. If you agree to take part, you will need to sign the assent form to show us that you are happy to do so. If you decide you do not want to take part, do not sign the assent form.

The people leading this research study are Professor Alison Elliott and Dr Ludoviko Zirimenya.

1. What this research is about, and the reason for doing this research.

This research is about how worm infections like Bilharzia "switch off" the body's defence systems and how this affects vaccine responses. **We want to find out whether treating Bilharzia much more frequently (a total of 7 times in a year) can get rid of it completely and whether this will improve vaccine responses.**

2. Why have I been asked to take part in this study?

You have been asked because you are attending primary school in Koome islands.

3. What is going to happen in this research study?

Only if you agree, a comprehensive health check-up that will involve taking stool, urine and blood samples will be done. If everything is okay, the following will be done:

You will be put into one of the two **Bilharzia treatment** groups by luck

- One group will be treated for Bilharzia using praziquantel (the recommended medicine for Bilharzia) seven times in the year, and an eighth time after the end of the study.
- The other group will be treated for Bilharzia using praziquantel once in the year, and a second time after the end of the study.

You will be immunised with **several vaccines**. These are the vaccines:

- BCG vaccine. This is intended to protect against tuberculosis.
- Yellow fever vaccine. This protects against Yellow Fever virus carried by mosquitoes.
- HPV vaccine. Human Papilloma Virus (HPV) causes growths in private parts and cancer in girls and boys.
- Tetanus and diphtheria vaccines. Tetanus and diphtheria are bacterial infections that cause very serious disease.
- Typhoid vaccine. Typhoid is a serious form of fever which is spread through contaminated food.

These immunisations will be given on three different days

POPVAC Project A, assent age 13-17 years v1.3, 15 July 2019 | Page 1 of 6





Most of the vaccines will be injected into your upper arm (either right or left side). This will be a bit painful, as for any injection of medicine that you may have had, and will feel a bit sore over the next week or so. The typhoid vaccine is an oral vaccine: it is in the form of capsules which are swallowed. You will be given one capsule per day for three alternating days.

You will be asked to give **blood samples**

We will ask you to give blood samples before and after you are given the vaccines. The amount of blood that we will take at each visit is completely safe and will vary between 4 and 27 millilitres (1 teaspoon and 5 and a half teaspoons) at the different visits

You will be asked to give **stool and urine samples**

At some visits we will ask you for additional stool and urine samples for testing to check whether the worm infections have responded to treatment or (for girls) whether you have become pregnant.

Some of you will be asked to take part in a special **sugar test** of absorption from the intestines

We think that worm infections may make our intestines a little bit leaky, and this might explain some of their effects. We can test this using a special sugar drink. We will ask about 200 people to do this additional test. If you are asked to do this, it will be done about 8 weeks after your first vaccine is given. You will be asked not to eat overnight or in the morning of the test day. Then you will be given a special drink that contains sugars early in the morning. Afterwards you will also be asked to drink a litre of water (equal to two Rwenzori bottles) over the following few hours. All the urine that you pass during the next five hours will be collected. The volume (amount) of urine will be measured and the amount of sugar in it will be tested, to find out how much of the sugar your body has absorbed.

4. What will the blood, stool and urine samples be used for?

The samples will be used to test for infections, including HIV, malaria and worms, to test for anaemia (the strength of your blood) and to test for pregnancy (among girls). The blood samples will also be used to test how your body responds to vaccines. For some participants, the sugar test will be used to test absorption from the intestines.

5. How many people will take part in the study, and how long will it last?

The whole study will enrol 480 people, and the study will last for about two years.

6. What are the risks of participating in this trial?

You will need to take time off classes during each visit by the study team.

Taking blood samples is not expected to cause any problem for you, apart from the discomfort or pain, bruising or bleeding, redness at the place where the needle goes into the skin, but this will go away in a few days.

During the sugar test you will be asked not to eat overnight and during the morning of the test so you may get hungry (although water and a snack will be provided). Most people have no problems with this test although a few may feel a little sick and stools may become a bit loose.

POPVAC Project A, assent age 13-17 years v1.3 | 1st July 2019 | Page 2 of 6



MRC/UVRI and LSHTM Uganda Research Unit



If you are a girl, even if you know you could not be pregnant. We have to be quite sure. We will test for pregnancy at the beginning of the study and on each immunisation day. You must as well promise that you can avoid getting pregnant while taking part in this study.

Treatment of Bilharzia with praziquantel may make you feel dizzy or sick, give you abdominal pain or diarrhoea, or occasionally cause an itchy rash. This is most likely caused by your body's response to the worms as they are being killed by the medicine. The research team will have medicines available to help you if you have a strong reaction to the treatment.

All the study vaccines are known to be safe though not often, may cause a serious reaction, such as an allergic reaction. The research doctors and nurses will be available to help if this happens.

7. What are the benefits of taking part in this trial?

The treatment for Bilharzia is likely to be good for you, whichever group you are put in. The vaccines are likely to help you by protecting you from infectious diseases.

Also, you will be helping us to find out why vaccines sometimes don't work so well in countries like Uganda, and whether vaccines work better if worms are treated first. This may help other people in the future.

8. What is the cost of taking part in the trial?

There is no cost to participate in this trial. You will receive a soft drink and the gift of a pen or other simple school material on days when blood samples are drawn, and the gift of a T-shirt at the end of the study when everything is done, in appreciation of your contribution to the work.

9. What happens if I refuse to take part?

It is very important for you to know that you do not have to take part in the research, the choice is yours. No-one will be upset if you decide not to take part. If you agree to take part and later decide that you do not want to take part anymore, that is also okay.

10. What happens if something goes wrong?

You will be making an important contribution to medical research. The researchers will make every effort to ensure your safety and well-being. MRC/UVRI and LSHTM has a specialist insurance policy in place which would help to pay for treatment if any harm came to you as a result of taking part in the research.

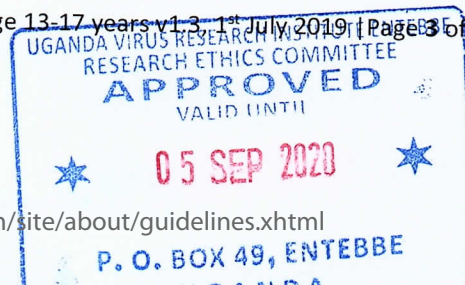
11. Who will have access to my information and samples from this research?

All our research records are stored securely in rooms with restricted access and on password protected computers. Only research staff trained to keep the information confidential will have access to the records. Your name will be removed from the records, so no-one will be able to find out information about you from our records.

12. Who has reviewed the trial?

This trial has been reviewed by the Uganda National Council for Science and Technology, Uganda National Drug Authority the Uganda Virus Research Institute Research and Ethics Committee the ethics committee of the London School of Hygiene and Tropical Medicine in the United Kingdom.

POPVAC Project A, assent age 13-17 years v1.3 - 1st JULY 2019 | Page 3 of 6





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You can find out more about the study at any time by asking one of the members of the research team. You may also contact Professor Alison Elliott (telephone: 0417 704000) who is in charge of the study, or the Chairperson of the Ethics Committee for the Uganda Virus Research Institute on 0414 321962.

POPVAC Project A, assent age 13-17 years v1.3, 1st July 2019 | Page 4 of 6



MRC/UVRI and LSHTM Uganda Research Unit



Uganda Virus Research Institute

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Population differences in vaccine responses: the effect of intensive treatment for schistosomiasis on response to vaccines among island adolescents

Short Title: POPVAC Project A

Participant Assent

A copy of this form will be given to you. Please keep a copy of the form because it contains the information that was discussed with you and you may want to look at it again.

AGREEMENT TO TAKE PART:

I have read and understood (or been read to and understand) the information sheet for this study. My questions have been answered. I understand that taking part in the study is voluntary. I understand that at any time I may withdraw from this study without giving a reason. I agree to participate in this study.

Name: _____ PVA ID: |A|_|_|_|_|_|_|_|_|_|_|
(please write your name in capital letters here if you agree)

Signature: _____ Date: _____
(please sign or write your name here if you agree; or use a thumbprint)

What if I have any questions?

If you have any questions about your participation in this study, please feel free to ask any member of the research team at any time. If you prefer, you may speak to the principal investigator for this study (Professor Alison Elliott, telephone 0417 704000).

What if we want to ask someone independent anything about this research, or have any questions about your rights as a research participant? You may speak with the Chairman of the Science and Ethics Committee at Uganda Virus Research Institute on 0414 321962.



PVA ID: |A|_|_|_|_|

Witness:

I have read the participant information sheet and the assent statement above

to:..... (PRINT NAME OF PARTICIPANT) in a language which he/she understands. I believe that he/she gives assent to take part in the study.

.....
Witness name	Signature	Date

Witness required only for those using a thumb print instead of the final signature, or unable to read the information and consent form, or if the person taking consent does not speak the participant's language. The witness must not be a member of the research staff or a study participant. The witness must be present for the whole consent process.

Person taking the assent:

.....
Researcher name	Signature	Date

Comment:



MRC/UVRI and LSHTM Uganda Research Unit



Population differences in vaccine responses: the effect of intensive treatment for schistosomiasis on response to vaccines among island adolescents

Short Title: POPVAC Project A

Information Sheet for emancipated or mature minors

We are inviting you to join in some research which is being done by a team from Uganda Virus Research Institute (UVRI), working together with colleagues from the London School of Hygiene and Tropical Medicine, UK. This form will tell you about the research. If you have any questions, please ask the person who is telling you about this research. Then you can decide whether you want to take part or not. If you agree to take part, you will need to sign the consent form to show us that you are happy to do so. If you decide that you do not want to take part, do not sign the consent form.

The people leading this research study are Professor Alison Elliott and Dr Ludoviko Zirimenya.

1. What is this research about?

This research is about how worm infections like Bilharzia “switch off” the body’s defence systems and how this affects vaccine responses. **We want to find out whether treating Bilharzia much more frequently (a total of 7 times in a year) can get rid of it completely and whether this will improve vaccine responses.**

2. Why have I been asked to take part in this study?

You have been asked because you are attending primary school in Koome islands.

3. What is going to happen in this research study?

Only if you agree, you will have a comprehensive health check-up that will involve taking stool, urine and blood samples. If everything is okay, the following will be done:

You will be put into one of the two Bilharzia treatment groups by chance

One group will be treated for Bilharzia using praziquantel (the recommended medicine for Bilharzia) seven times in the year, and an eighth time after the end of the study.

The other group will be treated for Bilharzia using praziquantel once in the year, and a second time after the end of the study.



POPVAC Project A, consent for emancipated minors v1.1, 1st July 2019 | Page 1 of 6

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You will be immunised with **five (5) vaccines**. These are:

- **BCG vaccine.** This is intended to protect against tuberculosis.
- **Yellow fever vaccine.** This protects against Yellow Fever an infection carried by mosquitoes.
- **HPV vaccine.** Human Papilloma Virus (HPV) causes growths in private parts and cancer in girls and boys.
- **Tetanus and diphtheria vaccines.** Tetanus and diphtheria are bacterial infections that cause very serious disease.
- **Typhoid vaccine.** Typhoid is a serious form of fever which is spread through contaminated food.

These immunisations will be given on different times during the entire duration of the study.

Most of the vaccines will be injected into your upper arm (either right or left side). The typhoid vaccine is an oral vaccine: it is in the form of capsules which are swallowed. You will be given one capsule per day for three alternating days.

You will be asked to give **blood samples**

We will ask you to give blood samples before and after you are given the vaccines. The amount of blood that we will take at each visit is completely safe and will vary between 4 and 27 millilitres (1 teaspoon and 5 and a half teaspoons) at the different visits.

You will be asked to give **stool and urine samples**

At some visits we will ask you for additional stool and urine samples for testing to check whether the worm infections have responded to treatment or (for girls) whether you have become pregnant.

Some of you will be asked to take part in a special **sugar test** of absorption from the intestines

We think that worm infections may make our intestines a little bit leaky, and this might explain some of their effects. We can test this using a special sugar drink. We will ask about 200 people to do this additional test. If you are asked to do this, it will be done about 8 weeks after your first vaccine is given. You will be asked not to eat overnight or in the morning of the test day. Then you will be given a special drink that contains sugars early in the morning. Afterwards you will also be asked to drink a litre of water (equal to two Rwenzori bottles) over the following few hours. All the urine that you pass during the next five hours will be collected. The volume (amount) of urine will be measured and the amount of sugar in it will be tested, to find out how much of the sugar your body has absorbed.

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HYGIENE
& TROPICAL
MEDICINE



4. What will the blood, stool and urine samples be used for?

The samples will be used to test for infections, including HIV, malaria and worms, to test for anaemia (the strength of your blood) and to test for pregnancy (among girls). The blood samples will also be used to test how your body responds to vaccines.

5. How many people will take part in the study, and how long will it last?

The whole study will enrol 480 people, and the study will last for about two years.

6. What are the risks of participating in this trial?

- You will need to take time off classes during each visit by the study team.
- Taking blood samples is not expected to cause any problem for you, apart from the discomfort or pain, bruising or bleeding, redness at the place where the needle goes into the skin, but this will go away in a few days.
- During the sugar test, you will be asked not to eat overnight and during the morning of the test so you may get hungry (although water and a snack will be provided). This may make you feel a little sick and stools may become a bit loose.
- If you are a girl, even if you know you could not be pregnant, we will do a pregnancy test. We have to be quite sure. You must as well promise that you can avoid getting pregnant while taking part in this study.
- Treatment of Bilharzia with praziquantel may make you feel dizzy or sick, give you abdominal pain or diarrhoea, or occasionally cause an itchy rash. The research team will have medicines available to help you if you have a strong reaction to the treatment.
- All the study vaccines are known to be safe. However, very occasionally even approved vaccines may cause a serious reaction, such as an allergic reaction. The research doctors and nurses will be available to help if this happens.

7. What are the benefits of taking part in this trial?

The treatment for Bilharzia is likely to be good for you, whichever group you are put in. The vaccines are likely to help you by protecting you from infectious diseases.

Also, you will be helping us to find out why vaccines sometimes don't work so well in countries like Uganda, and whether vaccines work better if worms are treated first. This may help other people in the future.



POPVAC Project A, consent for emancipated minors v1.1, 1st July 2019 | Page 3 of 6



8. What is the cost of taking part in the trial?

There is no cost to participate in this trial. You will receive a soft drink and the gift of a pen or other simple school material on days when blood samples are drawn, and the gift of a T-shirt at the end of the study when everything is done, in appreciation of your contribution to the work.

9. What happens if I refuse to take part?

It is very important for you to know that you do not have to take part in the research, the choice is yours. No-one will be upset if you decide not to take part. If you agree to take part and later decide that you do not want to take part anymore, that is also okay.

10. What happens if something goes wrong?

The researchers will make every effort to ensure your safety and well-being. MRC/UVRI and LSHTM has a specialist insurance policy in place which would help to pay for treatment if any harm came to you as a result of taking part in the research.

11. Who will have access to my information and samples from this research?

All our research records and samples are stored securely in rooms with restricted access and on password protected computers. Only research staff trained to keep the information confidential will have access to the them. Your name will be removed from the records, so no-one will be able to find out information about you from our records.

12. Who has reviewed the trial?

This trial has been reviewed by the Uganda National Council for Science and Technology, The Uganda National Drug Authority, the Uganda Virus Research Institute Research and Ethics Committee the ethics committee of the London School of Hygiene and Tropical Medicine in the United Kingdom.

You can find out more about the study at any time by asking one of the members of the research team. You may also contact Professor Alison Elliott (telephone: 0417 704000) who is in charge of the study, or the Chairperson of the Ethics Committee for the Uganda Virus Research Institute on 0414 321962.



PVA ID | A | ___ | ___ | ___ | ___ |

Witness:

I have read the participant information sheet and the consent statement above

to:..... (PRINT NAME OF PARTICIPANT) in a language which he/she understands. I believe that he/she gives consent to take part in the study.

.....
Witness name	Signature	Date

Witness required only for those using a thumb print instead of the final signature, or unable to read the information and consent form, or if the person taking consent does not speak the participant's language. The witness must not be a member of the research staff or a study participant. The witness must be present for the whole consent process.

Person taking the consent:

.....
Researcher name	Signature	Date

Comment:



MRC/UVRI and LSHTM Uganda Research Unit



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Population differences in vaccine responses: the effect of intensive treatment for schistosomiasis on response to vaccines among island adolescents

Short Title: POPVAC Project A

Information Sheet for parents/guardians

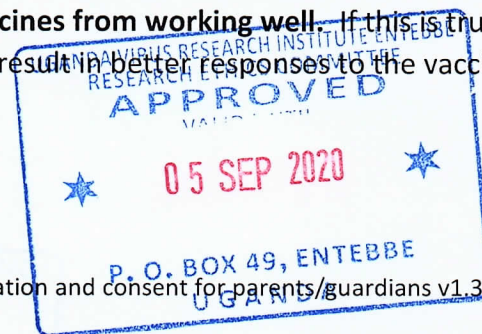
We are inviting your child to join in some research which is being done by a team from Uganda Virus Research Institute (UVRI), working together with colleagues from the London School of Hygiene and Tropical Medicine, UK. This form will tell you about the research. If you have any questions, please ask the person who is telling you about this research. Then you can decide whether you want your child to take part or not. There is no need for your child to take part unless you really want him/her to do so. If you agree for your child to take part, you will need to sign the consent form to show us that you are happy for him/her to do so. If you decide that you do not want your child to take part, do not sign the consent form.

The people leading this research study are Professor Alison Elliott and Dr Ludoviko Zirimenya.

1. What this research is about, and the reason for doing this research?

Vaccines are a very important tool for preventing infectious diseases. They have saved many lives. Vaccines are usually made from a weakened or killed strain of the bacteria or viruses that cause infectious diseases, or from a part of the bacteria or viruses. Vaccines are designed to help our body's defence system to recognise infectious diseases before we actually meet them, so that we can defeat them more easily. **However, some vaccines seem to work less well in hot countries, near the equator (such as Uganda), than in cooler countries (such as the United Kingdom, the UK).** We want to find out why this is so.

Worm infections are much more common in warm countries (such as Uganda) than in cooler countries (such as the UK). Bilharzia (schistosomiasis) is a worm infection which is very common in Koome islands. Almost everyone in Koome has Bilharzia. Worms can live in our bodies for many years. To do this they have to be able to switch off some of our body's defence systems so that they will not be killed. **We think that this "switching off" of defence systems may also prevent some vaccines from working well.** If this is true then treating worms before giving vaccines might result in better responses to the vaccines.



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1
2
3 Light infections with Bilharzia may not be noticed but heavy infections can cause serious
4 damage, especially to the liver. That is why the Ugandan government usually gives treatment
5 for Bilharzia once a year in Ugandan schools. This usually reduces the number of Bilharzia
6 worms but does not get rid of them. **We want to find out whether treating Bilharzia much
7 more frequently (a total of 7 times in a year) can get rid of it completely and whether this will
8 improve vaccine responses.**
9
10

11 **2. Why has my child been asked to take part in this study?**

12
13 Your child has been asked because he/she is attending primary school in Koome islands, in
14 primary 1 to 6. Bilharzia infection is often heaviest in people of primary school age. Also, the
15 government of Uganda offers vaccines to your child's age group that will help to protect them
16 from infectious diseases later on. So it is important to know whether Bilharzia can affect these
17 vaccines, and whether treating Bilharzia effectively is helpful.
18
19

20 **3. What is going to happen in this research study?**

21 Your child will have a health check-up

22
23 If you want your child to take part in this study, we will first check your child's health, and take
24 stool samples, urine samples and some blood from a vein in your child's arm and do some tests.
25 These will include tests for infections including HIV, malaria and worms, tests for anaemia (the
26 strength of blood in your child's body), and tests for pregnancy if your child is a girl.
27
28

29
30 If everything is okay then we will enrol your child in the study. If something is not okay then we
31 will either give your child the treatment that he/she needs, or tell you what to do.
32

33 Your child will be put into one of the two **Bilharzia treatment** groups

34
35 When your child is enrolled in the study we will put your child into one of two groups.
36

- 37 • One group will be treated for Bilharzia using praziquantel (the recommended medicine
38 for Bilharzia) seven times in the year, and an eighth time after the end of the study.
- 39 • The other group will be treated for Bilharzia using praziquantel once in the year, and a
40 second time after the end of the study.
41
42

43 The choice of groups will be done using a code generated by computer. This works so that your
44 child is put into one group or the other by luck – this is like a lottery.
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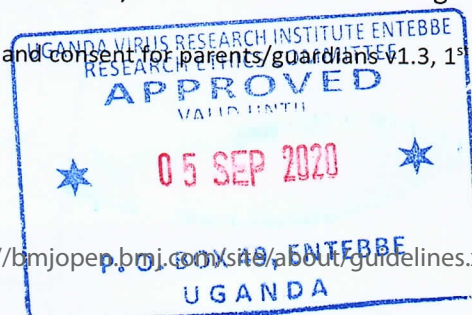
Your child will be immunised with **several vaccines**. These are the vaccines:

- BCG vaccine. This is intended to protect against tuberculosis. Tuberculosis is very common in Uganda. Many people receive the BCG vaccine as a baby. BCG can be given again at school age and may help to protect against tuberculosis later on although the level of protection varies a lot between countries. We do not know how well it works in school children in Uganda.
- Yellow fever vaccine. This protects against Yellow Fever. Yellow fever is a virus carried by mosquitoes. Yellow fever disease affects the liver and causes fever and jaundice (yellow eyes). Outbreaks occur in Uganda and neighbouring countries from time to time – an outbreak is when a group of people falls sick around the same time.
- HPV vaccine. Human Papilloma Virus (HPV) causes warts. As well, some strains of HPV can cause cancer in the genital areas, especially on the cervix (the opening of the womb) in girls. HPV can also cause cancer of the penis in boys, and other cancers. The HPV vaccine reduces the risk of infection with dangerous HPV strains that cause cancer. In this way it reduces the risk of cancers. The Ugandan government recently started offering this vaccine to girls in primary 4. We will give this vaccine to you if you are a girl (in any class) and you have not received it already. We will also give this vaccine to boys because it can protect them against some cancers too.
- Tetanus and diphtheria vaccines. Tetanus and diphtheria are bacterial infections. The tetanus and diphtheria bacteria produce chemicals called toxins which cause diseases. Tetanus bacteria infect deep wounds and produce tetanus toxin which causes very serious muscle spasms. Tetanus can affect young babies if the wound where the umbilical cord is cut becomes infected. Immunising young women can protect their future babies too. Diphtheria causes a very serious throat infection. Tetanus and diphtheria immunisation is given to babies but booster immunisation is recommended by the Uganda government for school children also.
- Typhoid vaccine. Typhoid is a serious form of fever which is spread through contaminated food. Outbreaks occur in Uganda. Immunisation against typhoid can prevent this illness.

These immunisations will be given at three different time points. BCG will be given first. HPV vaccine, oral typhoid vaccine and yellow fever vaccine will be given four weeks later. A second dose of HPV vaccine and first dose of Tetanus/diphtheria vaccine will be given at 28 weeks after the BCG immunisation.

Most of the vaccines will be injected into your child's upper arm (either right or left). This will be a bit painful, as for any injection of medicine that you may have had, and will feel a bit sore over the next week or so. The BCG vaccine is likely to form a small swelling and then an ulcer

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1
2
3 which may take quite some time (perhaps two to five months) to heal. The typhoid vaccine is
4 an oral vaccine: it is in the form of capsules which are swallowed. Your child will be given one
5 capsule per day for three alternating days.
6

7 Your child will be asked to give **blood samples**

8
9 To test your child's response to the vaccines we will ask your child to give blood samples before
10 and after they are given the vaccines. The amount of blood that we will take at each visit is
11 completely safe and will vary between 4 and 27 millilitres (1 teaspoon and 5 and a half
12 teaspoons) at the different visits. In total we will ask your child to give blood samples at 7
13 different times during the study. The study will last for about two years.
14

15 Your child will be asked to give **stool and urine samples**

16
17 At some visits we will ask your child for additional stool and urine samples for testing to check
18 whether the worm infections have responded to treatment or (for girls) whether your child has
19 become pregnant.
20

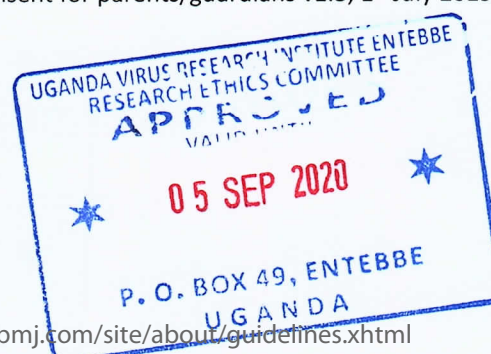
21 Some of the children will be asked to take part in a special **sugar test** of absorption from the 22 intestines

23
24 We think that worm infections may make our intestines a little bit leaky, and this might explain
25 some of their effects. We can test this using a special sugar drink. We will ask about 200
26 children to do this additional test. If your child is asked to do this, it will be done about 8 weeks
27 after the first vaccine is given. He/she will be asked not to eat overnight or in the morning of
28 the test day. Then he/she will be given a special drink that contains sugars early in the
29 morning. Afterwards he/she will also be asked to drink a litre of water (equal to two Rwenzori
30 bottles) over the following few hours. All the urine that he/she passes during the next five
31 hours will be collected. The volume (amount) of urine will be measured and the amount of
32 sugar in it will be tested, to find out how much of the sugar his/her body has absorbed.
33

34
35 **If you do not want your child to take part in the study, to have blood, urine and stool samples
36 taken or to receive the treatment and vaccines, you can say no to your child's taking part in
37 this study.**
38

39 **4. What will the blood, stool and urine samples be used for?**

40
41 The samples will be used to test for infections, including HIV, malaria and worms, to test for
42 anaemia (the strength of your child's blood) and to test for pregnancy (among girls). The blood
43 samples will also be used to test how your child's body responds to vaccines.
44



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7 **5. How many people will take part in the study, and how long will it last?**

8
9 The whole study will enrol 480 people, and the study will last for about two years.

10
11 **6. What are the risks of participating in this trial?**

12
13 Your child's time

14
15 Your child will need to take time off classes during each visit by the study team. There will be
16 12 visits altogether. Each visit will usually take about 30 minutes, but the first visit may take
17 about two hours to give time for everything to be explained, and for a thorough check-up. The
18 research team will work with teachers to avoid disturbing classes too much.

19
20
21 Blood samples

22
23 Taking blood samples is not expected to cause any problem for your child, apart from the
24 discomfort or pain, bruising or bleeding, redness at the place where the needle goes into the
25 skin, but this will go away in a few days. There is a very small chance that your child may get an
26 infection or some swelling at this place – this almost never happens. Some people faint when
27 their blood is taken. The person taking the blood will do all they can to prevent these things
28 from happening.

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30
31 Sugar test

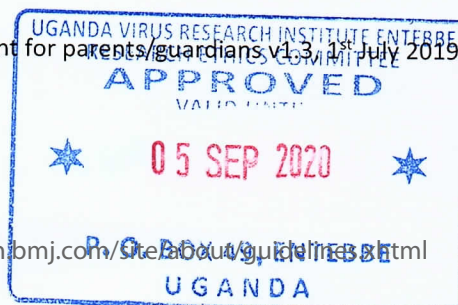
32
33 During the sugar test your child will be asked not to eat overnight and during the morning of
34 the test so he/she may get hungry (although water and a snack will be provided). Most people
35 have no problems with this test although a few may feel a little sick and stools may become a
36 bit loose.

37
38
39 Pregnancy

40
41 We do not know how some of these vaccines might affect a developing baby if they were given
42 to someone who was pregnant. That is why we will do a pregnancy test, if your child is a girl,
43 even if she knows she could not be pregnant. We have to be quite sure. We will test for
44 pregnancy at the beginning of the study and on each immunisation day. We will not enrol your
45 child in the study if she is pregnant at the start. We will not give your child the vaccines if she
46 falls pregnant later.

47
48
49 Bilharzia treatment

50
51 Treatment of Bilharzia with praziquantel may make your child feel dizzy or sick, give your child
52 abdominal pain or diarrhoea, or occasionally cause an itchy rash. This is most likely caused by



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3 the body's response to the worms as they are being killed by the medicine. The research team
4 will have medicines available to help your child if he/she has a strong reaction to the treatment.
5

6 Immunisations

7
8 All the vaccines that we will give have been used to protect very large numbers of people and
9 are known to be safe. They are not expected to cause any major problems for your child. As
10 mentioned, the BCG vaccine is expected to cause an ulcer and to heal slowly, leaving a scar. The
11 injections will cause some pain at the time of injection and your child's arm will feel a bit sore
12 for a day or two. Some people develop flu-like symptoms including headache and fever for a
13 day or two. It is fine for your child to take painkillers like paracetamol (Panadol) for these
14 symptoms. The typhoid vaccine, which your child will swallow as capsules, is not expected to
15 cause your child any problem at all, although your child may experience some stomach pain,
16 feeling sick, vomiting and (rarely) rash.
17
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19
20
21 Very occasionally any vaccine can cause a serious reaction, such as an allergic reaction. The
22 research doctors and nurses will be available to help if this happens.
23

24 **7. What are the benefits of taking part in this trial?**

25
26 The treatment for Bilharzia is likely to be good for your child, whichever group he or she is put
27 in. The vaccines are likely to help your child by protecting him/her from infectious diseases.
28

29
30 Also, you and your child will be helping us to find out why vaccines sometimes don't work so
31 well in countries like Uganda, and whether vaccines work better if worms are treated first. This
32 may help other people in the future.
33

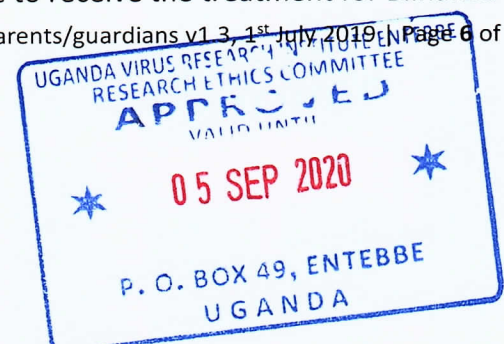
34 **8. What is the cost of taking part in the trial?**

35
36 There is no cost to participate in this trial. We will reimburse you (the parents) for the time you
37 spend at meetings at school, 20,000/= (twenty thousand shillings) for each visit. Your child will
38 receive a soft drink and the gift of a pen or other simple school material on days when blood
39 samples are drawn, and the gift of a T-shirt at the end of the study when everything is done, in
40 appreciation of his/her contribution to the work.
41
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44

45 **9. What happens if I refuse for my child to take part?**

46
47 It is very important for you to know that your child does not have to take part in the research,
48 the choice is yours and your child's. No-one will be upset if your child decides not to take part.
49 The teachers will not be upset and the research team will not be upset. If you agree for your
50 child to take part and later decide that you do not want him/her to take part anymore, that is
51 also okay. Whatever happens, your child will still be able to receive the treatment for Bilharzia
52

53 POPVAC Project A, information and consent for parents/guardians v1.3, 1st July 2019, Page 6 of 9



and the immunisations for HPV and tetanus and diphtheria when they are provided by the government, if you wish.

10. Who will be able to see the results of tests done on my child's samples?

The research team will keep your child's results private. Only members of the research team will be able to see results and to know that they belong to your child. Your child's samples will be given a special code so that anyone who is working on them in research laboratories will not know they came from your child.

11. What happens if something goes wrong?

You and your child will be making an important contribution to medical research. The researchers will make every effort to ensure your child's safety and well-being. MRC/UVRI and LSHTM has a specialist insurance policy in place which would help to pay for treatment if any harm came to your child as a result of taking part in the research.

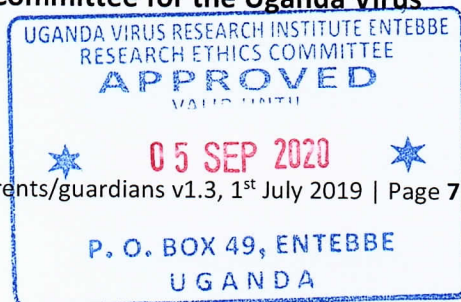
12. Who will have access to information from this research?

All our research records are stored securely in rooms with restricted access and on password protected computers. Only research staff trained to keep the information confidential will have access to the records. Your child's name will be removed from the records, so no-one will be able to find out information about your child from our records. The people who may review your child's records include Research Ethics Committees (Uganda Virus Research Institute Research Ethics Committee and the London School of Hygiene & Tropical Medicine Ethics Committee) the Uganda National Council Science and Technology, Study Monitors, Sponsor and the Uganda National Drug Authority. These organisations are there to ensure that your child's rights are protected and that the research is conducted properly and safely.

13. Who has reviewed the trial?

This trial has been reviewed by the Uganda National Council for Science and Technology, the Uganda Virus Research Institute Research and Ethics Committee, the ethics committee of the London School of Hygiene and Tropical Medicine in the United Kingdom. The Uganda National Drug Authority, which regulates the use of all medicines in Uganda, has granted permission to use the medicines and vaccines needed for this clinical trial.

You can find out more about the study at any time by asking one of the members of the research team. You may also contact Professor Alison Elliott (telephone: 0417 704000) who is in charge of the study, or the Chairperson of the Ethics Committee for the Uganda Virus Research Institute on 0414 321962.



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MRC/UVRI and LSHTM Uganda Research Unit



Population differences in vaccine responses: the effect of intensive treatment for schistosomiasis on response to vaccines among island adolescents

Short Title: POPVAC Project A

Parent/guardian consent

A copy of this form will be given to you. Please keep a copy of the form because it contains the information that was discussed with you and you may want to look at it again.

Participant (child) name: _____ **PVA ID|A|**____|____|____|____|
(please write your child's name in capital letters here if you agree)

AGREEMENT BY PARENT OR GUARDIAN:

I have read and/or been fully explained the information sheet concerning my child's participation in this study and I understand what will be required if he or she takes part. I understand that my child's participation is voluntary. My questions concerning this study have been answered. I understand that at any time, I may withdraw my child from this study without giving a reason and without affecting his or her entitlement to government health care. I agree for my child to take part in this study.

Name: _____
(please write your name in capital letters here if you agree)

Signature: _____ **Date:** _____
(please sign or write your name here if you agree; or use a thumbprint)

What if I have any questions?

If you have any questions about your participation in this study, please feel free to ask any member of the research team at any time. If you prefer, you may speak to the principal investigator for this study (Professor Alison Elliott, telephone 0417 704000).

What if we want to ask someone independent anything about this research, or have any questions about your rights as a research participant? You may speak with the Chairman of the Science and Ethics Committee at Uganda Virus Research Institute on 0414 321962.

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Reporting checklist for protocol of a clinical trial.

Based on the SPIRIT guidelines.

Instructions to authors

Complete this checklist by entering the page numbers from your manuscript where readers will find each of the items listed below.

Your article may not currently address all the items on the checklist. Please modify your text to include the missing information. If you are certain that an item does not apply, please write "n/a" and provide a short explanation.

Upload your completed checklist as an extra file when you submit to a journal.

In your methods section, say that you used the SPIRIT reporting guidelines, and cite them as:

Chan A-W, Tetzlaff JM, Altman DG, Laupacis A, Gøtzsche PC, Krleža-Jerić K, Hróbjartsson A, Mann H, Dickersin K, Berlin J, Doré C, Parulekar W, Summerskill W, Groves T, Schulz K, Sox H, Rockhold FW, Rennie D, Moher D. SPIRIT 2013 Statement: Defining standard protocol items for clinical trials. *Ann Intern Med.* 2013;158(3):200-207

	Reporting Item	Page Number
Administrative information		
Title	#1 Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	1
Trial registration	#2a Trial identifier and registry name. If not yet registered, name of intended registry	2
Trial registration: data set	#2b All items from the World Health Organization Trial Registration Data Set	n/a
Protocol version	#3 Date and version identifier	Information available at ISRCTN60517191
Funding	#4 Sources and types of financial, material, and other support	15
Roles and	#5a Names, affiliations, and roles of protocol	15

responsibilities: contributorship		contributors	
Roles and responsibilities: sponsor contact information	#5b	Name and contact information for the trial sponsor	Information available at ISRCTN60517191
Roles and responsibilities: sponsor and funder	#5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities	16
Roles and responsibilities: committees	#5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)	Information detailed in supplementary information file
Introduction			
Background and rationale	#6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention	4
Background and rationale: choice of comparators	#6b	Explanation for choice of comparators	8
Objectives	#7	Specific objectives or hypotheses	6
Trial design	#8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, non-inferiority, exploratory)	7

**Methods:
Participants,
interventions, and
outcomes**

1	Study setting	#9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained	7
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8	Eligibility criteria	#10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists)	7
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14	Interventions:	#11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	8
15	description			
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19				
20	Interventions:	#11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving / worsening disease)	Detailed in supplementary information file
21	modifications			
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27	Interventions:	#11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return; laboratory tests)	Detailed in supplementary information file
28	adherence			
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32	Interventions:	#11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	Detailed in supplementary information file
33	concomitant care			
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37	Outcomes	#12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended	10
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50	Participant timeline	#13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure)	14
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52				
53				Also detailed in supplementary information file, Table S1
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1	Sample size	#14	Estimated number of participants needed to achieve	11
2			study objectives and how it was determined,	
3			including clinical and statistical assumptions	
4			supporting any sample size calculations	
5				
6				
7				
8	Recruitment	#15	Strategies for achieving adequate participant	Detailed in
9			enrolment to reach target sample size	supplementary
10				information file
11				
12				

13 Methods:

14 Assignment of 15 interventions (for 16 controlled trials)

19				
20	Allocation: sequence	#16a	Method of generating the allocation sequence (eg,	8
21	generation		computer-generated random numbers), and list of	
22			any factors for stratification. To reduce predictability	
23			of a random sequence, details of any planned	
24			restriction (eg, blocking) should be provided in a	
25			separate document that is unavailable to those who	
26			enrol participants or assign interventions	
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31	Allocation	#16b	Mechanism of implementing the allocation sequence	9
32	concealment		(eg, central telephone; sequentially numbered,	
33	mechanism		opaque, sealed envelopes), describing any steps to	
34			conceal the sequence until interventions are assigned	
35				
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38	Allocation:	#16c	Who will generate the allocation sequence, who will	8, 9
39	implementation		enrol participants, and who will assign participants to	
40			interventions	
41				
42				
43	Blinding (masking)	#17a	Who will be blinded after assignment to	9
44			interventions (eg, trial participants, care providers,	
45			outcome assessors, data analysts), and how	
46				
47				
48				
49	Blinding (masking):	#17b	If blinded, circumstances under which unblinding is	Detailed in
50	emergency		permissible, and procedure for revealing a	supplementary
51	unblinding		participant's allocated intervention during the trial	information file
52				
53				

54 Methods: Data 55 collection, 56 management, and 57 analysis 58 59

1	Data collection plan	#18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol	10. More details in supplementary information file
2				
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14	Data collection plan: retention	#18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols	Detailed in supplementary information file
15				
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21	Data management	#19	Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol	13. More information in the statistical analysis plan found at ISRCTN60517191
22				
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31	Statistics: outcomes	#20a	Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol	13. More information in the statistical analysis plan found at ISRCTN60517191
32				
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40	Statistics: additional analyses	#20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	11. More information in the statistical analysis plan found at ISRCTN60517191
41				
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49	Statistics: analysis population and missing data	#20c	Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation)	Information in the statistical analysis plan found at ISRCTN60517191
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Methods: Monitoring

1	Data monitoring:	#21a	Composition of data monitoring committee (DMC);	Detailed in
2	formal committee		summary of its role and reporting structure;	supplementary
3			statement of whether it is independent from the	information file
4			sponsor and competing interests; and reference to	
5			where further details about its charter can be found,	
6			if not in the protocol. Alternatively, an explanation of	
7			why a DMC is not needed	
8				
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12	Data monitoring:	#21b	Description of any interim analyses and stopping	Detailed in
13	interim analysis		guidelines, including who will have access to these	supplementary
14			interim results and make the final decision to	information file
15			terminate the trial	
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19	Harms	#22	Plans for collecting, assessing, reporting, and	Detailed in
20			managing solicited and spontaneously reported	supplementary
21			adverse events and other unintended effects of trial	information file
22			interventions or trial conduct	
23				
24				
25				
26	Auditing	#23	Frequency and procedures for auditing trial conduct,	Detailed in
27			if any, and whether the process will be independent	supplementary
28			from investigators and the sponsor	information file
29				
30				
31	Ethics and			
32	dissemination			
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35	Research ethics	#24	Plans for seeking research ethics committee /	12
36	approval		institutional review board (REC / IRB) approval	
37				
38				
39	Protocol	#25	Plans for communicating important protocol	11
40	amendments		modifications (eg, changes to eligibility criteria,	
41			outcomes, analyses) to relevant parties (eg,	
42			investigators, REC / IRBs, trial participants, trial	
43			registries, journals, regulators)	
44				
45				
46				
47	Consent or assent	#26a	Who will obtain informed consent or assent from	12.
48			potential trial participants or authorised surrogates,	
49			and how (see Item 32)	
50				Also detailed in
51				supplementary
52				information file
53				
54				
55	Consent or assent:	#26b	Additional consent provisions for collection and use	Detailed in
56	ancillary studies		of participant data and biological specimens in	supplementary
57			ancillary studies, if applicable	information file
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1	Confidentiality	#27	How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial	Detailed in supplementary information file
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8	Declaration of interests	#28	Financial and other competing interests for principal investigators for the overall trial and each study site	15
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11	Data access	#29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators	n/a
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17	Ancillary and post trial care	#30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation	Detailed in supplementary information file
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22	Dissemination policy: trial results	#31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions	2, 13
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32	Dissemination policy: authorship	#31b	Authorship eligibility guidelines and any intended use of professional writers	n/a
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36	Dissemination policy: reproducible research	#31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	13
37				
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41	Appendices			
42				
43	Informed consent materials	#32	Model consent form and other related documentation given to participants and authorised surrogates	Supplementary files provided
44				
45				
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47	Biological specimens	#33	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable	n/a
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BMJ Open

The effect of intensive treatment for schistosomiasis on immune responses to vaccines among rural Ugandan island adolescents: randomised controlled trial protocol A for the 'POPulation differences in VACcine responses' (POPVAC) programme

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Date Submitted by the Author:	09-Dec-2020
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Primary Subject Heading:	Infectious diseases
Secondary Subject Heading:	Epidemiology, Immunology (including allergy), Public health
Keywords:	Infection control < INFECTIOUS DISEASES, PARASITOLOGY, Public health < INFECTIOUS DISEASES, Immunology < TROPICAL MEDICINE, Epidemiology < TROPICAL MEDICINE, Paediatric infectious disease & immunisation < PAEDIATRICS

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3 1 **The effect of intensive treatment for schistosomiasis on immune responses to vaccines among**
4 **rural Ugandan island adolescents: randomised controlled trial protocol A for the ‘POPulation**
5 **differences in VACCine responses’ (POPVAC) programme**
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10 Kabuubi¹, Emmanuel Niwagaba¹, Gloria Oduru¹, Grace Kabami¹, Rebecca Amongin¹, Alex Mutebe¹,
11 Milly Namutebi¹, Christopher Zziwa¹, Susan Amongi¹, Caroline Ninsiima¹, Caroline Onen¹, Florence
12 Akello¹, Moses Sewankambo¹, Samuel Kiwanuka¹, Robert Kizindo¹, James Kaweesa², Stephen Cose^{1,3},
13 Emily L Webb⁴, Alison M Elliott^{1,3} **for the POPVAC trial team**
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3 **20 Abstract**
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5 **21 Introduction**
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8 Several licensed and investigational vaccines have lower efficacy, and induce impaired immune
9
10 responses, in low-income versus high-income countries and in rural, versus urban, settings.
11
12 Understanding these population differences is essential to optimising vaccine effectiveness in the
13
14 tropics. We suggest that repeated exposure to and immunomodulation by chronic helminth
15
16 infections partly explains population differences in vaccine response.
17

18 **27 Methods and analysis**
19

20
21 We have designed an individually randomised, parallel group trial of intensive versus standard
22
23 praziquantel (PZQ) intervention against schistosomiasis, to determine effects on vaccine response
24
25 outcomes among school-going adolescents (9 to 17 years) from rural *Schistosoma mansoni* (*Sm*)-
26
27 endemic Ugandan islands. Vaccines to be studied comprise BCG on day 'zero'; yellow fever, oral
28
29 typhoid and Human Papilloma Virus (HPV) vaccines at week 4; and HPV and Tetanus/diphtheria
30
31 booster vaccine at week 28. The intensive arm will receive PZQ doses three times, each two weeks
32
33 apart, before BCG immunisation, followed by a dose at week 8 and quarterly thereafter. The
34
35 standard arm will receive PZQ at week 8 and week 52. We expect to enrol 480 participants, with 80%
36
37 *Sm* infected at the outset.

38
39 Primary outcomes are BCG-specific IFN- γ ELISpot responses eight weeks after BCG immunisation and
40
41 for other vaccines, antibody responses to key vaccine antigens at four weeks after immunisation.
42
43 Secondary analyses will determine effects of intensive anthelmintic treatment on correlates of
44
45 protective immunity, on vaccine response waning, on priming vs boosting immunisations, and on *Sm*
46
47 infection status and intensity. Exploratory immunology assays using archived samples will enable
48
49 assessment of mechanistic links between helminths and vaccine responses.
50

51 **43 Ethics and dissemination**
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53
54 Ethics approval has been obtained from relevant Ugandan and UK ethics committees. Results will be
55
56 shared with Uganda Ministry of Health, relevant district councils, community leaders and study
57
58 participants. Further dissemination will be done through conference proceedings and publications.
59

60 **47 Trial registration**

48 Current Controlled Trials identifier: ISRCTN60517191.
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3 50 **Article summary**
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5 51 *Strengths and limitations of this study*
6

- 7 52
- 8 • This will be the first adequately powered intervention study to investigate effects of
9 schistosomiasis treatment on vaccine responses in adolescents.
10
 - 11 54 • Effects on both live-attenuated and inert vaccines will be studied.
12
 - 13 55 • Our strong immunoepidemiological design and nested immunological studies will address
14 specific hypotheses regarding pathways of effects.
15
 - 16 57 • The sample archives developed will provide a major asset for exploration of new leads
17 arising from this hypothesis-driven work, or for an alternative, “systems biology” approach
18 58 investigating (for example) transcriptome, microbiome and virome.
19 59
 - 20 60 • Even with intensive anthelmintic intervention, it may be difficult to “successfully” treat
21 61 *Schistosoma* infection in our endemic setting due to re-infections; however, we still expect a
22 62 substantial difference in intensity between the two trial arms.
23
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27 63
28 64 **Word count**
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30 65 3347
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32 66 **Keywords**
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34 67 Vaccine; Schistosomiasis; Praziquantel; Immunization
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68 Introduction

69 Vaccine-specific immune responses are often impaired, and vaccine efficacy and effectiveness lower,
70 in tropical low-income countries (LICs) compared to temperate high-income countries and in rural,
71 compared to urban, LIC settings.¹⁻⁸ This has been recognised for both live vaccines (such as BCG,^{2,3,5,9}
72 polio,¹ yellow fever⁴ vaccines) and non-live vaccines (such as influenza¹⁰ and tetanus¹¹).

73 Investigational malaria⁷ and viral-vectored tuberculosis⁶ and Ebola¹² vaccines are also affected.

74 Previous exposure to the target pathogen (or related organisms) may mask the benefit of the
75 vaccine.^{13,14} However, pre-vaccination exposure does not explain why Ebola trial vaccine-specific

76 responses differ between healthy UK and Senegalese adults,¹² as the target organism is rare.

77 Therefore, environmentally-dependent mechanisms may play an important role.⁵

78 A long-held hypothesis is that parasites, particularly helminths, modulate vaccine responses through

79 profound pre- and post-immunisation bystander effects on immunological activation and

80 regulation.¹⁵⁻¹⁷ Helminths might also impact vaccines responses through interactions with the

81 complex ecosystem of mammalian gut bacteria, fungi, protozoa and viruses (the “trans-kingdom”

82 concept¹⁸ detailed elsewhere in this journal [bmjopen-2020-040425]). Helminth-induced gut mucosa

83 damage, the associated translocation of microbial products into the systemic circulation¹⁹⁻²¹ and

84 systemic immune activation or regulation mediated by microbial products might contribute to

85 modulation of responses to vaccines and other infections.

86 Helminth-mediated modulation of vaccine responses has not been substantiated in human

87 populations. No appropriately powered trials have been conducted to evaluate reversibility of their

88 effects. In animal models, helminths generally impair priming and accelerate waning of vaccine

89 responses, although effects vary with helminth species, vaccine type and the timing of infection and

90 immunisation.²² Most observational studies in humans also suggest suppressed or biased responses

91 during helminth infection, especially during systemic infections, such as schistosomiasis and the

92 filariases. There is modest evidence that treating geohelminths in humans improves responses to

93 BCG^{23,24} or oral cholera vaccine²⁵ and we found that schistosomiasis treatment improved the

94 measles-booster response in pre-school children.²⁶ There is therefore a strong case for a

95 comprehensive assessment of the effects of helminths and their treatment on vaccine responses.

96 The extent to which helminths and related “trans-kingdom” mediators causally and reversibly

97 impact immunological characteristics associated with vaccine responses may best be determined by

98 intervention studies. This trial protocol A of the ‘Population differences in Vaccine responses’

99 programme (POPVAC A; Current Controlled Trials identifier: ISRCTN60517191) has been designed to

100 evaluate the effect of *Schistosoma mansoni* and its treatment on vaccine responses. This study is

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101 one of three parallel trials whose designs and cross-cutting analyses are described separately in this
102 journal (bmjopen-2020-040425, bmjopen-2020-040427 and bmjopen-2020-040430).

For peer review only

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3 103 **Hypothesis**
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5 104 The overarching goal of the POPVAC programme is to understand population differences in vaccine
6
7 105 responses in Uganda, in order to identify strategies through which vaccine effectiveness can be
8
9 106 optimised for the low-income, tropical settings where they are especially needed. For this Trial A, we
10
11 107 focus on the hypothesis that *Schistosoma mansoni* infection suppresses responses to unrelated
12
13 108 vaccines; and that this effect can be reversed, at least in part, by intensive praziquantel treatment
14
15 109 intervention.

16 110 **Objective**

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18 111 To determine whether there are reversible effects of chronic *Schistosoma mansoni* infection on
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20 112 vaccine response in adolescents, using an intervention study.
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113 **Methods and analysis**

114 ***Setting and participants***

115 SPIRIT reporting guidelines²⁷ have been used. We will conduct an individually randomised, parallel
116 group trial of intensive versus standard intervention against schistosomiasis (described below) in the
117 *S. mansoni*-endemic Koome islands of Lake Victoria, Mukono district, Uganda.²⁸ We aim to enroll 480
118 participants, randomising 240 to each intervention arm. The study cohort will recruit participants
119 aged 9 to 17 years in primary school years 1 to 6. Adolescents²⁹ in this study setting bear a heavy
120 parasite burden.³⁰ In addition, this age-group is a target group for vaccines against sexually
121 transmitted infections (currently human papilloma virus [HPV] – in future, it is hoped, for vaccines
122 against HIV) and for booster immunisations.

123 ***Recruitment criteria***

124 *Inclusion criteria*

- 125 i. Attending the selected school and planning to continue to attend the school for the duration
126 of the study
- 127 ii. Aged 9 to 17 years and enrolled in primary 1 to 6 (to avoid primary leaving examinations in
128 late year 7, and loss to follow up of children leaving after primary 7)
- 129 iii. Written informed assent by participant and consent by parent or guardian
- 130 iv. Females agree to avoid pregnancy for the duration of the trial
- 131 v. Willing to provide locator information and to be contacted during the course of the trial
- 132 vi. Able and willing (in the investigator's opinion) to comply with all the study requirements

133 *Exclusion criteria*

- 134 i. Clinically significant history of immunodeficiency (including HIV), cancer, cardiovascular
135 disease, gastrointestinal disease, liver disease, renal disease, endocrine disorder and
136 neurological illness
- 137 ii. History of serious psychiatric condition or disorder
- 138 iii. Moderate or severe acute illness characterised by any of the following symptoms: fever,
139 impaired consciousness, convulsions, difficulty in breathing, vomiting; or as otherwise
140 determined by the attending project clinician.
- 141 iv. Concurrent oral or systemic steroid medication or the concurrent use of other
142 immunosuppressive agents within 2 months prior to enrolment
- 143 v. History of allergic reaction to immunisation or any allergy likely to be exacerbated by any
144 component of the study vaccines including egg or chicken proteins

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3 145 vi. History of previous immunisation with Yellow Fever (YF), oral typhoid or Human Papillomavirus
4 146 (HPV) vaccine; previous immunisation with BCG or Tetanus and diphtheria vaccine (Td) at age
5 147 ≥ 5 years
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8 148 vii. Tendency to develop keloid scars
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10 149 viii. Haemoglobin less than 82g/L
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12 150 ix. Positive HIV serology
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14 151 x. Positive pregnancy test
15 152 xi. Female currently lactating, confirmed pregnancy or intention to become pregnant during the
16 153 trial period
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18 154 xii. Use of an investigational medicinal product or non-registered drug, live vaccine, or medical
19 155 device other than the study vaccines for 30 days prior to dosing with the study vaccine, or
20 156 planned use during the study period
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22 157 xiii. Administration of immunoglobulins and/or any blood products within the three months
23 158 preceding the planned trial immunisation date
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27 159 Further information on recruitment criteria can be found in Supplementary information.
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29 160 **Interventions**

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31 161 We will individually randomise participants to intensive or standard praziquantel (PZQ) treatment, in
32 162 a 1:1 ratio. The intensive arm will receive three doses of PZQ (approximately 40mg/kg, assessed by
33 163 height pole³¹) each two weeks apart prior to the first immunisation (the last of these 2-3 weeks before
34 164 immunisation), followed by PZQ at 8 weeks (after primary endpoint sampling) and thereafter quarterly
35 165 PZQ (approximately; timings adjusted to accommodate school terms) during follow up. The standard
36 166 arm will receive their first dose of PZQ at week 8 (after immunisation and after primary endpoint
37 167 sampling) and a second dose at week 52 (to conform to Uganda Ministry of Health policy, which is
38 168 annual treatment) (**Figure 1**). No placebo will be used in this trial because all participants will be
39 169 treated (albeit at different frequencies) and participants are unlikely to seek additional treatments
40 170 outside the trial schedule: praziquantel treatment is not popular because of the recognised (although
41 171 temporary) adverse effects (described in Supplementary information).
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50 172 **Randomisation and allocation to treatment arm**

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52 173 A randomisation code will be generated by an independent statistician using a randomly permuted
53 174 block size (sizes 4, 6, 8 and 10) and used to allocate participants to either receive quarterly PZQ
54 175 (intensive arm) or annual PZQ (standard arm). A set of envelopes will be prepared, labelled
55 176 sequentially with the randomisation numbers and containing a card indicating the corresponding
56 177 allocation (to intensive or standard treatment). The randomisation code will be kept securely by the
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178 trial statistician and made available only to those responsible for providing or preparing the trial
 179 interventions. A second copy will be held by a data manager or statistician not otherwise involved in
 180 the trial at the MRC/UVRI and LSHTM Uganda Research Unit. At enrolment, eligibility criteria will be
 181 checked and eligible participants will be allocated sequentially to the next randomisation number
 182 until the required sample size is achieved. Randomisation implementation will be done by a clinician
 183 using the sequentially numbered opaque sealed envelopes. When the next randomisation number in
 184 the sequence is allocated, the envelope bearing that number will be opened to reveal the allocation.

185 **Blinding**

186 Clinicians and participants will not be blinded to the treatment allocation since they will not
 187 participate in outcome ascertainment; only immunology laboratory staff who are assessing trial
 188 outcomes will be blinded.

189 **Immunisations**

190 We will study a portfolio of licensed vaccines (live and inert, oral and parental, priming and boosting)
 191 expected to be beneficial (in some cases, already given) to adolescents in Uganda. Our schedule
 192 (**Table 1**, supplementary **Table S1**) will comprise three main immunisation days (week 0, week 4 and
 193 week 28). Additional HPV immunisation will be provided for girls aged 14 years or above, and a
 194 second Tetanus/diphtheria boost will be given after completion of the study, to accord with the
 195 national Expanded Programme on Immunisation (EPI) routines, but the response to these will not
 196 specifically be assessed. Further rationale for the selection of vaccines is detailed in the
 197 Supplementary information.

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Table 1. Immunisation schedule

	Immunisation week 0	Immunisation week 4	[Immunisation week 8]	Immunisation week 28	[Immunisation week 52]
Live vaccines	BCG vaccination / re-vaccination ¹	Yellow fever (YF-17D) Oral typhoid (Ty21a)			
Non-live vaccines		HPV prime ²	HPV boost for girls aged ≥ 14 years ^{3,4}	HPV boost ² and Tetanus/ diphtheria (Td) boost	Tetanus/ diphtheria (Td) boost ^{4,5}
1. Prior BCG status may vary (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) 2. Both girls and boys will receive the HPV vaccine 3. The National EPI programme recommends three doses of HPV vaccine for older girls 4. These doses will be given to comply with guidelines but outcomes specifically relating to these doses will not be assessed 5. Priming by immunisation in infancy is assumed					

199

200 ***Schedule of immunisation and sampling***

201 The schedule of immunisation and sampling is outlined in **Figure 1** and **Table S1**. Pre-immunisation
202 vaccine responses will be assessed in baseline samples. While optimal timings for outcome measures
203 vary between vaccines, sampling at 8 weeks post BCG and 4 weeks post YF-17D, Ty21a, HPV and Td
204 is proposed for the primary endpoints, targeting the establishment of memory responses and
205 approximate peak of antibody responses. A secondary endpoint at one year will assess waning.
206 Immunisation postponement criteria are detailed in Supplementary information.

207 ***Outcomes***

208 *Primary outcomes*

209 These will be assessed in all participants.

- 210 i. **BCG:** BCG-specific IFN- γ ELISpot response eight weeks post BCG immunisation.
- 211 ii. **YF-17D:** neutralising antibody titres (plaque-reduction neutralisation test) at four weeks post
212 YF immunisation.
- 213 iii. **Ty21a:** *Salmonella typhi* lipopolysaccharide (LPS)-specific immunoglobulin (Ig)G concentration
214 at four weeks post Ty21a immunisation.
- 215 iv. **HPV:** IgG specific for L1-proteins of HPV-16/18 at four weeks post HPV priming immunisation.
- 216 v. **Td:** Tetanus and diphtheria toxoid-specific IgG concentration at four weeks post Td
217 immunisation.

218 *Secondary outcomes*

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

- 222 i. **Protective immunity.** Proportions with protective neutralising antibody (YF); protective IgG
223 levels (TT);³² seroconversion rates (Ty21a) at four weeks post the corresponding
224 immunisation.
- 225 ii. **Response waning.** Primary outcome measures (all vaccines) repeated at week 52, and area-
226 under-the curve (AUC) analyses. Parasitic infection may accelerate,³³ and anti-parasitic
227 interventions delay, waning.
- 228 iii. **Priming versus boosting.** Effects on priming versus boosting will be examined for HPV only,
229 comparing outcomes four weeks after the first, and four weeks after the second vaccine dose.

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3 230 iv. **Current *S. mansoni* infection status and intensity** will be determined by serum/plasma levels
4 231 of circulating anodic antigen (CAA). The method is quantitative, highly specific for *Schistosoma*
5 232 infection, and much more sensitive than the conventional Kato Katz method.³⁴ CAA will be
6 233 assessed retrospectively on stored samples collected at baseline, on immunisation days, and
7 234 on primary and secondary endpoint days.

11
12 235 Furthermore, our sample collection will offer opportunities for an array of exploratory
13 236 immunological evaluations on stored samples, focusing mainly on vaccine antigen specific outcomes.
14 237 Exploratory assays will provide further detail on the role of immunological profiles and trans-
15 238 kingdom effects in mediating helminth modulation of vaccine-specific responses.

19 239 ***Evaluation of parasite infection exposure***

20 240 The following measures will also be assessed in all participants, and will be used to describe the
21 241 general infection-exposure experience of the study participants.

- 22 242 i. **Prior exposure to schistosomiasis** will be evaluated by ELISA for IgG to schistosome egg
23 243 antigen using stored blood samples collected at baseline.
- 24 244 ii. **The presence of other helminth infections** will be determined retrospectively using stool
25 245 PCR of samples collected at baseline and at weeks 28 and 52.³⁰ In accordance with national
26 246 guidelines, all participants will be treated with albendazole or mebendazole after collection
27 247 of samples for primary endpoints at week 8 and 28, and after collection of samples for
28 248 secondary endpoints at week 52.
- 29 249 iii. **Current malaria infection status and intensity** will be assessed retrospectively by PCR on
30 250 stored samples collected on immunisation days and at week 52. Individuals presenting with
31 251 fever will be investigated using rapid diagnostic tests for malaria and treated based on the
32 252 results and according to prevailing national guidelines.
- 33 253 iv. **Prior malaria exposure** will be evaluated by ELISA for IgG to malaria antigen using stored
34 254 samples collected at baseline.

47 255 ***Sample size considerations***

48 256 Based on the literature^{4 35 36} and preliminary data, we anticipate that, following log to base 10
49 257 transformations that will be applied to normalise primary outcome measures, standard deviations
50 258 (SDs) of primary outcome measures will lie between 0.3 and 0.6 on this log scale, and that effective
51 259 treatment may increase responses by approximately 0.2 on the log scale (based on Tweyongyere *et*
52 260 *al.*²⁶). We have therefore powered our study to detect differences of this magnitude (0.2 on the log
53 261 scale) or (in some cases) smaller (**Table 2**). We assume *S. mansoni* prevalence of $\geq 80\%$.

262 Based on these assumptions and a two independent samples t-test, we plan to include 480
 263 participants in total (240 quarterly PZQ, 240 annual PZQ); of whom 384 are expected to be *S.*
 264 *mansoni* infected,²⁸ giving 192 participants in each trial arm who are infected at baseline.

265 **Table 2** shows power estimates, for 5% type-1 error rate for each primary outcome measure and
 266 assuming 20% loss to follow-up.

267

Table 2. Power estimates (5% type-1 error rate for each primary outcome measure)

Standard deviation (log ₁₀)	Difference in mean log ₁₀ transformed outcome, between trial arms						
	0.08	0.10	0.12	0.14	0.16	0.18	0.20
192 intensive PZQ vs 192 standard PZQ (<i>S. mansoni</i> 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%
Cells highlighted in grey correspond to >80% power; differences in mean log ₁₀ transformed outcome of 0.08, 0.10, 0.12, 0.14, 0.16, 0.18 and 0.20 are equivalent to geometric mean ratios for untransformed outcomes of 1.20, 1.26, 1.32, 1.38, 1.45, 1.51 and 1.59, respectively.							

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269 ***Ethics and dissemination***

270 Ethical approval has been granted from the Research Ethics Committee of the Uganda Virus
 271 Research Institute (UVRI REC, reference: GC/127/19/05/664) and the London School of Hygiene and
 272 Tropical Medicine (LSHTM, reference: 16032), and from the Uganda National Council for Science and
 273 Technology (UNCST, reference: HS2486) and the Uganda National Drug Authority (NDA, reference:
 274 CTA0093). Any protocol amendments will be submitted to ethics committees and regulatory bodies
 275 for approval before implementation.

276 Participants are adolescents and therefore a vulnerable human population. Care will be taken to
 277 provide adequate, age and education-status appropriate information and to ensure that it is
 278 understood; and to emphasise that participation is voluntary. Participants will be enrolled only when
 279 they have given their own assent and when consent has been given by the parent or guardian.
 280 Model consent and assent forms are shown in Supplementary file 2. No major risks to the
 281 participants are anticipated since all the treatments and vaccines to be given are licensed and known
 282 to be safe. The main risk to participants will be time lost from school work: we will work with
 283 teachers and parents to minimise disruption to classes, and will avoid enrolment of primary 7
 284 students since these classes are involved in national examinations. Further risks are discussed in
 285 Supplementary information.

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3 286 Study findings will be published through open access peer-reviewed journals, presentations at local,
4
5 287 national and international conferences and to the local community through community meetings.
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7 288 Anonymised participant level datasets generated will be available upon request.

8 9 289 ***Patient and public involvement***

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11 290 Concepts involved in this work have been discussed with colleagues at the Vector Control Division
12
13 291 and Expanded Programme on Immunisation in the Ministry of Health (Uganda), with the Mukono
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15 292 District Council and with community leaders and Village Health Teams from Koome subcounty. We
16
17 293 also have held meetings to explain the proposed work to teachers, parents, participants and village
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19 294 members, and to address their questions about issues such as study length, the study's ethical
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21 295 approval status, why adults were excluded from the study, and to explain to them why boys will also
22
23 296 receive the HPV vaccine. Study findings will be shared with these stakeholders and with participants.

23 24 297 ***Data management and analysis***

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26 298 Socio-demographic information and clinical and laboratory measurements will be recorded and
27
28 299 managed using Research Electronic Data Capture (REDCap) tools,^{37 38} with paper-based forms as
29
30 300 back-up. All data will be recorded under a unique study ID number. When paper forms must be
31
32 301 used, data will be double entered in a study-specific database, with standard checks for
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34 302 discrepancies. All data for analysis will be anonymised and stored on a secure and password-
35
36 303 protected server, with access limited to essential research personnel.

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38 304 Baseline characteristics including age, sex, school, location of birth, prior vaccination status,
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40 305 helminth infection and prior exposure status and malaria infection and prior exposure status will be
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42 306 summarised by trial arm. The effect of intensive (compared to standard) praziquantel treatment on
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44 307 the outcomes will be analysed. Information on infection status will only be available after
45
46 308 randomisation. The primary analysis will be done on individuals identified as infected at baseline
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48 309 (through randomisation, these will be balanced between treatment arms); this will test the
49
50 310 hypothesis that treating the infection (and subsequent reinfections) reverses the parasite's effects
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52 311 on vaccine responses. If treating *S. mansoni* reverses adverse parasite effects on vaccine responses,
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54 312 this may be a beneficial public health intervention. However, routine screening for parasite infection
55
56 313 before immunisation would be laborious. Secondary analyses will include all randomised individuals;
57
58 314 this will provide insight into the broader benefit of the interventions as public health measures. The
59
60 315 effect of intensive versus standard praziquantel treatment on primary outcomes will be assessed
61
62 316 using unpaired t-tests, with results presented as a mean difference in vaccine response measure
63
64 317 together with 95% confidence interval and p-value. For all outcomes, we will investigate adjusting
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66 318 for corresponding baseline vaccine responses as this may improve the precision of effect estimates;

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3 319 this will be done using multivariable regression. We anticipate that outcomes will be positively
4
5 320 skewed, and will apply log transformations to normalise distributions before analysis if required. The
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7 321 detailed analytical plan is available on the online trial registration site
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9 322 (<http://www.isrctn.com/ISRCTN60517191>).

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For peer review only

323 Discussion

324 This will be the first adequately powered intervention study to investigate the effect of
325 schistosomiasis treatment on vaccine responses in adolescents. This study will determine whether *S.*
326 *mansoni* infection has a causal, reversible, impact on the response to live or inert vaccines, including
327 effects on vaccine replication, immune response profile, priming, boosting and waning. The results
328 will add to understanding of population differences in vaccine responses and on interventions that
329 may enhance responses. If treating helminths improves vaccine responses in adolescents, combined
330 parasite-control/immunisation programmes offer an attractive, practical public health intervention
331 for schools and communities.

332 There are risks associated with our approach to addressing the trial objective. **First**, there is a risk of
333 failure to clear *S. mansoni* infections, and repeated reinfection during the trial. This issue can be
334 challenging because of incomplete cure or maturation of immature worms after treatment, and
335 lifestyles in endemic communities that result in repeated exposure. To mitigate this, we will
336 administer three PZQ treatments over a six-week period before the first immunisations, and
337 continuing quarterly treatment in the intensive arm. **Second**, there is a risk that *S. mansoni* infection
338 has long-term effects, not removed by treatment, mediated, for example, by epigenetic change.³⁹
339 However, studies show that parasite treatment results in immunological changes,^{40,41} and our data
340 suggest at least partial recovery of the measles vaccine response among young children treated for
341 schistosomiasis.²⁶ By initiating intervention six to eight weeks before the first immunisations, and
342 providing repeated intervention in the intensive arms, we hope to achieve significant resolution of *S.*
343 *mansoni* effects.

344 We are interested in the effects of removing *S. mansoni*. Treating parasites can induce acute
345 immunological change due to release of previously hidden antigens.^{42,43} To minimise such effects,
346 immunisations will be given at least 2 weeks after PZQ (the longest practicable interval; **Figure 1**).

347 Laboratory analyses will also highlight immune parameters and cellular populations that link
348 environmental exposures to vaccine responses. Identifying processes associated with poor or good
349 outcomes will inform strategies in vaccine design (for example, the genetic modification of vaccines,
350 or innovative use of adjuvants, to counter any adverse immunological milieu, currently an area of
351 intense research for cancer vaccines⁴⁴); ultimately supporting the development of effective vaccines
352 tailored to the low-income settings that most need them.

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354 Study timeline

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3 355 Applications for ethical approval were submitted in May 2018, with approval received in September
4 356 2018 (Uganda Virus Research Institute Research Ethics Committee), May 2019 (National Drug
5 357 Authority and Uganda National Council for Science and Technology), June 2019 (London School of
6 358 Hygiene and Tropical Medicine). Collaborator/investigator/trial steering committee meetings were
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8 359 also held during the initial 12-month planning period. The study began recruitment in July 2019.
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10 360 Intervention will be up to 12 months, with completion of the project scheduled for September 2020.
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362 **Competing interests**

363 Alison Elliott reports a grant from the Medical research Council, UK (POPVAC programme funding).
364 The rest of the authors declare that they have no conflicts of interest.

365 **Author contributions**

366 AME conceived the study. AME, GN, ELW, AN, JN, SC, LZ and JK contributed to study design. LZ, GO,
367 PNK, EN, GK, RA, CN, CO, MN, CZ, SA and FA are site clinicians/nurses/clinical laboratory technicians
368 providing valuable input on clinical considerations of the intervention. MS, SK and RK are field
369 workers handling the organisational integration of the intervention. AN, AM and ELW are involved in
370 organisation of the databases, trial randomization, treatment allocation and drawing up of analytical
371 plans. GN, LZ, JN, AN, SC, ELW and AME drafted the manuscript. All authors reviewed the
372 manuscript, contributed to it and approved the final version.

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376 diphtheria vaccines were kind donations from the Serum Institute of India. We thank the Vector
377 Control Division of the Ministry of Health and the Mukono district local government for their
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379 Richard Hayes) and the Data and Safety Monitoring Board (Dr David Meya, Prof Andrew Prendergast
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8 392 and is also part of the EDCTP2 programme supported by the European Union.

9
10 393 The study sponsor (London School of Hygiene and Tropical Medicine) and funders had no role in
11 394 study design; collection, management, analysis, and interpretation of data; writing of the protocol;
12 395 and the decision to submit the protocol for publication.

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17 396 **POPVAC trial team**

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19 397 **Principal investigator:** Alison Elliott; **Project leader:** Ludoviko Zirimenya; **laboratory staff:** Gyaviira
20 398 Nkurunungi, Stephen Cose, Rebecca Amongin, Beatrice Nassanga, Jacent Nassuuna, Irene Nambuya,
21 399 Prossy Kabuubi, Emmanuel Niwagaba, Gloria Oduru, Grace Kabami; **statisticians and data**
22 400 **managers:** Emily Webb, Agnes Natukunda, Helen Akurut, Alex Mutebe; **clinicians:** Anne Wajja, Milly
23 401 Namutebi, Christopher Zziwa, Joel Serubanja; **nurses:** Caroline Onen, Esther Nakazibwe, Josephine
24 402 Tumusiime, Caroline Ninsiima, Susan Amongi, Florence Akello; **internal monitor:** Mirriam
25 403 Akello; **field workers:** Robert Kizindo, Moses Sewankambo, Denis Nsubuga, Samuel Kiwanuka, Fred
26 404 Kiwudhu; **boatman:** David Abiriga; **administrative management:** Moses Kizza, Samsi Nansukusa;
27 405 **internal and external collaborators:** Pontiano Kaleebu, Hermelijn Smits, Maria Yazdanbakhsh,
28 406 Govert van Dam, Paul Corstjens, Sarah Staedke, Henry Luzze, James Kaweesa, Edridah Tukahebwa,
29 407 Elly Tumushabe, Moses Muwanga
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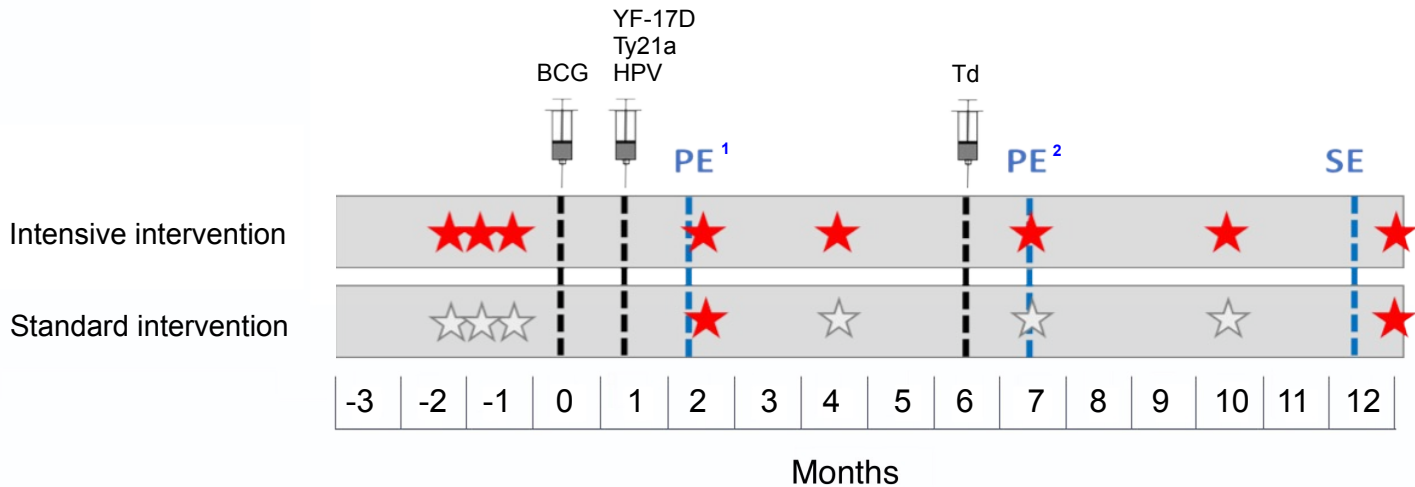
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15 542 **FIGURE LEGENDS**

16
17 543 **Figure 1. Outline of immunisations and anthelmintic intervention**

18
19 544 ¹Primary endpoints will be at eight weeks post BCG and four weeks post Yellow fever (YF-17D), oral
20 545 typhoid (Ty21a), Human Papilloma Virus (HPV) and Tetanus/diphtheria (Td) vaccination.

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22 546 ²Primary endpoint for responses to Td given at 28 weeks.
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★ praziquantel; - - - immunisations; PE - - - primary endpoint; SE - - - secondary endpoint
★ standard arm, no praziquantel

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3 1 **SUPPLEMENTARY INFORMATION**
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7 3 **The effect of intensive treatment for schistosomiasis on immune responses to vaccines among**
8 **rural Ugandan island adolescents: randomised controlled trial protocol A for the ‘POPulation**
9 **differences in VACCine responses’ (POPVAC) programme**
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14 7 Kabuubi¹, Emmanuel Niwagaba¹, Gloria Oduru¹, Grace Kabami¹, Rebecca Amongin¹, Alex Mutebe¹,
15 8 Milly Namutebi¹, Christopher Zziwa¹, Susan Amongi¹, Caroline Ninsiima¹, Caroline Onen¹, Florence
16 9 Akello¹, Moses Sewankambo¹, Samuel Kiwanuka¹, Robert Kizindo¹, James Kaweesa², Stephen Cose^{4,3},
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33 17 Kingdom
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Table S1. Schedule of visits and procedures

VISIT NUMBER	1	2 & 3	4	5 ⁹	5.2	6	7	8	9	10	11
WEEKS FROM 1 ST IMMUNISATION	-8 ¹	-6 ¹⁰ , -4, -2	0	4	4 weeks +4 days	8	20	28	32	44	52
	Screening	Treatment (Rx) only	Immunisations	Immunisations		Primary Endpoint (PE), Immunisations	Rx only	Immunisations	PE/Secondary Endpoint (SE) ²	Rx only	SE, Immunisations
RANDOMISED INTENSIVE VS STANDARD PRAZIQUANTEL INTERVENTION											
PZQ intensive arm (x)		x				x ³	x		x ³	x	x ³
PZQ standard arm						x ³					x ³
Albendazole						x ³			x ³		x ³
VACCINES											
BCG			x								
YF-17D				x							
Ty21a				x							
HPV				x		[x] ⁴		x			
Td								x			[x] ⁵
INVESTIGATIONS/PROCEDURES											
Inclusion/exclusion criteria	x										
Informed consent	x										
Questionnaire	x		x	x	x	x		x	x		x
Examination	x		(x)	(x)	(x)	(x)		(x)	(x)		(x)
Urine β-HCG test (female only) 1mL	x		x	x				x			
Urine YF viral load					x						
Stool for PCR and storage	x							x			x
Stool for coproantibody and storage	x					x					
BLOOD SAMPLES											
Malaria PCR (1ml)			x	x				x			x
Serology for HIV, prior malaria and <i>S. mansoni</i> (0.5ml)	x										
Mansonella perstans (1ml)	x										
Serum/plasma CAA (1ml)	x		x	x		x		x	x		x
Hb ⁸ / Full blood count (0.5ml)	x		x	x				x			
Assessments of pre-immunisation responses, and/or vaccine response outcomes and/or exploratory immunology; storage ⁷ (10 – 20 mls)			x	x		x		x	x		x
Blood for gene expression (2mls)			x	x				x			
Blood vol (mL)	4		27	17		10-20		27	10		14
Cumulative blood vol (mL) ⁷	4		31	48		68		95	105		119

PE: primary endpoint; **SE:** secondary endpoint; **Rx only:** treatment only

Immunisation days highlighted in green, primary end point days in red, days for treatment only in grey

(x) performed if clinically indicated

1. Screening and enrolment into Project A will take place about 8 weeks before immunisation 0 to allow initiation of the praziquantel intervention.
2. Week 32 is primary endpoint for responses to Td given at 28 weeks; secondary endpoint for HPV booster
3. Treatments given after sampling when schedules coincide
4. Week 8 HPV dose will be given for previously-unvaccinated girls aged ≥ 14 years
5. Week 52 Td booster dose will be provided as a service
6. Exploratory immunology blood volume will be guided by guidelines from Harvard Mass General, where a maximum of 3ml/kg body weight is taken at any one time point and not more than 3ml/kg is taken over any 8-week period (ref http://www.drgreene.com/21_1616.html.) These guidelines have been followed in a previous study vaccinating adolescents with investigational tuberculosis vaccine MVA85A (in Uganda).¹ The total blood volume planned is 68 ml over the initial intensive sampling period of 8 weeks. Revision of sample volumes based on weight will only be required for participants who weigh less than 22 kg; the average weight of children aged 9 years is expected to be 28kg (with 21kg the 3rd centile) with greater weights for older children.²
7. At baseline, it will only be Hb estimation by Haemocue
8. Oral typhoid vaccine doses will be administered on three alternate days namely visit 5, 5.1 and 5.2.
9. The first PZQ treatment at week -6 will be administered at the end of the screening visit

22

23 **Further information on recruitment criteria**

- 24 • Participants who are excluded from the trial because they have been discovered (during
25 screening procedures) to be suffering from a previously undiagnosed condition thought to
26 require further medical attention will be referred appropriately for further investigation and
27 treatment.
- 28 • Participants discovered to have severe anaemia will be excluded from the trial and treated
29 for anaemia
- 30 • Participants discovered to be HIV-positive will be counselled and offered a CD4 T-cell count
31 and referred to a provider of antiretroviral treatment (“Test and Treat” – i.e. initiation of
32 treatment regardless of CD4 count is recommended for these high-risk communities).
- 33 • Participants discovered to be pregnant will be counselled and referred to an antenatal clinic
34 of their choice.

35 This trial proposes to recruit all participants within a short time-frame. It will not, therefore, be
36 possible to reconsider enrolment of potential participants with temporary exclusion criteria after
37 treatment and resolution of the condition.

38 **Further rationale for the selection of vaccines**

39 *Bacillus Calmette–Guérin (BCG)*

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

44 Worldwide, TB is among the top 10 causes of death; Uganda has an estimated incidence of
45 202/100,000 people.³ Infectious, sputum positive, pulmonary TB classically emerges in adolescence,
46 driving the on-going epidemic.⁴ Thus adolescent booster immunisation is a key TB control strategy.⁵
47 However, BCG vaccine response and efficacy are often impaired in tropical and rural settings⁶⁻⁸ and
48 new TB vaccines are similarly affected.⁹ In the past, the WHO has been hesitant to recommend BCG
49 re-vaccination. However, in 2017 WHO’s Strategic Advisory Group of Experts (SAGE) recommended:
50 “Further research is warranted to explore whether certain sub-groups of age, geographic or *M.*
51 *tuberculosis* exposure categories would benefit from re-vaccination.”¹⁰ Recent results suggest that,
52 despite the variability of BCG efficacy between populations, BCG vaccination in adolescence offers
53 benefit in some tropical settings, especially for individuals who are not yet infected with
54 *Mycobacterium tuberculosis*, and may also be cost-effective.^{7,11} Also, BCG vaccine is currently being
55 used among adolescents in South Africa as a comparator in a trial of a novel TB vaccine (trial

1
2
3 56 registration NCT02075203). BCG efficacy in Ugandan adolescents, and differences in BCG vaccine
4
5 57 responses between urban and rural Ugandan populations, have not been tested. Information
6
7 58 obtained from this study is expected to further inform the use of BCG in adolescents, and also to
8
9 59 inform the development of new vaccines for tuberculosis.

10 60 *Yellow fever vaccine*

11
12 61 Yellow fever vaccine YF-17D is a live replicating parenteral vaccine. The vaccine (Stamaril; Sanofi
13
14 62 Pasteur) is available for purchase in Uganda. Yellow Fever (YF) causes outbreaks in Uganda and the
15
16 63 wider region¹² and YF-17D is a candidate for Uganda's expanded programme on immunisation (EPI).
17
18 64 Lower vaccine replication, lower neutralising antibody induction, and greater waning, are described
19
20 65 in Uganda compared to Switzerland.¹³ YF-17D is a potential vector for novel vaccine constructs,¹⁴
21
22 66 adding relevance to vaccine development.

23 67 *Typhoid vaccine Ty21a*

24
25 68 Typhoid vaccine Ty21a is a live replicating oral vaccine and also a potential vector for new vaccine
26
27 69 constructs.¹⁵ The Ty21a vaccine will be purchased from PaxVax, Redwood City, California.
28
29 70 Substantial, multi-year typhoid outbreaks occur in Uganda and immunisation campaigns have been
30
31 71 advocated as cost effective.¹⁶ Schistosomiasis has been associated with prolonged *S. typhi* infection¹⁷
32
33 72 and impaired antibody responses to killed typhoid vaccines.¹⁸

34
35 73 Ty21a was developed in the 1970s. Although not routinely used in Uganda, it has been (and is
36
37 74 currently) registered in many countries. It was first registered in the United States and United
38
39 75 Kingdom in the 1980s, and is recommended by the WHO for both endemic and epidemic settings.¹⁹
40
41 76 It has comparable efficacy to the parenteral Vi polysaccharide typhoid vaccine, good durability and
42
43 77 minimal adverse effects.¹⁹ It is proposed for use in this study to model effects of study exposures
44
45 78 and intervention on the response to a live oral vaccine.

46
47 79 The Ty21a vaccine is given as a three-dose regimen on alternate days.

48 80 *Human Papilloma Virus (HPV) vaccine*

49
50 81 The Human Papilloma Virus (HPV) vaccine is a protein virus-like particle. The quadrivalent HPV
51
52 82 Vaccine Gardasil (Merck) is available for purchase in Uganda and is the vaccine used by the national
53
54 83 EPI programme. Studies after three vaccine doses have found somewhat enhanced responses in the
55
56 84 presence of malaria, but no effect of helminths.²⁰ No study has previously investigated parasite
57
58 85 effects on the priming response, but recent results for tetanus suggest that priming may be more
59
60 86 susceptible than boosting to adverse effects.²¹ This will be important if forthcoming trials support
87
88 89 single-dose HPV immunisation (NCT02834637). HPV immunisation is being rolled out among girls to

1
2
3 88 prevent cervical neoplasia, the most common cancer among Ugandan women and we will
4
5 89 coordinate provision with the national HPV immunisation programme.²² HPV immunisation is also
6
7 90 beneficial for boys since HPV infection is associated with anogenital warts, anal cancer and
8
9 91 oropharyngeal cancers in both males and females, and with penile cancer in men,²³ and we will
10
11 92 include boys in these studies.

12 93 *Tetanus and diphtheria vaccines*

14 94 Tetanus and diphtheria (Td) vaccines comprise inert toxoids. Schistosomiasis is associated with a Th2
15
16 95 biased response to tetanus toxoid²⁴ and with suppressed antibody responses among those with low
17
18 96 pre-immunisation antibody levels.²¹ Booster immunisation is recommended for young women to
19
20 97 prevent maternal and neonatal tetanus. Recent evidence emphasises the need to protect young
21
22 98 men also.²⁵

23 99 ***Immunisation Postponement Criteria***

25 100 If any one of the following is identified at the time scheduled for immunisation, the participant may
26
27 101 be immunised at a later date, or withdrawn, at the discretion of the Investigator. The participant
28
29 102 must be followed until resolution of the event as with any adverse event:

- 31 103 • Acute disease at the time of immunisation. Acute disease is defined as the presence of a
32
33 104 moderate or severe illness with or without fever. All vaccines can be administered to persons
34
35 105 with a minor illness such as diarrhoea or mild upper respiratory infection with or without low-
36
37 106 grade fever, i.e. temperature of $\leq 37.5^{\circ}\text{C}$ (99.5°F)
- 38 107 • Temperature of $>37.5^{\circ}\text{C}$ (99.5°F) at the time of immunisation
- 40 108 • Taking antibiotics or antimalarials currently, or within the past 7 days, of the date of Ty21a
41
42 109 administration (ascertained verbally)

44 110 ***Vaccine storage and transport***

46 111 In order to maintain a reliable vaccine cold chain, the vaccines and diluents to be used will be stored
47
48 112 and transported within the recommended temperature range of $+2^{\circ}\text{C}$ to $+8^{\circ}\text{C}$. Care will be taken to
49
50 113 ensure that the vaccines are not frozen. BCG, being sensitive to light, will be kept in the dark
51
52 114 (normally within its secondary packaging) for as long as possible to protect it during storage and
53
54 115 transportation. All vaccines will be kept in appropriate refrigeration equipment with a temperature
55
56 116 monitoring device to ensure temperatures remain between $+2^{\circ}\text{C}$ and $+8^{\circ}\text{C}$. Cold boxes/vaccines
57
58 117 carriers with temperature monitors will be used to transport vaccines and the diluents from the
59
60 118 MRC/UVRI and LSHTM Uganda Research Unit (Entebbe) to Koome island and while transporting
119
vaccines to immunisation sessions. Designated staff will be given responsibility for managing the

1
2
3 120 vaccine cold chain. All cold chain equipment including the temperature monitoring devices used for
4
5 121 this project will comply with relevant technical specifications as defined by the EPI standards. Basic
6
7 122 routine maintenance will be regularly carried out on all cold chain equipment.

8 9 123 ***Additional laboratory measurements***

10
11 124 Additional assays will comprise HIV serology, pregnancy testing and full blood counts. HIV testing
12
13 125 and pregnancy testing will be accompanied by appropriate counselling by trained staff.

- 14
15 126 • HIV serology will be done on blood samples using rapid tests and according to prevailing
16
17 127 national algorithms.²⁶ This will be done at baseline.
- 18
19 128 • Pregnancy testing will be done using urine samples and standard operating procedures for
20
21 129 assessment of urine β -human chorionic gonadotropin (β hCG). This will be done at baseline
22
23 130 and before immunisation on each immunisation day.
- 24
25 131 • Full blood counts will be conducted using a haematology analyser. Mild, moderate and
26
27 132 severe anaemia will be defined according to WHO guidelines, by age.²⁷ This will be done at
28
29 133 baseline to test for anaemia as part of the eligibility assessment, and pre-immunisation as
30
31 134 part of the assessment of immunological profile.

32
33 135 Individuals found to be HIV positive or pregnant will be referred to appropriate providers for further
34
35 136 care. Individuals with severe anaemia (haemoglobin $<82\text{g/L}$) will be excluded from the randomised
36
37 137 intervention (since the intervention might be beneficial in management of anaemia). They will be
38
39 138 treated for anaemia.

40 41 139 ***Sample handling and archive***

42
43 140 Blood and other samples will be processed according to local laboratory standard operating
44
45 141 procedures (SOPs). All samples will reach the laboratory in anonymised form.

46
47 142 A sample archive will be developed. Although our current programme of work will address specific
48
49 143 hypotheses regarding pathways of effects of parasites and interventions, the sample archive will
50
51 144 provide a major asset for exploration of new leads arising from this work, or for an alternative,
52
53 145 “systems biology” approach employing (for example) proteomic, genomic, epigenetic and
54
55 146 transcriptomic analyses, and investigating the microbiome and virome. Information provided to
56
57 147 participants, and consent forms, will include considerations of sample storage, and the possibility of
58
59 148 sample analysis in laboratories within and outside Uganda. Participants will be able to decide if they
60
149 will permit such future use of any leftover samples. We plan to store the samples for up to 20 years.
150
151 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 ethical review committees.

1
2
3 152 If they elect not to permit this, all of those leftover samples will be discarded after the completion of
4
5 153 the work included in the current protocol.

6
7 154 ***Operational considerations***

8
9 155 *Programme governance*

10
11 156 A Programme Steering Committee has been set up to guide progress across all projects. This
12
13 157 comprises the following:

- 14
15 158 • An independent chair
- 16
17 159 • Representatives from the Ministry of Health programmes for immunisation and for vector
18
19 160 borne disease control
- 20
21 161 • Representatives of district authorities (Mukono and Jinja districts)
- 22
23 162 • Community representatives
- 24
25 163 • Principal investigator and co-investigators
- 26
27 164 • Project leader and post-doctoral immunologist
- 28
29 165 • Trial statistician
- 30
31 166 • Laboratory manager
- 32
33 167 • Medical Research Council observer

34
35 168 *Informed consent*

36
37 169 Both written informed assent from the participants and written informed consent from a parent or
38
39 170 guardian will be required for participation, although these may not necessarily be obtained at the
40
41 171 same time. Information will be provided in both English and the appropriate local language. For
42
43 172 individuals who cannot speak the languages used, or who cannot read or write, a witness who can
44
45 173 read the information sheet and translate the information to the participant or parent/guardian will
46
47 174 be used. Two different types of age specific assent forms will be used for the group of participants
48
49 175 aged 9 – 12 years and for the group aged 13 – 17 years. Informed consent by emancipated or mature
50
51 176 minors will be obtained using a designated consent form for these categories of participants.

52
53 177 The aims of the study, all tests, treatments and immunisations to be carried out and potential risks
54
55 178 will be explained. The participant will be given the opportunity to ask about details of the trial, and
56
57 179 will then have time to consider whether or not to participate. If they do decide to participate, they
58
59 180 and their parent/guardian will sign and date two copies of the assent and consent forms, one for
60
181 them to take away and keep, and one to be stored securely by the research team. Separate
182
information and consent forms will be provided (i) for consent for storage of samples for future

183 studies and for anonymous sharing of data from this study and (ii) for possible genetic studies; the
184 information sheet will explain that these data may be used in analyses related to this protocol.

185 *Screening and Eligibility Assessment*

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

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

194 *Enrolment*

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

197 *Discontinuation/withdrawal criteria*

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

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

210 Any participant who becomes pregnant during the trial will be followed up until the end of the
211 pregnancy but no further immunisations will be given unless indicated during pregnancy (as is the
212 case for tetanus toxoid). The trial allocation for this participant will be unblinded and the participant
213 will only be given further treatment if clinically indicated. The babies will also be followed up and

1
2
3 214 examined for any adverse effects. We will not routinely perform venepuncture in a pregnant
4
5 215 participant.

6
7 216 The reason for withdrawal will be recorded in the case report form (CRF). If withdrawal is due to an
8
9 217 AE, appropriate follow-up visits or medical care will be arranged, with the agreement of the
10
11 218 participant, until the AE has resolved, stabilised or a non-trial related causality has been assigned.

12
13 219 If a participant withdraws from the study samples collected before their withdrawal from the trial
14
15 220 will be used/ stored unless the participant specifically requests otherwise.

16 221 *Trial discontinuation*

17
18 222 The trial will be discontinued in the event of new scientific information that renders continuation
19
20 223 futile or unethical, or for any other reason, at the discretion of the Programme Steering Committee.

21
22 224 *End of study definition*

23
24 225 The trial will be completed when the last participant enrolled into the trial has completed their final
25
26 226 follow up visit.

27
28 227 *Safety assessments and oversight*

29
30 228 No new investigational drug or product will be used in the proposed trial. However, standard
31
32 229 approaches for monitoring safety and reporting of serious adverse events will be followed.

33
34 230 *Monitoring*

35
36 231 The trial will be monitored by both internal and external monitors according to a pre-defined
37
38 232 monitoring plan which will include a site initiation visit, monitoring visits at least annually, and a
39
40 233 close-out visit. The monitors will assess patient safety, data integrity, and adherence to the protocol
41
42 234 and to Good Clinical Research Practice procedures.

43
44 235 ***Considerations regarding standard of care***

45
46 236 *S. mansoni* infection status will be determined retrospectively through assays conducted in bulk on
47
48 237 stored samples (plasma CAA). These results will not, therefore, be useful to determine management
49
50 238 of individual participants.

51 239 Participants in the standard treatment arm will receive lower levels of anthelmintic treatment.

52
53 240 However, all trial arms will receive a minimum of well-implemented national standard of care.

54
55 241 Standard of care will comprise annual praziquantel treatment. Our own results from the Lake

56
57 242 Victoria Island Intervention Study on Worms and Allergy-related diseases (LaVIISWA),²⁸ which

58
59 243 compared annual versus quarterly intervention for schistosomiasis at community level over three
60

1
2
3 244 years, showed no advantage of quarterly treatment for morbidity outcomes attributed to
4
5 245 schistosomiasis.
6
7 246 Schistosomiasis can cause anaemia. To manage the expected differential benefits of the
8
9 247 interventions for anaemia, a full blood count will be performed at baseline, as discussed above;
10
11 248 anaemic children will be managed appropriately and severely anaemic children excluded.
12
13 249 Albendazole will be provided twice a year to manage nematode infections (after collection of
14
15 250 primary and secondary endpoint samples).

16 251 ***Procedures to be followed in the event of abnormal findings***

17
18 252 Abnormal clinical findings from medical history, examination or blood tests will be assessed as to
19
20 253 their clinical significance throughout the trials. If an abnormal test result is deemed clinically
21
22 254 significant, it may be repeated. If a test remains clinically significant, the participant will be informed
23
24 255 and appropriate medical care arranged as appropriate and with the permission of the participant.
25
26 256 Specific details regarding findings, discussion with participants and resulting actions will be recorded
27
28 257 in the clinical records. Decisions to exclude the participant from enrolling in the trial or to withdraw
29
30 258 a participant from the trial will be at the discretion of the Investigator.

31 259 ***Data and Safety Monitoring Board (DSMB)***

32
33 260 A data and safety monitoring board (DSMB) has been appointed to provide real-time safety
34
35 261 oversight. The DSMB will be notified within 7 days of the Investigators' being aware of the
36
37 262 occurrence of SAEs. The DSMB may recommend the Investigators to place the trial on hold if
38
39 263 deemed necessary following an intervention-related SAE. The DSMB will be chaired by a clinician
40
41 264 experienced in clinical trials. There will be a minimum of two other appropriately qualified
42
43 265 committee members. In the case of events related to a blinded intervention, the DSMB can request
44
45 266 unblinding. Membership will include a statistician, and at least one Ugandan member. All
46
47 267 correspondence between Investigators and the DSMB will be conveyed by the Principal Investigator
48
49 268 to the trial Sponsor. The Chair of the DSMB will be contacted for advice and independent review by
50
51 269 the Investigator or trial Sponsor in the following situations:

- 52 270 • The occurrence of any SAE
- 53 271 • Any other situation where the Investigator or trial Sponsor feels independent advice or
- 54 272 review is important

55 273 ***Ethical and regulatory considerations***

56
57
58 274 *Further information regarding risks*
59
60

1
2
3 275 The immunisations to be given have recognised side effects which are usually mild and resolve
4
5 276 spontaneously in a few days to one week. Parenteral vaccines are likely to result in pain and
6
7 277 swelling at the site of injection and mild fever; very occasionally pain and swelling can be severe and
8
9 278 associated with difficulty in moving the shoulder. Sometimes headache and tiredness occur. Rarely
10
11 279 a vaccine may cause a severe allergic reaction. For most vaccines this is estimated at less than one
12
13 280 in a million doses (but 1 in 55,000 for Yellow Fever vaccine).²⁹ Individuals with a history of a
14
15 281 possible allergic reaction to drugs or vaccines, or to vaccine components including eggs or chicken
16
17 282 proteins, will be excluded from the studies. The research team will be trained and prepared to
18
19 283 manage severe allergic reactions.

20
21 284 Adverse reactions to Yellow Fever vaccine include severe nervous system reaction (about 1 person in
22
23 285 125,000) and severe, life-threatening illness with organ failure (about 1 person in 250,000). The
24
25 286 mortality for this severe, life-threatening adverse effect is reported as about 50%.²⁹

26
27 287 BCG immunisation is likely to induce a scar in many cases. This may develop over several weeks,
28
29 288 starting as a small papule at the injection site which may become ulcerated and then heal over a
30
31 289 period of 2 to 5 months; and lymphadenopathy may develop. Occasionally a more severe local
32
33 290 reaction occurs (estimated at 1 per 1,000-10,000 doses): for example, an abscess develops and scars
34
35 291 may develop into keloids. Rarely BCG can cause disseminated disease (1 per 230,000 to 640,000
36
37 292 doses), or disease in sites remote from the immunisation site. Disseminated BCG disease usually
38
39 293 occurs in immunocompromised people: HIV positive people will be excluded from these studies.³⁰
40
41 294 BCG "pre-immunisation" may interfere with the response to the subsequent live vaccines; indeed
42
43 295 our hypothesis, and published results, suggest that it may suppress replication of YF 17D vaccine.³¹
44
45 296 However, this reduced replication has not been shown to correlate with, or result in, reduced levels
46
47 297 of neutralising antibody titres (which are the desired protective outcome).^{13 31}

48
49 298 Oral typhoid vaccine (Ty21a) may occasionally be associated with stomach pain, nausea, vomiting
50
51 299 and (rarely) rash.²⁹

52
53 300 Praziquantel has been in use for about 30 years. It has a well-recognised profile of side effects
54
55 301 including dizziness, nausea, vomiting, abdominal pain, diarrhoea (sometimes with blood) and
56
57 302 urticarial rash. The symptoms are considered to arise largely from the effects of killing worms and to
58
59 303 be more severe in people with heavy infections. Symptoms are better tolerated when the drugs are
60
304 given after food and we will provide treatment after a meal or snack. Simple medications, such as
305 paracetamol and cetirizine, can alleviate symptoms and these will be available on treatment days.

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MRC/UVRI and LSHTM Uganda Research Unit



Population differences in vaccine responses: the effect of intensive treatment for schistosomiasis on response to vaccines among island adolescents

Short Title: POPVAC Project A

Information Sheet for participants 9 – 12 years

1. Why are we meeting with you?

We are inviting you to take part in a research which is being done by a team from Uganda Virus Research Institute (UVRI), working together with colleagues from the London School of Hygiene and Tropical Medicine, UK. We are meeting you because you are aged between 9 and 12 years and study in a primary school on Koome island. After we tell you about it, we will ask if you'd like to be in this study or not. Only If you agree to take part, will you sign the assent form to show us that you are happy to do so.

The people leading this research study are Professor Alison Elliott and Dr Ludoviko Zirimenya.

2. Why are we doing this research?

We want to **find out whether treating Bilharzia much more frequently (a total of 7 times in a year) can get rid of it completely and whether this will improve vaccine responses.**

In the whole study, there will be about 480 children from schools of Koome islands.

3. What is going to happen in this research study?

Only if you agree, a full health check-up that will involve taking stool, urine and blood samples will be done. If everything is okay, the following will be done:

You will be put into one of the two **Bilharzia treatment** groups by chance

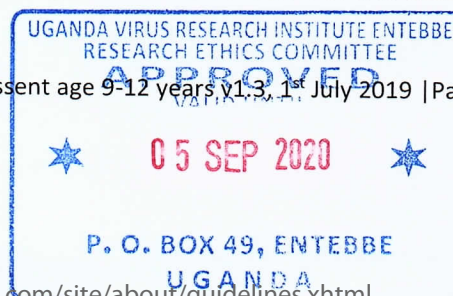
- One group will be treated for Bilharzia using praziquantel (the recommended medicine for Bilharzia) seven times in the year, and an eighth time after the end of the study.
- The other group will be treated for Bilharzia using praziquantel once in the year, and a second time after the end of the study.

You will be immunised with **five (5) vaccines**. These are:

- BCG vaccine. This is intended to protect against tuberculosis.
- Yellow fever vaccine. This protects against Yellow Fever an infection carried by mosquitoes.
- HPV vaccine. Human Papilloma Virus (HPV) causes growths in private parts and cancer in girls and boys.
- Tetanus and diphtheria vaccines. Tetanus and diphtheria are bacterial infections that cause very serious disease.
- Typhoid vaccine. Typhoid is a serious form of fever which is spread through contaminated food.

These immunisations will be given on different times during the entire duration of the study.

POPVAC Project A, assent age 9-12 years v1.3, 1st July 2019 | Page 1 of 5



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Most of the vaccines will be injected into your upper arm (either right or left side). The typhoid vaccine is an oral vaccine: it is in the form of capsules which are swallowed. You will be given one capsule per day for three alternating days.

You will be asked to give **blood samples**

We will ask you to give blood samples before and after you are given the vaccines. The amount of blood that we will take at each visit is completely safe and will vary between 4 and 27 millilitres (1 teaspoon and 5 and a half teaspoons) at the different visits.

You will be asked to give **stool and urine samples**

At some visits we will ask you for additional stool and urine samples for testing to check whether the worm infections have responded to treatment or (for girls) whether you have become pregnant.

Some of you will be asked to take part in a special **sugar test** of absorption from the intestines

We think that worm infections may make our intestines a little bit leaky, and this might explain some of their effects. We can test this using a special sugar drink. We will ask about 200 people to do this additional test. If you are asked to do this, it will be done about 8 weeks after your first vaccine is given. You will be asked not to eat overnight or in the morning of the test day. Then you will be given a special drink that contains sugars early in the morning. Afterwards you will also be asked to drink a litre of water (equal to two Rwenzori bottles) over the following few hours. All the urine that you pass during the next five hours will be collected. The volume (amount) of urine will be measured and the amount of sugar in it will be tested, to find out how much of the sugar your body has absorbed.

4. What will the blood, stool and urine samples be used for?

The samples will be used to test for infections, including HIV, malaria and worms, to test for anaemia (the strength of your blood) and to test for pregnancy (among girls). The blood samples will also be used to test how your body responds to vaccines. For some participants, the sugar test will be used to test absorption from the intestines.

5. Will taking part in this study harm me?

We do not expect this to harm you, though you will experience the following:

- You will need to take time off classes during each visit by the study team.
- Taking blood samples is not expected to cause any problem for you, apart from the discomfort or pain, bruising or bleeding, redness at the place where the needle goes into the skin, but this will go away in a few days.
- During the sugar test, you will be asked not to eat overnight and during the morning of the test so you may get hungry (although water and a snack will be provided). This may make you feel a little sick and stools may become a bit loose.
- If you are a girl, even if you know you could not be pregnant, we will do a pregnancy test. We have to be quite sure. You must as well promise that you can avoid getting pregnant while taking part in this study.

POPVAC Project A - assent age 9-12 years v1.3, 1st July 2019 | Page 2 of 5



MRC/UVRI and LSHTM Uganda Research Unit



- Treatment of Bilharzia with praziquantel may make you feel dizzy or sick, give you abdominal pain or diarrhoea, or occasionally cause an itchy rash. The research team will have medicines available to help you if you have a strong reaction to the treatment.
- All the study vaccines are known to be safe. However, even approved vaccines may very occasionally cause a serious reaction, such as an allergic reaction. The research doctors and nurses will be available to help if this happens.

6. Will the study help me?

The treatment for Bilharzia is likely to be good for you, whichever group you are put in. The vaccines are likely to help you by protecting you from infectious diseases. The research results may help all people in the world, because in the end we may get a better understanding whether treating Bilharzia much more frequently (a total of 7 times in a year) can get rid of it completely and whether this will improve vaccine responses.

7. What is the cost of taking part in the trial?

There is no cost to participate in this trial. You will receive a soft drink and the gift of a pen or other simple school material on days when blood samples are drawn, and the gift of a T-shirt at the end of the study when everything is done, in appreciation of your contribution to the work.

8. What happens if I refuse to take part?

It is very important for you to know that you do not have to take part in the research, the choice is yours. No-one will be upset if you decide not to take part. If you agree to take part and later decide that you do not want to take part anymore, that is also okay.

9. What happens if something goes wrong?

The researchers will make every effort to ensure your safety and well-being. MRC/UVRI and LSHTM has a specialist insurance policy in place which would help to pay for treatment if any harm came to you as a result of taking part in the research.

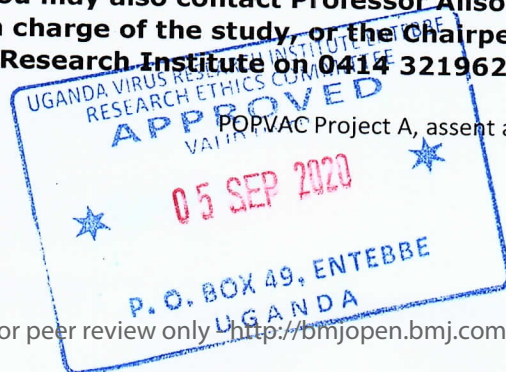
10. Who will have access to your information and samples from this research?

Only research staff trained to keep the information confidential will have access to the records. Your name will be removed from the records and samples, so no-one will be able to find out information about you from our records.

11. Who has reviewed the trial?

This trial has been reviewed by the Uganda National Council for Science and Technology, The Uganda National Drug Authority, the Uganda Virus Research Institute Research and Ethics Committee the ethics committee of the London School of Hygiene and Tropical Medicine in the United Kingdom.

You can find out more about the study at any time by asking one of the members of the research team. You may also contact Professor Alison Elliott (telephone: 0417 704000) who is in charge of the study, or the Chairperson of the Ethics Committee for the Uganda Virus Research Institute on 0414 321962.



POPVAC Project A, assent age 9-12 years v1.3, 1st July 2019 | Page 3 of 5



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Population differences in vaccine responses: the effect of intensive treatment for schistosomiasis on response to vaccines among island adolescents

Short Title: POPVAC Project A

Participant Assent

A copy of this form will be given to you. Please keep a copy of the form because it contains the information that was discussed with you and you may want to look at it again.

AGREEMENT TO TAKE PART:

I have read and understood (or been read to and understand) the information sheet for this study. My questions have been answered. I understand that taking part in the study is voluntary. I understand that at any time I may withdraw from this study without giving a reason. I agree to participate in this study.

Name: _____ **PVA ID:** |A|_|_|_|_|_|_|_|_|
(please write your name in capital letters here if you agree)

Signature: _____ **Date:** _____
(please sign or write your name here if you agree; or use a thumbprint)

What if I have any questions?

If you have any questions about your participation in this study, please feel free to ask any member of the research team at any time. If you prefer, you may speak to the principal investigator for this study (Professor Alison Elliott, telephone 0417 704000).

What if we want to ask someone independent anything about this research, or have any questions about your rights as a research participant? You may speak with the Chairman of the Science and Ethics Committee at Uganda Virus Research Institute on 0414 321962.



PVA ID: |A|_|_|_|_|

Witness:

I have read the participant information sheet and the assent statement above

to:..... (PRINT NAME OF PARTICIPANT) in a language which he/she understands. I believe that he/she gives assent to take part in the study.

.....
Witness name

.....
Signature

.....
Date

Witness required only for those using a thumb print instead of the final signature, or unable to read the information and consent form, or if the person taking consent does not speak the participant's language. The witness must not be a member of the research staff or a study participant. The witness must be present for the whole consent process.

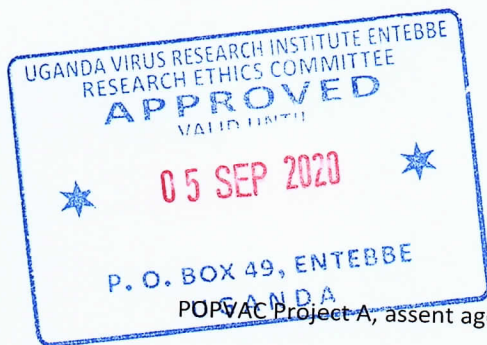
Person taking the assent:

.....
Researcher name

.....
Signature

.....
Date

Comment:



POPVAC Project A, assent age 9-12 years v1.3, 1st July 2019 | Page 5 of 5

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MRC/UVRI and LSHTM Uganda Research Unit



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Population differences in vaccine responses: the effect of intensive treatment for schistosomiasis on response to vaccines among island adolescents

Short Title: POPVAC Project A

Information Sheet for participants aged 13 to 17 years

We are inviting you to join in some research which is being done by a team from Uganda Virus Research Institute (UVRI), working together with colleagues from the London School of Hygiene and Tropical Medicine, UK. This form will tell you about the research. If you have any questions, please ask the person who is telling you about this research. Then you can decide whether you want to take part or not. There is no need to take part unless you really wish to. If you agree to take part, you will need to sign the assent form to show us that you are happy to do so. If you decide you do not want to take part, do not sign the assent form.

The people leading this research study are Professor Alison Elliott and Dr Ludoviko Zirimenya.

1. What this research is about, and the reason for doing this research.

This research is about how worm infections like Bilharzia "switch off" the body's defence systems and how this affects vaccine responses. **We want to find out whether treating Bilharzia much more frequently (a total of 7 times in a year) can get rid of it completely and whether this will improve vaccine responses.**

2. Why have I been asked to take part in this study?

You have been asked because you are attending primary school in Koome islands.

3. What is going to happen in this research study?

Only if you agree, a comprehensive health check-up that will involve taking stool, urine and blood samples will be done. If everything is okay, the following will be done:

You will be put into one of the two **Bilharzia treatment** groups by luck

- One group will be treated for Bilharzia using praziquantel (the recommended medicine for Bilharzia) seven times in the year, and an eighth time after the end of the study.
- The other group will be treated for Bilharzia using praziquantel once in the year, and a second time after the end of the study.

You will be immunised with **several vaccines**. These are the vaccines:

- BCG vaccine. This is intended to protect against tuberculosis.
- Yellow fever vaccine. This protects against Yellow Fever virus carried by mosquitoes.
- HPV vaccine. Human Papilloma Virus (HPV) causes growths in private parts and cancer in girls and boys.
- Tetanus and diphtheria vaccines. Tetanus and diphtheria are bacterial infections that cause very serious disease.
- Typhoid vaccine. Typhoid is a serious form of fever which is spread through contaminated food.

These immunisations will be given on three different days

POPVAC Project A, assent age 13-17 years v1.3, 15 July 2019 | Page 1 of 6





Most of the vaccines will be injected into your upper arm (either right or left side). This will be a bit painful, as for any injection of medicine that you may have had, and will feel a bit sore over the next week or so. The typhoid vaccine is an oral vaccine: it is in the form of capsules which are swallowed. You will be given one capsule per day for three alternating days.

You will be asked to give **blood samples**

We will ask you to give blood samples before and after you are given the vaccines. The amount of blood that we will take at each visit is completely safe and will vary between 4 and 27 millilitres (1 teaspoon and 5 and a half teaspoons) at the different visits

You will be asked to give **stool and urine samples**

At some visits we will ask you for additional stool and urine samples for testing to check whether the worm infections have responded to treatment or (for girls) whether you have become pregnant.

Some of you will be asked to take part in a special **sugar test** of absorption from the intestines

We think that worm infections may make our intestines a little bit leaky, and this might explain some of their effects. We can test this using a special sugar drink. We will ask about 200 people to do this additional test. If you are asked to do this, it will be done about 8 weeks after your first vaccine is given. You will be asked not to eat overnight or in the morning of the test day. Then you will be given a special drink that contains sugars early in the morning. Afterwards you will also be asked to drink a litre of water (equal to two Rwenzori bottles) over the following few hours. All the urine that you pass during the next five hours will be collected. The volume (amount) of urine will be measured and the amount of sugar in it will be tested, to find out how much of the sugar your body has absorbed.

4. What will the blood, stool and urine samples be used for?

The samples will be used to test for infections, including HIV, malaria and worms, to test for anaemia (the strength of your blood) and to test for pregnancy (among girls). The blood samples will also be used to test how your body responds to vaccines. For some participants, the sugar test will be used to test absorption from the intestines.

5. How many people will take part in the study, and how long will it last?

The whole study will enrol 480 people, and the study will last for about two years.

6. What are the risks of participating in this trial?

You will need to take time off classes during each visit by the study team.

Taking blood samples is not expected to cause any problem for you, apart from the discomfort or pain, bruising or bleeding, redness at the place where the needle goes into the skin, but this will go away in a few days.

During the sugar test you will be asked not to eat overnight and during the morning of the test so you may get hungry (although water and a snack will be provided). Most people have no problems with this test although a few may feel a little sick and stools may become a bit loose.

POPVAC Project A, assent age 13-17 years v1.3 | 1st July 2019 | Page 2 of 6



MRC/UVRI and LSHTM Uganda Research Unit



If you are a girl, even if you know you could not be pregnant. We have to be quite sure. We will test for pregnancy at the beginning of the study and on each immunisation day. You must as well promise that you can avoid getting pregnant while taking part in this study.

Treatment of Bilharzia with praziquantel may make you feel dizzy or sick, give you abdominal pain or diarrhoea, or occasionally cause an itchy rash. This is most likely caused by your body's response to the worms as they are being killed by the medicine. The research team will have medicines available to help you if you have a strong reaction to the treatment.

All the study vaccines are known to be safe though not often, may cause a serious reaction, such as an allergic reaction. The research doctors and nurses will be available to help if this happens.

7. What are the benefits of taking part in this trial?

The treatment for Bilharzia is likely to be good for you, whichever group you are put in. The vaccines are likely to help you by protecting you from infectious diseases.

Also, you will be helping us to find out why vaccines sometimes don't work so well in countries like Uganda, and whether vaccines work better if worms are treated first. This may help other people in the future.

8. What is the cost of taking part in the trial?

There is no cost to participate in this trial. You will receive a soft drink and the gift of a pen or other simple school material on days when blood samples are drawn, and the gift of a T-shirt at the end of the study when everything is done, in appreciation of your contribution to the work.

9. What happens if I refuse to take part?

It is very important for you to know that you do not have to take part in the research, the choice is yours. No-one will be upset if you decide not to take part. If you agree to take part and later decide that you do not want to take part anymore, that is also okay.

10. What happens if something goes wrong?

You will be making an important contribution to medical research. The researchers will make every effort to ensure your safety and well-being. MRC/UVRI and LSHTM has a specialist insurance policy in place which would help to pay for treatment if any harm came to you as a result of taking part in the research.

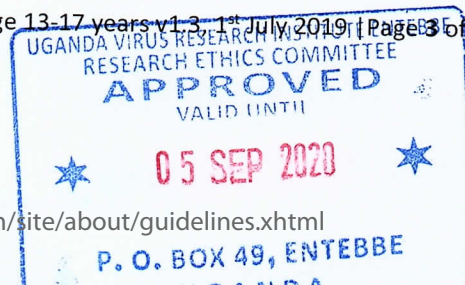
11. Who will have access to my information and samples from this research?

All our research records are stored securely in rooms with restricted access and on password protected computers. Only research staff trained to keep the information confidential will have access to the records. Your name will be removed from the records, so no-one will be able to find out information about you from our records.

12. Who has reviewed the trial?

This trial has been reviewed by the Uganda National Council for Science and Technology, Uganda National Drug Authority the Uganda Virus Research Institute Research and Ethics Committee the ethics committee of the London School of Hygiene and Tropical Medicine in the United Kingdom.

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You can find out more about the study at any time by asking one of the members of the research team. You may also contact Professor Alison Elliott (telephone: 0417 704000) who is in charge of the study, or the Chairperson of the Ethics Committee for the Uganda Virus Research Institute on 0414 321962.

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PVA ID: |A|_|_|_|_|_|

Witness:

I have read the participant information sheet and the assent statement above

to:..... (PRINT NAME OF PARTICIPANT) in a language which he/she understands. I believe that he/she gives assent to take part in the study.

.....
Witness name	Signature	Date

Witness required only for those using a thumb print instead of the final signature, or unable to read the information and consent form, or if the person taking consent does not speak the participant's language. The witness must not be a member of the research staff or a study participant. The witness must be present for the whole consent process.

Person taking the assent:

.....
Researcher name	Signature	Date

Comment:



MRC/UVRI and LSHTM Uganda Research Unit



Population differences in vaccine responses: the effect of intensive treatment for schistosomiasis on response to vaccines among island adolescents

Short Title: POPVAC Project A

Information Sheet for emancipated or mature minors

We are inviting you to join in some research which is being done by a team from Uganda Virus Research Institute (UVRI), working together with colleagues from the London School of Hygiene and Tropical Medicine, UK. This form will tell you about the research. If you have any questions, please ask the person who is telling you about this research. Then you can decide whether you want to take part or not. If you agree to take part, you will need to sign the consent form to show us that you are happy to do so. If you decide that you do not want to take part, do not sign the consent form.

The people leading this research study are Professor Alison Elliott and Dr Ludoviko Zirimenya.

1. What is this research about?

This research is about how worm infections like Bilharzia “switch off” the body’s defence systems and how this affects vaccine responses. **We want to find out whether treating Bilharzia much more frequently (a total of 7 times in a year) can get rid of it completely and whether this will improve vaccine responses.**

2. Why have I been asked to take part in this study?

You have been asked because you are attending primary school in Koome islands.

3. What is going to happen in this research study?

Only if you agree, you will have a comprehensive health check-up that will involve taking stool, urine and blood samples. If everything is okay, the following will be done:

You will be put into one of the two Bilharzia treatment groups by chance

One group will be treated for Bilharzia using praziquantel (the recommended medicine for Bilharzia) seven times in the year, and an eighth time after the end of the study.

The other group will be treated for Bilharzia using praziquantel once in the year, and a second time after the end of the study.



POPVAC Project A, consent for emancipated minors v1.1, 1st July 2019 | Page 1 of 6

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You will be immunised with **five (5) vaccines**. These are:

- **BCG vaccine.** This is intended to protect against tuberculosis.
- **Yellow fever vaccine.** This protects against Yellow Fever an infection carried by mosquitoes.
- **HPV vaccine.** Human Papilloma Virus (HPV) causes growths in private parts and cancer in girls and boys.
- **Tetanus and diphtheria vaccines.** Tetanus and diphtheria are bacterial infections that cause very serious disease.
- **Typhoid vaccine.** Typhoid is a serious form of fever which is spread through contaminated food.

These immunisations will be given on different times during the entire duration of the study.

Most of the vaccines will be injected into your upper arm (either right or left side). The typhoid vaccine is an oral vaccine: it is in the form of capsules which are swallowed. You will be given one capsule per day for three alternating days.

You will be asked to give **blood samples**

We will ask you to give blood samples before and after you are given the vaccines. The amount of blood that we will take at each visit is completely safe and will vary between 4 and 27 millilitres (1 teaspoon and 5 and a half teaspoons) at the different visits.

You will be asked to give **stool and urine samples**

At some visits we will ask you for additional stool and urine samples for testing to check whether the worm infections have responded to treatment or (for girls) whether you have become pregnant.

Some of you will be asked to take part in a special **sugar test** of absorption from the intestines

We think that worm infections may make our intestines a little bit leaky, and this might explain some of their effects. We can test this using a special sugar drink. We will ask about 200 people to do this additional test. If you are asked to do this, it will be done about 8 weeks after your first vaccine is given. You will be asked not to eat overnight or in the morning of the test day. Then you will be given a special drink that contains sugars early in the morning. Afterwards you will also be asked to drink a litre of water (equal to two Rwenzori bottles) over the following few hours. All the urine that you pass during the next five hours will be collected. The volume (amount) of urine will be measured and the amount of sugar in it will be tested, to find out how much of the sugar your body has absorbed.

POPVAC Project A, consent for emancipated minors v1.1, 1st July 2019 | Page 2 of 6



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4. What will the blood, stool and urine samples be used for?

The samples will be used to test for infections, including HIV, malaria and worms, to test for anaemia (the strength of your blood) and to test for pregnancy (among girls). The blood samples will also be used to test how your body responds to vaccines.

5. How many people will take part in the study, and how long will it last?

The whole study will enrol 480 people, and the study will last for about two years.

6. What are the risks of participating in this trial?

- You will need to take time off classes during each visit by the study team.
- Taking blood samples is not expected to cause any problem for you, apart from the discomfort or pain, bruising or bleeding, redness at the place where the needle goes into the skin, but this will go away in a few days.
- During the sugar test, you will be asked not to eat overnight and during the morning of the test so you may get hungry (although water and a snack will be provided). This may make you feel a little sick and stools may become a bit loose.
- If you are a girl, even if you know you could not be pregnant, we will do a pregnancy test. We have to be quite sure. You must as well promise that you can avoid getting pregnant while taking part in this study.
- Treatment of Bilharzia with praziquantel may make you feel dizzy or sick, give you abdominal pain or diarrhoea, or occasionally cause an itchy rash. The research team will have medicines available to help you if you have a strong reaction to the treatment.
- All the study vaccines are known to be safe. However, very occasionally even approved vaccines may cause a serious reaction, such as an allergic reaction. The research doctors and nurses will be available to help if this happens.

7. What are the benefits of taking part in this trial?

The treatment for Bilharzia is likely to be good for you, whichever group you are put in. The vaccines are likely to help you by protecting you from infectious diseases.

Also, you will be helping us to find out why vaccines sometimes don't work so well in countries like Uganda, and whether vaccines work better if worms are treated first. This may help other people in the future.



POPVAC Project A, consent for emancipated minors v1.1, 1st July 2019 | Page 3 of 6



8. What is the cost of taking part in the trial?

There is no cost to participate in this trial. You will receive a soft drink and the gift of a pen or other simple school material on days when blood samples are drawn, and the gift of a T-shirt at the end of the study when everything is done, in appreciation of your contribution to the work.

9. What happens if I refuse to take part?

It is very important for you to know that you do not have to take part in the research, the choice is yours. No-one will be upset if you decide not to take part. If you agree to take part and later decide that you do not want to take part anymore, that is also okay.

10. What happens if something goes wrong?

The researchers will make every effort to ensure your safety and well-being. MRC/UVRI and LSHTM has a specialist insurance policy in place which would help to pay for treatment if any harm came to you as a result of taking part in the research.

11. Who will have access to my information and samples from this research?

All our research records and samples are stored securely in rooms with restricted access and on password protected computers. Only research staff trained to keep the information confidential will have access to the them. Your name will be removed from the records, so no-one will be able to find out information about you from our records.

12. Who has reviewed the trial?

This trial has been reviewed by the Uganda National Council for Science and Technology, The Uganda National Drug Authority, the Uganda Virus Research Institute Research and Ethics Committee the ethics committee of the London School of Hygiene and Tropical Medicine in the United Kingdom.

You can find out more about the study at any time by asking one of the members of the research team. You may also contact Professor Alison Elliott (telephone: 0417 704000) who is in charge of the study, or the Chairperson of the Ethics Committee for the Uganda Virus Research Institute on 0414 321962.



PVA ID | A | ___ | ___ | ___ | ___ |

Witness:

I have read the participant information sheet and the consent statement above

to:..... (PRINT NAME OF PARTICIPANT) in a language which he/she understands. I believe that he/she gives consent to take part in the study.

.....
Witness name	Signature	Date

Witness required only for those using a thumb print instead of the final signature, or unable to read the information and consent form, or if the person taking consent does not speak the participant's language. The witness must not be a member of the research staff or a study participant. The witness must be present for the whole consent process.

Person taking the consent:

.....
Researcher name	Signature	Date

Comment:



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Population differences in vaccine responses: the effect of intensive treatment for schistosomiasis on response to vaccines among island adolescents

Short Title: POPVAC Project A

Information Sheet for parents/guardians

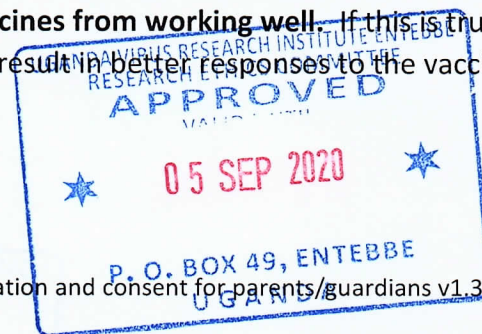
We are inviting your child to join in some research which is being done by a team from Uganda Virus Research Institute (UVRI), working together with colleagues from the London School of Hygiene and Tropical Medicine, UK. This form will tell you about the research. If you have any questions, please ask the person who is telling you about this research. Then you can decide whether you want your child to take part or not. There is no need for your child to take part unless you really want him/her to do so. If you agree for your child to take part, you will need to sign the consent form to show us that you are happy for him/her to do so. If you decide that you do not want your child to take part, do not sign the consent form.

The people leading this research study are Professor Alison Elliott and Dr Ludoviko Zirimenya.

1. What this research is about, and the reason for doing this research?

Vaccines are a very important tool for preventing infectious diseases. They have saved many lives. Vaccines are usually made from a weakened or killed strain of the bacteria or viruses that cause infectious diseases, or from a part of the bacteria or viruses. Vaccines are designed to help our body's defence system to recognise infectious diseases before we actually meet them, so that we can defeat them more easily. **However, some vaccines seem to work less well in hot countries, near the equator (such as Uganda), than in cooler countries (such as the United Kingdom, the UK).** We want to find out why this is so.

Worm infections are much more common in warm countries (such as Uganda) than in cooler countries (such as the UK). Bilharzia (schistosomiasis) is a worm infection which is very common in Koome islands. Almost everyone in Koome has Bilharzia. Worms can live in our bodies for many years. To do this they have to be able to switch off some of our body's defence systems so that they will not be killed. **We think that this "switching off" of defence systems may also prevent some vaccines from working well.** If this is true then treating worms before giving vaccines might result in better responses to the vaccines.



POPVAC Project A, information and consent for parents/guardians v1.3, 1st July 2019 | Page 1 of 9

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3 Light infections with Bilharzia may not be noticed but heavy infections can cause serious
4 damage, especially to the liver. That is why the Ugandan government usually gives treatment
5 for Bilharzia once a year in Ugandan schools. This usually reduces the number of Bilharzia
6 worms but does not get rid of them. **We want to find out whether treating Bilharzia much
7 more frequently (a total of 7 times in a year) can get rid of it completely and whether this will
8 improve vaccine responses.**
9
10

11 **2. Why has my child been asked to take part in this study?**

12
13 Your child has been asked because he/she is attending primary school in Koome islands, in
14 primary 1 to 6. Bilharzia infection is often heaviest in people of primary school age. Also, the
15 government of Uganda offers vaccines to your child's age group that will help to protect them
16 from infectious diseases later on. So it is important to know whether Bilharzia can affect these
17 vaccines, and whether treating Bilharzia effectively is helpful.
18
19

20 **3. What is going to happen in this research study?**

21 Your child will have a health check-up

22
23 If you want your child to take part in this study, we will first check your child's health, and take
24 stool samples, urine samples and some blood from a vein in your child's arm and do some tests.
25 These will include tests for infections including HIV, malaria and worms, tests for anaemia (the
26 strength of blood in your child's body), and tests for pregnancy if your child is a girl.
27
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29
30 If everything is okay then we will enrol your child in the study. If something is not okay then we
31 will either give your child the treatment that he/she needs, or tell you what to do.
32

33 Your child will be put into one of the two Bilharzia treatment groups

34
35 When your child is enrolled in the study we will put your child into one of two groups.
36

- 37 • One group will be treated for Bilharzia using praziquantel (the recommended medicine
38 for Bilharzia) seven times in the year, and an eighth time after the end of the study.
- 39 • The other group will be treated for Bilharzia using praziquantel once in the year, and a
40 second time after the end of the study.
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43 The choice of groups will be done using a code generated by computer. This works so that your
44 child is put into one group or the other by luck – this is like a lottery.
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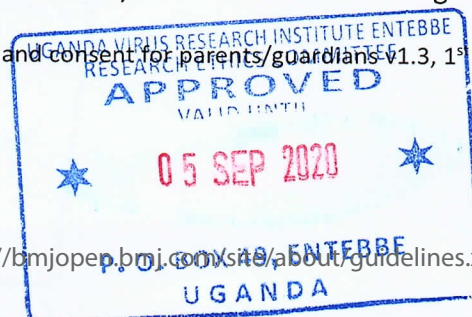
Your child will be immunised with **several vaccines**. These are the vaccines:

- BCG vaccine. This is intended to protect against tuberculosis. Tuberculosis is very common in Uganda. Many people receive the BCG vaccine as a baby. BCG can be given again at school age and may help to protect against tuberculosis later on although the level of protection varies a lot between countries. We do not know how well it works in school children in Uganda.
- Yellow fever vaccine. This protects against Yellow Fever. Yellow fever is a virus carried by mosquitoes. Yellow fever disease affects the liver and causes fever and jaundice (yellow eyes). Outbreaks occur in Uganda and neighbouring countries from time to time – an outbreak is when a group of people falls sick around the same time.
- HPV vaccine. Human Papilloma Virus (HPV) causes warts. As well, some strains of HPV can cause cancer in the genital areas, especially on the cervix (the opening of the womb) in girls. HPV can also cause cancer of the penis in boys, and other cancers. The HPV vaccine reduces the risk of infection with dangerous HPV strains that cause cancer. In this way it reduces the risk of cancers. The Ugandan government recently started offering this vaccine to girls in primary 4. We will give this vaccine to you if you are a girl (in any class) and you have not received it already. We will also give this vaccine to boys because it can protect them against some cancers too.
- Tetanus and diphtheria vaccines. Tetanus and diphtheria are bacterial infections. The tetanus and diphtheria bacteria produce chemicals called toxins which cause diseases. Tetanus bacteria infect deep wounds and produce tetanus toxin which causes very serious muscle spasms. Tetanus can affect young babies if the wound where the umbilical cord is cut becomes infected. Immunising young women can protect their future babies too. Diphtheria causes a very serious throat infection. Tetanus and diphtheria immunisation is given to babies but booster immunisation is recommended by the Uganda government for school children also.
- Typhoid vaccine. Typhoid is a serious form of fever which is spread through contaminated food. Outbreaks occur in Uganda. Immunisation against typhoid can prevent this illness.

These immunisations will be given at three different time points. BCG will be given first. HPV vaccine, oral typhoid vaccine and yellow fever vaccine will be given four weeks later. A second dose of HPV vaccine and first dose of Tetanus/diphtheria vaccine will be given at 28 weeks after the BCG immunisation.

Most of the vaccines will be injected into your child's upper arm (either right or left). This will be a bit painful, as for any injection of medicine that you may have had, and will feel a bit sore over the next week or so. The BCG vaccine is likely to form a small swelling and then an ulcer

POPVAC Project A, information and consent for parents/guardians v1.3, 1st July 2019 | Page 3 of 9



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3 which may take quite some time (perhaps two to five months) to heal. The typhoid vaccine is
4 an oral vaccine: it is in the form of capsules which are swallowed. Your child will be given one
5 capsule per day for three alternating days.
6

7 Your child will be asked to give **blood samples**

8
9 To test your child's response to the vaccines we will ask your child to give blood samples before
10 and after they are given the vaccines. The amount of blood that we will take at each visit is
11 completely safe and will vary between 4 and 27 millilitres (1 teaspoon and 5 and a half
12 teaspoons) at the different visits. In total we will ask your child to give blood samples at 7
13 different times during the study. The study will last for about two years.
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15

16 Your child will be asked to give **stool and urine samples**

17
18 At some visits we will ask your child for additional stool and urine samples for testing to check
19 whether the worm infections have responded to treatment or (for girls) whether your child has
20 become pregnant.
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23 Some of the children will be asked to take part in a special **sugar test** of absorption from the 24 intestines

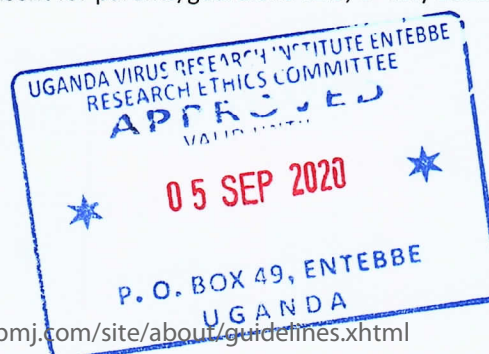
25
26 We think that worm infections may make our intestines a little bit leaky, and this might explain
27 some of their effects. We can test this using a special sugar drink. We will ask about 200
28 children to do this additional test. If your child is asked to do this, it will be done about 8 weeks
29 after the first vaccine is given. He/she will be asked not to eat overnight or in the morning of
30 the test day. Then he/she will be given a special drink that contains sugars early in the
31 morning. Afterwards he/she will also be asked to drink a litre of water (equal to two Rwenzori
32 bottles) over the following few hours. All the urine that he/she passes during the next five
33 hours will be collected. The volume (amount) of urine will be measured and the amount of
34 sugar in it will be tested, to find out how much of the sugar his/her body has absorbed.
35
36
37
38

39 **If you do not want your child to take part in the study, to have blood, urine and stool samples**
40 **taken or to receive the treatment and vaccines, you can say no to your child's taking part in**
41 **this study.**
42
43

44 **4. What will the blood, stool and urine samples be used for?**

45
46 The samples will be used to test for infections, including HIV, malaria and worms, to test for
47 anaemia (the strength of your child's blood) and to test for pregnancy (among girls). The blood
48 samples will also be used to test how your child's body responds to vaccines.
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53 POPVAC Project A, information and consent for parents/guardians v1.3, 1st July 2019 | Page 4 of 9



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7 **5. How many people will take part in the study, and how long will it last?**

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9 The whole study will enrol 480 people, and the study will last for about two years.

10
11 **6. What are the risks of participating in this trial?**

12
13 Your child's time

14
15 Your child will need to take time off classes during each visit by the study team. There will be
16 12 visits altogether. Each visit will usually take about 30 minutes, but the first visit may take
17 about two hours to give time for everything to be explained, and for a thorough check-up. The
18 research team will work with teachers to avoid disturbing classes too much.

19
20
21 Blood samples

22
23 Taking blood samples is not expected to cause any problem for your child, apart from the
24 discomfort or pain, bruising or bleeding, redness at the place where the needle goes into the
25 skin, but this will go away in a few days. There is a very small chance that your child may get an
26 infection or some swelling at this place – this almost never happens. Some people faint when
27 their blood is taken. The person taking the blood will do all they can to prevent these things
28 from happening.

29
30
31 Sugar test

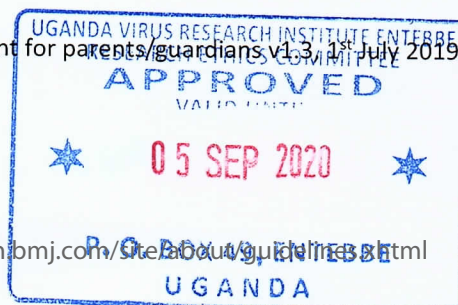
32
33 During the sugar test your child will be asked not to eat overnight and during the morning of
34 the test so he/she may get hungry (although water and a snack will be provided). Most people
35 have no problems with this test although a few may feel a little sick and stools may become a
36 bit loose.

37
38
39 Pregnancy

40
41 We do not know how some of these vaccines might affect a developing baby if they were given
42 to someone who was pregnant. That is why we will do a pregnancy test, if your child is a girl,
43 even if she knows she could not be pregnant. We have to be quite sure. We will test for
44 pregnancy at the beginning of the study and on each immunisation day. We will not enrol your
45 child in the study if she is pregnant at the start. We will not give your child the vaccines if she
46 falls pregnant later.

47
48
49 Bilharzia treatment

50
51 Treatment of Bilharzia with praziquantel may make your child feel dizzy or sick, give your child
52 abdominal pain or diarrhoea, or occasionally cause an itchy rash. This is most likely caused by



1
2
3 the body's response to the worms as they are being killed by the medicine. The research team
4 will have medicines available to help your child if he/she has a strong reaction to the treatment.
5

6 Immunisations

7
8 All the vaccines that we will give have been used to protect very large numbers of people and
9 are known to be safe. They are not expected to cause any major problems for your child. As
10 mentioned, the BCG vaccine is expected to cause an ulcer and to heal slowly, leaving a scar. The
11 injections will cause some pain at the time of injection and your child's arm will feel a bit sore
12 for a day or two. Some people develop flu-like symptoms including headache and fever for a
13 day or two. It is fine for your child to take painkillers like paracetamol (Panadol) for these
14 symptoms. The typhoid vaccine, which your child will swallow as capsules, is not expected to
15 cause your child any problem at all, although your child may experience some stomach pain,
16 feeling sick, vomiting and (rarely) rash.
17
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19
20
21 Very occasionally any vaccine can cause a serious reaction, such as an allergic reaction. The
22 research doctors and nurses will be available to help if this happens.
23

24 **7. What are the benefits of taking part in this trial?**

25
26 The treatment for Bilharzia is likely to be good for your child, whichever group he or she is put
27 in. The vaccines are likely to help your child by protecting him/her from infectious diseases.
28

29
30 Also, you and your child will be helping us to find out why vaccines sometimes don't work so
31 well in countries like Uganda, and whether vaccines work better if worms are treated first. This
32 may help other people in the future.
33

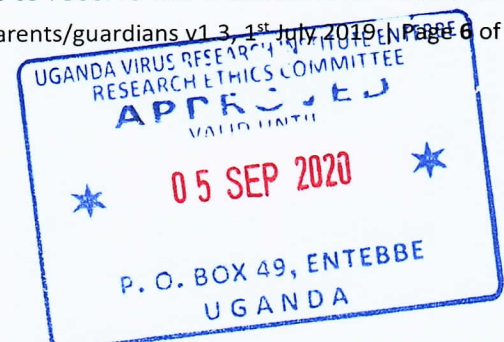
34 **8. What is the cost of taking part in the trial?**

35
36 There is no cost to participate in this trial. We will reimburse you (the parents) for the time you
37 spend at meetings at school, 20,000/= (twenty thousand shillings) for each visit. Your child will
38 receive a soft drink and the gift of a pen or other simple school material on days when blood
39 samples are drawn, and the gift of a T-shirt at the end of the study when everything is done, in
40 appreciation of his/her contribution to the work.
41
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45 **9. What happens if I refuse for my child to take part?**

46
47 It is very important for you to know that your child does not have to take part in the research,
48 the choice is yours and your child's. No-one will be upset if your child decides not to take part.
49 The teachers will not be upset and the research team will not be upset. If you agree for your
50 child to take part and later decide that you do not want him/her to take part anymore, that is
51 also okay. Whatever happens, your child will still be able to receive the treatment for Bilharzia
52

53 POPVAC Project A, information and consent for parents/guardians v1.3, 1st July 2019, Page 6 of 9



and the immunisations for HPV and tetanus and diphtheria when they are provided by the government, if you wish.

10. Who will be able to see the results of tests done on my child's samples?

The research team will keep your child's results private. Only members of the research team will be able to see results and to know that they belong to your child. Your child's samples will be given a special code so that anyone who is working on them in research laboratories will not know they came from your child.

11. What happens if something goes wrong?

You and your child will be making an important contribution to medical research. The researchers will make every effort to ensure your child's safety and well-being. MRC/UVRI and LSHTM has a specialist insurance policy in place which would help to pay for treatment if any harm came to your child as a result of taking part in the research.

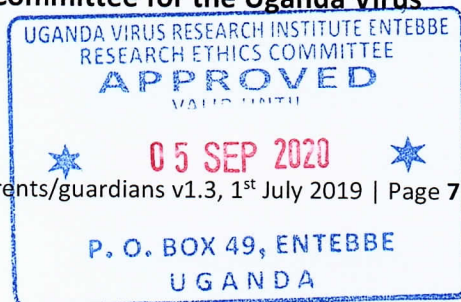
12. Who will have access to information from this research?

All our research records are stored securely in rooms with restricted access and on password protected computers. Only research staff trained to keep the information confidential will have access to the records. Your child's name will be removed from the records, so no-one will be able to find out information about your child from our records. The people who may review your child's records include Research Ethics Committees (Uganda Virus Research Institute Research Ethics Committee and the London School of Hygiene & Tropical Medicine Ethics Committee) the Uganda National Council Science and Technology, Study Monitors, Sponsor and the Uganda National Drug Authority. These organisations are there to ensure that your child's rights are protected and that the research is conducted properly and safely.

13. Who has reviewed the trial?

This trial has been reviewed by the Uganda National Council for Science and Technology, the Uganda Virus Research Institute Research and Ethics Committee, the ethics committee of the London School of Hygiene and Tropical Medicine in the United Kingdom. The Uganda National Drug Authority, which regulates the use of all medicines in Uganda, has granted permission to use the medicines and vaccines needed for this clinical trial.

You can find out more about the study at any time by asking one of the members of the research team. You may also contact Professor Alison Elliott (telephone: 0417 704000) who is in charge of the study, or the Chairperson of the Ethics Committee for the Uganda Virus Research Institute on 0414 321962.



POPVAC Project A, information and consent for parents/guardians v1.3, 1st July 2019 | Page 7 of 9

MRC/UVRI and LSHTM Uganda Research Unit



Population differences in vaccine responses: the effect of intensive treatment for schistosomiasis on response to vaccines among island adolescents

Short Title: POPVAC Project A

Parent/guardian consent

A copy of this form will be given to you. Please keep a copy of the form because it contains the information that was discussed with you and you may want to look at it again.

Participant (child) name: _____ **PVA ID|A|**____|____|____|____|
(please write your child's name in capital letters here if you agree)

AGREEMENT BY PARENT OR GUARDIAN:

I have read and/or been fully explained the information sheet concerning my child's participation in this study and I understand what will be required if he or she takes part. I understand that my child's participation is voluntary. My questions concerning this study have been answered. I understand that at any time, I may withdraw my child from this study without giving a reason and without affecting his or her entitlement to government health care. I agree for my child to take part in this study.

Name: _____
(please write your name in capital letters here if you agree)

Signature: _____ **Date:** _____
(please sign or write your name here if you agree; or use a thumbprint)

What if I have any questions?

If you have any questions about your participation in this study, please feel free to ask any member of the research team at any time. If you prefer, you may speak to the principal investigator for this study (Professor Alison Elliott, telephone 0417 704000).

What if we want to ask someone independent anything about this research, or have any questions about your rights as a research participant? You may speak with the Chairman of the Science and Ethics Committee at Uganda Virus Research Institute on 0414 321962.

POPVAC Project A, information and consent for parents/guardians v1.3, 1st July 2019 | Page 8 of 9



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PVA ID | A | | | | |

Witness:

I have read the participant information sheet and the assent statement above

to:..... (PRINT NAME OF PARTICIPANT) in a language which he/she understands. I believe that he/she gives consent for his/her child to take part in the study.

.....
Witness name

.....
Signature

.....
Date

Witness required only for those using a thumb print instead of the final signature, or unable to read the information and consent form, or if the person taking consent does not speak the participant's language. The witness must not be a member of the research staff or a study participant. The witness must be present for the whole consent process.

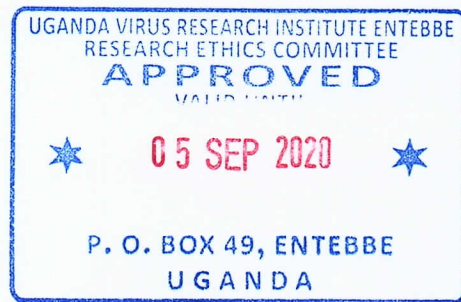
Person taking the consent:

.....
Researcher name

.....
Signature

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Date

Comment:



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Reporting checklist for protocol of a clinical trial.

Based on the SPIRIT guidelines.

Instructions to authors

Complete this checklist by entering the page numbers from your manuscript where readers will find each of the items listed below.

Your article may not currently address all the items on the checklist. Please modify your text to include the missing information. If you are certain that an item does not apply, please write "n/a" and provide a short explanation.

Upload your completed checklist as an extra file when you submit to a journal.

In your methods section, say that you used the SPIRIT reporting guidelines, and cite them as:

Chan A-W, Tetzlaff JM, Altman DG, Laupacis A, Gøtzsche PC, Krleža-Jerić K, Hróbjartsson A, Mann H, Dickersin K, Berlin J, Doré C, Parulekar W, Summerskill W, Groves T, Schulz K, Sox H, Rockhold FW, Rennie D, Moher D. SPIRIT 2013 Statement: Defining standard protocol items for clinical trials. *Ann Intern Med.* 2013;158(3):200-207

	Reporting Item	Page Number
Administrative information		
Title	#1 Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	1
Trial registration	#2a Trial identifier and registry name. If not yet registered, name of intended registry	2
Trial registration: data set	#2b All items from the World Health Organization Trial Registration Data Set	n/a
Protocol version	#3 Date and version identifier	Information available at ISRCTN60517191
Funding	#4 Sources and types of financial, material, and other support	15
Roles and	#5a Names, affiliations, and roles of protocol	15

responsibilities: contributorship		contributors	
Roles and responsibilities: sponsor contact information	#5b	Name and contact information for the trial sponsor	Information available at ISRCTN60517191
Roles and responsibilities: sponsor and funder	#5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities	16
Roles and responsibilities: committees	#5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)	Information detailed in supplementary information file
Introduction			
Background and rationale	#6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention	4
Background and rationale: choice of comparators	#6b	Explanation for choice of comparators	8
Objectives	#7	Specific objectives or hypotheses	6
Trial design	#8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, non-inferiority, exploratory)	7

**Methods:
Participants,
interventions, and
outcomes**

1	Study setting	#9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained	7
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8	Eligibility criteria	#10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists)	7
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14	Interventions:	#11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	8
15	description			
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20	Interventions:	#11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving / worsening disease)	Detailed in supplementary information file
21	modifications			
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27	Interventions:	#11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return; laboratory tests)	Detailed in supplementary information file
28	adherence			
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32	Interventions:	#11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	Detailed in supplementary information file
33	concomitant care			
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37	Outcomes	#12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended	10
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50	Participant timeline	#13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure)	14
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53				Also detailed in supplementary information file, Table S1
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1	Sample size	#14	Estimated number of participants needed to achieve	11
2			study objectives and how it was determined,	
3			including clinical and statistical assumptions	
4			supporting any sample size calculations	
5				
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7				
8	Recruitment	#15	Strategies for achieving adequate participant	Detailed in
9			enrolment to reach target sample size	supplementary
10				information file
11				
12				

13 **Methods:**

14 **Assignment of** 15 **interventions (for** 16 **controlled trials)**

19				
20	Allocation: sequence	#16a	Method of generating the allocation sequence (eg,	8
21	generation		computer-generated random numbers), and list of	
22			any factors for stratification. To reduce predictability	
23			of a random sequence, details of any planned	
24			restriction (eg, blocking) should be provided in a	
25			separate document that is unavailable to those who	
26			enrol participants or assign interventions	
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31	Allocation	#16b	Mechanism of implementing the allocation sequence	9
32	concealment		(eg, central telephone; sequentially numbered,	
33	mechanism		opaque, sealed envelopes), describing any steps to	
34			conceal the sequence until interventions are assigned	
35				
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38	Allocation:	#16c	Who will generate the allocation sequence, who will	8, 9
39	implementation		enrol participants, and who will assign participants to	
40			interventions	
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43	Blinding (masking)	#17a	Who will be blinded after assignment to	9
44			interventions (eg, trial participants, care providers,	
45			outcome assessors, data analysts), and how	
46				
47				
48				
49	Blinding (masking):	#17b	If blinded, circumstances under which unblinding is	Detailed in
50	emergency		permissible, and procedure for revealing a	supplementary
51	unblinding		participant's allocated intervention during the trial	information file
52				
53				

54 **Methods: Data** 55 **collection,** 56 **management, and** 57 **analysis**

1	Data collection plan	#18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol	10. More details in supplementary information file
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14	Data collection plan: retention	#18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols	Detailed in supplementary information file
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21	Data management	#19	Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol	13. More information in the statistical analysis plan found at ISRCTN60517191
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31	Statistics: outcomes	#20a	Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol	13. More information in the statistical analysis plan found at ISRCTN60517191
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40	Statistics: additional analyses	#20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	11. More information in the statistical analysis plan found at ISRCTN60517191
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49	Statistics: analysis population and missing data	#20c	Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation)	Information in the statistical analysis plan found at ISRCTN60517191
50				
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Methods: Monitoring

1	Data monitoring:	#21a	Composition of data monitoring committee (DMC);	Detailed in
2	formal committee		summary of its role and reporting structure;	supplementary
3			statement of whether it is independent from the	information file
4			sponsor and competing interests; and reference to	
5			where further details about its charter can be found,	
6			if not in the protocol. Alternatively, an explanation of	
7			why a DMC is not needed	
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12	Data monitoring:	#21b	Description of any interim analyses and stopping	Detailed in
13	interim analysis		guidelines, including who will have access to these	supplementary
14			interim results and make the final decision to	information file
15			terminate the trial	
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19	Harms	#22	Plans for collecting, assessing, reporting, and	Detailed in
20			managing solicited and spontaneously reported	supplementary
21			adverse events and other unintended effects of trial	information file
22			interventions or trial conduct	
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26	Auditing	#23	Frequency and procedures for auditing trial conduct,	Detailed in
27			if any, and whether the process will be independent	supplementary
28			from investigators and the sponsor	information file
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31	Ethics and			
32	dissemination			
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35	Research ethics	#24	Plans for seeking research ethics committee /	12
36	approval		institutional review board (REC / IRB) approval	
37				
38				
39	Protocol	#25	Plans for communicating important protocol	11
40	amendments		modifications (eg, changes to eligibility criteria,	
41			outcomes, analyses) to relevant parties (eg,	
42			investigators, REC / IRBs, trial participants, trial	
43			registries, journals, regulators)	
44				
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47	Consent or assent	#26a	Who will obtain informed consent or assent from	12.
48			potential trial participants or authorised surrogates,	
49			and how (see Item 32)	
50				Also detailed in
51				supplementary
52				information file
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55	Consent or assent:	#26b	Additional consent provisions for collection and use	Detailed in
56	ancillary studies		of participant data and biological specimens in	supplementary
57			ancillary studies, if applicable	information file
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1	Confidentiality	#27	How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial	Detailed in supplementary information file
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8	Declaration of interests	#28	Financial and other competing interests for principal investigators for the overall trial and each study site	15
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11	Data access	#29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators	n/a
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17	Ancillary and post trial care	#30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation	Detailed in supplementary information file
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22	Dissemination policy: trial results	#31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions	2, 13
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32	Dissemination policy: authorship	#31b	Authorship eligibility guidelines and any intended use of professional writers	n/a
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36	Dissemination policy: reproducible research	#31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	13
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41	Appendices			
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43	Informed consent materials	#32	Model consent form and other related documentation given to participants and authorised surrogates	Supplementary files provided
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47	Biological specimens	#33	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable	n/a
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