THE LANCET
Global Health

Supplementary appendix 2

This appendix formed part of the original submission and has been peer reviewed. We post it as supplied by the authors.

Supplement to: Nkurunungi G, Nassuuna J, Natukunda A, et al. The effect of intensive praziquantel administration on vaccine-specific responses among schoolchildren in Ugandan schistosomiasis-endemic islands (POPVAC A): an open-label, randomised controlled trial. *Lancet Glob Health* 2024; **12:** e1826–37.

SUPPLEMENTARY INFORMATION FOR

The effect of intensive praziquantel administration on vaccine-specific responses among schoolchildren in Ugandan schistosomiasis-endemic islands: results of the POPVAC A randomised, controlled trial

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Supplementary tables

Table S1. Praziquantel administration

Weeks from BCG vaccination	**-6**	**-4**	**-2**	**8**	**20**	**32[a]**	**44**	**52**
	n/N(%)[§]	n/N(%)[§]	n/N(%)[§]	n/N(%)[§]	n/N(%)[§]	n/N(%)[§]	n/N(%)[§]	n/N(%)[§]
Intensive arm	219/239 (91.6)	213/239 (89.1)	201/239 (84.1)	192/239 (80.3)	172/239 (72.0)	_	166/239 (69.5)	184/239 (77.0)
Standard arm				199/239 (83.3)				166/239 (69.5)

[§] n/N (%) Number received treatment/Total number (percent who received treatment)

a Praziquantel (PZQ) at week 32 was not given due to Covid-19 lockdown.

173/239(72.4%) participants in the intensive arm received all three doses of PZQ before BCG vaccination.

237/239 (99.2%) participants in the intensive arm received at least one dose of PZQ before BCG vaccination.

Table S2. Adverse events

S2A. Summary of adverse events

	Intensive arm N=239		**Standard arm N=239**	
Type of event	**All AEs**	**Possibly, Probably or Definitely Related Adverse Events[a]**	**All AEs**	**Possibly, Probably or Definitely Related Adverse Events[a]**
Subjects with at least one AE [n (%)]	46(19.2%)	43(18.0%)	12(5.0%)	10(4.2%)
Total # AEs [n (number with grade 3)]	62(0)	53(0)	12(0)	10(0)
Average # of AEs per subject experiencing AEs.*	1.3	1.2	1.0	1.0
Subjects experiencing a SAE [n (%)]	0	0	0	0
Total # of SAEs	0	0	0	0

AE: adverse event; [a]relation is to any study intervention. *Calculated as the number of AEs divided by the number of subjects experiencing any AE.

S2B. Details of adverse events

	Intensive arm N=239	**Standard arm N=239**
Adverse event	**Related events**	**Related events**
Abdominal pains		
Mild	23	2
Moderate	9	5
Dizziness		
Mild	4	
Moderate		
Diarrhoea		
Mild	3	2
Moderate	7	1
Vomiting		

Mild	1	
Moderate		
Swelling at injection site		
Mild	2	
Moderate		
Tenderness or pain at injection site		
Mild	1	
Moderate		
Headache		
Mild	3	
Moderate		

Table S3. Number of participants vaccinated in each treatment arm.

	Week 0 BCG	**Week 4** HPV, YF-17D and Ty21a	**Week 8** HPV*	**Week 28** HPV and Td	**Week 52** Td**
	n/N(%)[§]	n/N(%)[§]	n/N(%)[§]	n/N(%)[§]	n/N(%)[§]
Intensive arm	228/239 (95.4)		1/4 (25.0)	183/239 (76.6)	186/239 (77.8)
Standard arm	219/239 (91.6)		5/9 (55.6)	171/239 (71.5)	167/239 (69.9)

	Week 4 HPV	**Week 4** YF-17D	**Week 4*** ** Ty21a		
	n/N(%)[§]	**n/N(%)[§]**	**n/N(%)[§]**		
			Dose 1	**Dose 2**	**Dose 3**
Intensive arm	205/239 (85.8)	205/239 (85.8)	201/239 (84.1)	183/239 (76.6)	155/239 (64.9)
Standard arm	206/239 (86.2)	206/239 (86.2)	202/239 (84.5)	184/239 (77.0)	161/239 (67.4)

[§] n/N (%) Number vaccinated/Number expected at the time of vaccination (Percent who were vaccinated); at each visit.

*Among girls aged >14 years who had not received HPV vaccine before this trial

**Td at week 52 provided as a service

***140/239(58.6%) participants in the intensive arm received all three doses of oral typhoid vaccination.

***152/239(63.6%) participants in the standard arm received all three doses of oral typhoid vaccination.

***203/239(84.9%) participants in the intensive arm received at least one dose of oral typhoid vaccination.

***202/239(84.5%) participants in the standard arm received at least one dose of oral typhoid vaccination.

Table S4. Baseline vaccine responses

Antigen specific response	**n**	**Intensive arm** (N=239) Median (IQR)	**n**	**Standard arm** (N=239) Median (IQR)	**p value**
BCG-specific IFN-ɣ (SFUs/1 million PBMCs)	143	71.6 (35.0-113.3)	142	62.5 (35.0-103.3)	0.60
Yellow fever $PRNT_{50}$ titres	228	<10 (<10 - <10)	219	<10 (<10 - <10)	NA
Yellow fever $PRNT_{90}$ titres	228	<10 (<10 - <10)	219	<10 (<10 - <10)	NA
S. Typhi O:LPS-specific IgG (EU/ml)	228	123.4 (60.0-227.8)	219	115.8 (50.6-282.9)	0.89
HPV-16-specific IgG (EU/ml)	228	3.5 (2.4-5.6)	219	3.7 (2.5-5.5)	0.74
HPV-18-specific IgG (EU/ml)	228	62.5 (41.4- 87.1)	219	62.0 (41.4-87.6)	0.92
Tetanus toxoid-specific IgG (IU/ml)	183	0.075 (.0375-0.135)	172	0.062 (0.032-0.149)	0.66
Diphtheria toxoid-specific IgG (IU/ml)	183	0.051 (0.015-0.153)	172	0.044 (0.008-0.198)	0.30

IQR: interquartile range; NA: not applicable

Table S5. Impact of intensive vs standard praziquantel administration on vaccine responses in participants who were *Sm* infected at baseline but were *Sm* uninfected by the time of first vaccination in the intensive arm.

Arm	n	GM(SE)	GMR (95% CI)	P value
BCG-specific IFN-ɣ (8 weeks post-vaccination)				
Intensive	43	225.817(1.117)	1.293 (0.979-1.708)	0.07
Standard[§]	83	174.657(1.087)	Ref.	
Yellow fever $PRNT_{50}$ titres (4 weeks post-vaccination)				
Intensive	53	1872.634(1.182)	1.194 (0.782-1.822)	0.41
Standard[§]	103	1568.924(1.136)	Ref.	
Yellow fever $PRNT_{90}$ titres (4 weeks post-vaccination)				
Intensive	53	182.340(1.173)	1.132 (0.759-1.688)	0.54
Standard[§]	103	161.121(1.127)	Ref.	
***S.* Typhi O:LPS-specific IgG (4 weeks post-vaccination)**				
Intensive	53	428.675(1.161)	0.969 (0.662-1.419)	0.87
Standard[§]	101	442.465(1.123)	Ref.	
HPV-16-specific IgG (4 weeks post-vaccination)				
Intensive	53	**71.646(1.187)**	**0.595 (0.409-0.867)**	**0.007**
Standard[§]	103	**120.378(1.110)**	**Ref.**	
HPV-18-specific IgG (4 weeks post-vaccination)				
Intensive	**53**	**419.965(1.116)**	**0.746 (0.555-1.004)**	**0.053**
Standard[§]	**103**	**562.963(1.096)**	**Ref.**	
Tetanus toxoid-specific IgG (24 weeks post-vaccination)*				
Intensive	24	4.827(1.246)	1.135 (0.716-1.800)	0.58
Standard[§]	54	4.253(1.127)	Ref.	
Diphtheria toxoid-specific IgG (24 weeks post-vaccination)*				
Intensive	24	1.625(1.087)	0.932 (0.741-1.172)	0.54
Standard[§]	54	1.744(1.069)	Ref.	

CI: confidence interval; GM: geometric mean; GMR: geometric mean ratio; SE: standard error

[§]Table compares the intensive arm participants who were initially *Sm* infected (CAA>30pg/ml) and later reduced their CAA levels to less than 30pg/ml, to the standard arm individuals who were initially infected and remained infected at the time they received the first vaccination.

*We compared the intensive arm participants who were initially *S. mansoni* infected (CAA>30pg/ml) but later reduced their CAA levels to less than 30pg/ml by the time of vaccination with Td (week 28), to the standard arm individuals who were initially infected and remained infected by week 28.

Table S6. Differences in vaccine responses between intensive and standard arms (area under the curve for weeks 8 to 52; primary analysis population)

Trial arm	n	GM (AUC) (SE)	GMR (AUC) (95% CI)	P value
BCG-specific IFN-ɣ				
Intensive	85	8591(1.072)	1.165 (0.949, 1.430)	0.14

Standard	81	7373(1.080)	Ref.	
Yellow fever $PRNT_{50}$ titres				
Intensive	108	109572(1.089)	1.049 (0.826, 1.331)	0.70
Standard	102	104494(1.090)	Ref.	
Yellow fever $PRNT_{90}$ titres				
Intensive	108	11945(1.087)	1.056 (0.828, 1.347)	0.66
Standard	102	11314(1.096)	Ref.	
***S.* Typhi O:LPS-specific IgG**				
Intensive	108	17828(1.103)	1.198 (0.895, 1.602)	0.22
Standard	101	14886(1.117)	Ref.	
HPV-16-specific IgG				
Intensive	101	12082(1.126)	0.764 (0.547, 1.066)	0.11
Standard	91	15818(1.128)	Ref.	
HPV-18-specific IgG				
Intensive	101	33019(1.094)	0.872 (0.674, 1.129)	0.30
Standard	91	37864(1.099)	Ref.	

AUC: area under the curve; CI: confidence interval; GM: geometric mean; GMR: geometric mean ratio; SE: standard error

Table S7. Proportions with protective vaccine-specific responses compared between intensive and standard arm participants

	Intensive arm	**Standard arm**	**Proportion difference for protection (95% CI)**	**P value***
	n/N (%)	n/N (%)		
Yellow fever seropositivity ($PRNT_{50}$ titre≥10)[#]	184/184 (100.0)	194/194 (100.0)		
Protective tetanus toxoid-specific IgG levels (≥0.1IU/ml)[¶]	166/168 (98.8)	142/146 (97.3)	0.015 (-0.016, 0.047)	0.32
Four-fold increase in *S.* Typhi O:LPS-specific IgG from baseline[#]	83/181 (45.9)	69/185 (37.3)	0.086 (-0.015, 0.186)	0.097

*P values reported from chi square test
[#]4 weeks post-vaccination
[¶]24 weeks post-vaccination
Reference for the risk difference is the standard arm.

Table S8. Priming versus boosting: Comparison of HPV responses between trial arms after priming and boosting doses.

Intensive: n=138; Standard: n=131	**GMR (95%) CI**	**P value**	**Interaction p**
HPV-16-specific IgG – intensive arm			0.78
Week 52 vs week 8	3.90 (3.11- 4.89)	<0.0001	
HPV-16-specific IgG – standard arm			
Week 52 vs week 8	3.73 (2.95-4.70)	<0.0001	
HPV-18-specific IgG – intensive arm			0.67
Week 52 vs week 8	1.53 (1.28-1.85)	<0.0001	
HPV-18-specific IgG – standard arm			

Week 52 vs week 8	1.45 (1.20-1.75)	<0.0001	

Includes those who received 2 doses of HPV during the study and had responses at weeks 8 and 52. CI: confidence interval; GMR: geometric mean ratio

Supplementary figures

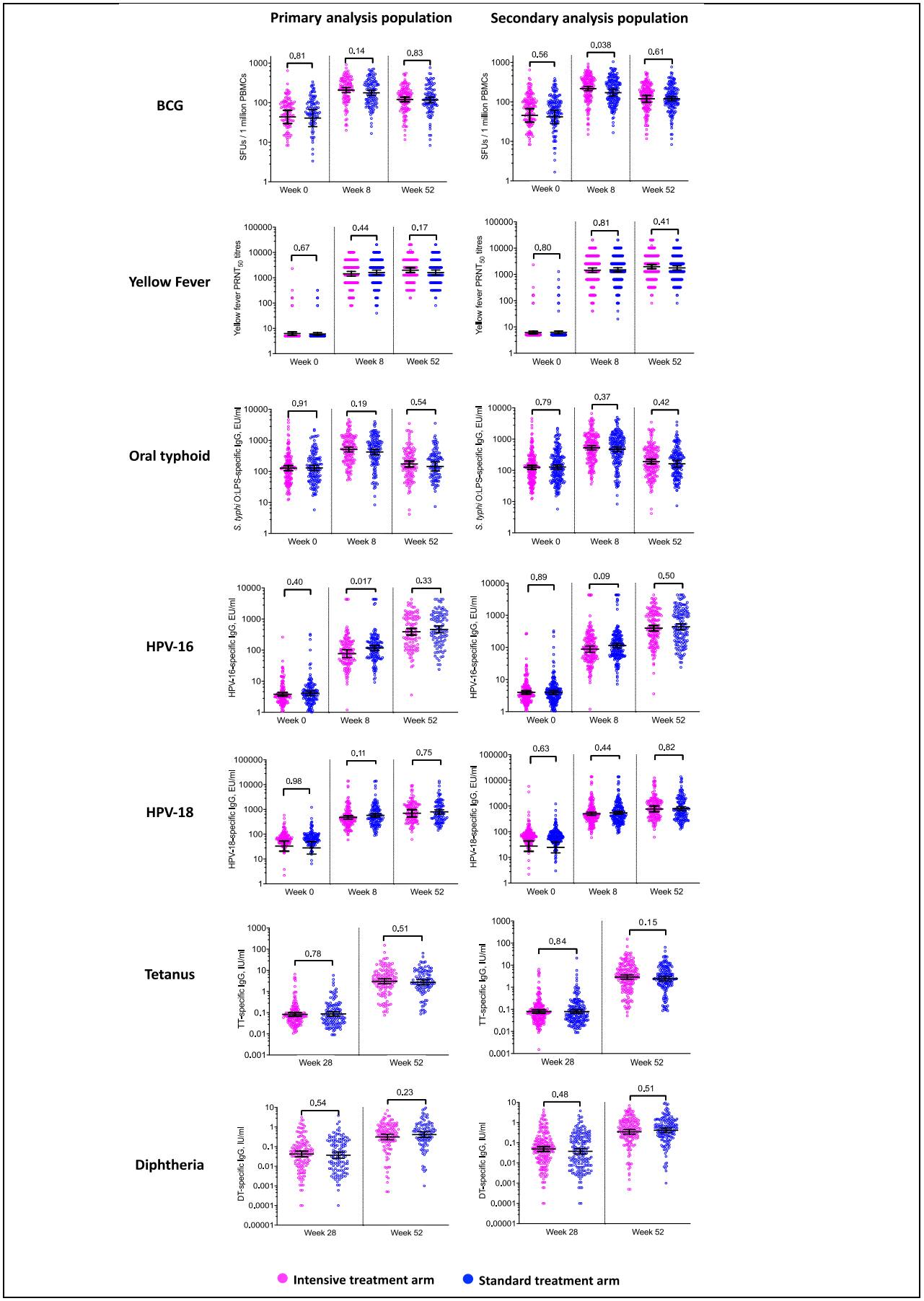

Figure S1. Impact of intensive versus standard praziquantel administration on vaccine responses. Plots show individual data points, a horizontal line and whiskers denoting the geometric mean and 95% CI, respectively. The primary analysis population was participants who had *Schistosoma mansoni* circulating anodic antigen (CAA)≥30pg/ml or who were PCR positive at baseline; the secondary analysis population was all randomised participants. **SFUs**: ELISpot assay spot forming units; **PBMCs**: peripheral blood mononuclear cells; **$PRNT_{50}$**: plaque reduction neutralizing reference tests at 50% neutralization; **HPV-16**: Human Papillomavirus type 16; **HPV-18**: Human Papillomavirus type 18; **TT**: tetanus toxoid; **DT**: diphtheria toxoid.

Supplementary methods

Plasma detection of *Schistosoma* circulating anodic antigen

Plasma samples collected at screening, week 0, week 8, week 28 and week 52 were retrospectively analysed for *Schistosoma* circulating anodic antigen (CAA) using the up-converting phosphor lateral flow (UCP-LF) assay with a SCAA20 test format, with a 30 pg/ml positivity threshold.[1] This limit of detection was previously determined by spiking CAA (standard series) in negative serum from non-endemic healthy individuals and analyzed against a large set of confirmed CAA negative controls from different endemic regions. Based on this, quality control was conducted on test materials provided for the study to guarantee a lower limit of detection of 30 pg/ml. All samples above this level (≥30 pg/ml) are regarded as CAA-positive.

For the current study, human negative serum (obtained from the Uganda blood bank, Nakasero) was spiked with a known concentration of CAA standard (100,000 pg/ml) and diluted up to eight standard points, with two negative controls. These were used to generate a standard curve to quantify the plasma sample CAA levels. Previous work has shown that there is no difference between standard curves with spiked CAA in serum and in plasma.[1] Therefore, plasma and standards (50 µl) were extracted with an equal volume of 4% w/v trichloroacetic acid (TCA; Merck Life Science NV, the Netherlands), vortexed and incubated at ambient temperature for five minutes. Thereafter, samples and standards were briefly vortexed and spun at 13,000 g for five minutes. The resulting supernatant (20µl) was added to the wells containing 100 ng dry UCP particles[2] (400 nm Y2O2S:Yb3+, Er3+) coated with mouse monoclonal anti-CAA antibodies[3] hydrated with 100µl of high salt lateral flow buffer (HSLF: 200 mM Tris pH8, 270 mM NaCl, 0.5% (v/v) Tween-20, 1% (w/v) BSA). These were incubated for one hour at 37°C while shaking at 900 rpm. The CAA lateral flow strips[4] were labeled with the standard and sample identifications and then placed in the wells on the UCP plate. The samples and standards were allowed to flow and left to dry overnight. The strips were then analysed using the Labrox Upcon reader (Labrox Oy, Turku, Finland). The test line signals (T; relative fluorescent units, peak area) were normalized to the flow control signals (FC) of the individual strips and the results were expressed as ratio values.

Stool PCR detection of *Schistosoma mansoni*, *Necator americanus* and *Strongyloides stercoralis* DNA

Stool samples collected at screening, week 28, and week 52 were retrospectively analysed for *helminth* DNA by PCR.

Stool samples stored at -80^{0}C in 95% molecular grade ethanol were retrieved and thawed at room temperature. Total DNA (and hence helminth DNA, if present) was extracted from the stool samples using the Fast DNA Spin Kit for Feces (catalogue number 116570200 , MP Biomedicals Germany

GmbH) to determine (using multiplex real-time PCR) *S. mansoni, Strongyloides stercoralis* and hookworm (*Necator americanus*) infections.

The DNA extraction procedure was conducted with minor changes to the manufacturer's instructions as follows: samples were left to thaw at room temperature (RT) and then vortexed for five seconds to homogenise the ethanol-stool mixture. The homogenized mixture (0.5 ml) was transferred into a safe-lock microcentrifuge (Eppendorf®) tube and centrifuged at 13000 rpm for 3 minutes to get rid of the ethanol. The pellet was re-suspended in a 2ml lysing matrix E tube, containing 825 µl of sodium phosphate buffer and 275 µl of PLS buffer. For solid samples, 300-500mg of stool were transferred to the lysis tube using a sterile loop. The samples were centrifuged for 5 minutes at 14000 g and the supernatant was decanted. 978 µl Sodium Phosphate Buffer and 122 µL MT Buffer were added to the lysing matrix E tube and vortexed briefly to mix the contents. The samples were then homogenised in the FastPrep® 24 instrument (MP Biomedicals Germany GmbH) at setting 6.0 m/s for 40 seconds and thereafter centrifuged at 14000 g for 5 minutes. The supernatant was then transferred to a clean 2.0 ml centrifuge tube and 250 µl of PPS solution was added. The samples were then shaken vigorously to mix, incubated at 4°C for 10 minutes, and centrifuged at 14000g for 2 minutes. During the centrifugation step, we added 1 ml of binding matrix solution to a clean 15 ml conical tube. The supernatant was then transferred to the 15 ml tube containing the binding matrix. These were mixed gently by hand for 3 to 5 minutes. The samples were then centrifuged at 14000 g for 2 minutes and the supernatant discarded. The binding mixture pellet was then washed by gently re-suspending it in 1 mL Wash Buffer #1. Two spins were performed by first transferring 600 µL of the binding mixture to a filter tube and centrifuged at 14000 g for 1 minute. The catch tube was then emptied and the remaining binding mixture was added to the filter tube and centrifuged as before. The catch tube was emptied again and 500 µL of prepared Wash Buffer #2 (concentrated salt wash solution, reconstituted with absolute molecular grade ethanol) was added to the filter tube and re-suspended by gently pipetting up and down to dislodge the pellet. The samples were centrifuged at the same speed for 2 minutes and the flow-through discarded. The samples were centrifuged again for 2 minutes to extract residual ethanol from the binding matrix and dry the sample. The filter bucket was transferred to a clean 1.9 mL Catch Tube and 100 µL TES elution buffer added. The tube was stirred with a pipette tip to resuspend the pellet. The samples were then centrifuged for 2 minutes to elute purified DNA into the clean catch tube. The samples were stored at -20°C until used for PCR (below).

The multiplex real-time PCR was adapted from existing procedures.[5,6] Below are the specific forward (F) and reverse (R) primers and TaqMan® probes that were used to simultaneously detect DNA from three helminth species:

Necator americanus

Na58F: 5'-CTGTTTGTCGAACGGTACTTGC-3'

Na158R: 5'- ATAACAGCGTGCACATGTTGC-3'

Nec-2-FAM (MGB): FAM-5'-CTGTACTACGCATTGTATAC-3'-XS

Nec-3-FAM (MGB): 5'-CTGTACTACGCATTGTATGT-3'

Schistosoma mansoni

Ssp48F: 5'-GGTCTAGATGACTTGATYGAGATGCT-3'

Ssp124R: 5'-TCCCGAGCGYGTATAATGTCATTA-3'

Ssp78T-RT: Texas Red-5'-TGGGTTGTGCTCGAGTCGTGGC-3'-BHQ2

Strongyloides stercoralis

Stro18S-1530F: 5'-GAATTCCAAGTAAACGTAAGTCATTAGC-3'

Stro18S-1630R: 5'-TGCCTCTGGATATTGCTCAGTTC-3'

Stro-4-TRBhq2-VIC- 5'-ACACACCGSCCGTCGCTGC-3'

Phocine herpes virus (PhHV) DNA, extracted from the Phocine herpes virus (kindly provided by Dr. Martin Schutten, Erasmus Medical Center, Rotterdam, the Netherlands), was included in the PCR master mix, thus distributed to all reaction wells as an internal control to check for PCR inhibition. The PhHV forward primer PhHV-267s (5'-GGGCGAATCACAGATTGAATC-3'), reverse primer PhHV-337as (5'-GCGGTTCCAAACGTACCAA-3') and probe PhHV-305tq (Cy5-5' TTTTTATGTGTCCGCCACCATCTGGATC-3'-BHQ2) were used for Phocin herpes virus DNA detection. A positive pool was included on the plate for every run as a test control. The positive pool was made up of a mixture of DNA from samples (from among the study samples) that were highly positive for *S. mansoni* and *N. americanus* on Kato-Katz, and for *S. stercoralis* by PCR (conducted on samples from a previous study). The amplification conditions were 15 minutes at 95^{o}C, 50 cycles of 15 seconds at 95^{o}C, 30s at 60^{o}C and 30s at 72^{o}C. DNA amplification, detection and data analysis were attained with the ABI 7500 Fast Real time machine and 7500 Fast systems software version 1.5.1.

***Ex vivo* interferon-γ ELISpot assays to quantify BCG-specific responses**

To quantify BCG-specific responses, we conducted *ex vivo* interferon (IFN)-γ ELISpot assays, using a Human IFN-γ (ALP) ELISpot Flex kit (Mabtech, Sweden) and multiscreen-IP 0.45μm filter 96-well plates (Merck Millipore). ELISpot plates were coated overnight at 4°C with 50 μl of anti-IFN-γ capture antibody (15 μg/ml) dissolved in 0.05 M carbonate-bicarbonate buffer (Sigma Aldrich). The plates

were then washed 5 times with sterile 1X PBS (Sigma Aldrich), and blocked (2-5 hours, 37°C) by adding 100 μl/well of R10 medium (10% fetal bovine serum [Sigma Adrich] in RPMI 1640 medium [Thermofisher scientific] supplemented with L-glutamine, streptomycin, HEPES buffer and penicillin [all from Life technologies, UK]). During plate blocking, peripheral blood mononuclear cells (PBMCs) were isolated from heparinised whole blood by density gradient centrifugation with Histopaque® (Sigma Aldrich). For each study sample, PBMCs (300,000 per test well) were stimulated in duplicate for 18-20 hours at 37°C, 5% CO_2, with BCG (Moscow strain, Serum Institute of India) at a concentration of 200,000 colony forming units per ml, or left unstimulated. Staphylococcal enterotoxin B (SEB; Sigma Aldrich) was used at a final concentration of 10 μg/ml as a positive control, and a 1:1 mix of the 6-kDa early secretory antigenic target and 10-kDa culture filtrate protein (ESAT-6 and CFP-10 recombinant proteins from BEI Resources, USA) used at a final concentration of 2.5 μg/ml for exploratory assessment of tuberculosis infection. Following the 18-20 hour incubation, plates were washed 5 times with 200ul/well PBS containing 0.05% Tween 20 (Sigma Aldrich) and incubated for 2 hours at room temperature with 50 μl per well of a 1/1000 PBS dilution of biotin anti-IFN-γ antibody from the ELISpot kit. After another washing step with PBS-0.05% Tween 20, plates were incubated for 1 hour at room temperature with 50 μl per well of a 1/1000 PBS dilution of a streptavidin-alkaline phosphatase conjugate from the ELISpot kit. Plates were washed, developed for 3-10 minutes with 50 μl per well of 5-Bromo-4-chloro-3-indoxyl phosphate/Nitro blue tetrazolium (BCIP/NBT; Europa Bioproducts), and the reaction stopped by washing the plate under tap water. Plates were allowed to dry in the dark at room temperature and read using an ELISpot reader (Autoimmun Diagnostika Gmbh iSpot , Strassberg, Germany) running AID ELISpot software v.7.0. Spot-forming units (SFUs) per well were manually verified to remove artefacts.

We performed QC through a number of steps: 1) for each sample, we checked to ascertain whether the PBMC isolation procedure was conducted within eight hours after sample collection; 2) we inspected each ELISpot plate visually for quality and completeness of labelling (sample IDs, date, time point and antigens) and compared the plate picture to the exported spot count spreadsheet to ensure the correct data had been exported; 3) we checked the calculated data in the exported database to ensure the background subtraction, average of duplicate wells and multiplication up to spot forming units per million PBMC had been performed correctly; 4) we assessed whether the unstimulated well and SEB well controls for each assay were within the accepted range.

Results were reported as SFUs per a million PBMCs, calculated sequentially by 1) subtracting mean SFUs of unstimulated wells from mean SFUs of duplicate antigen wells, and 2) correcting for the number of PBMCs per well (300,000). Samples that had more than 83.3 SFUs per a million PBMCs in the unstimulated well were considered invalid and not included in the final analysis.

Yellow Fever plaque reduction neutralizing reference test (PRNT)

A plaque reduction assay as described by Beaty *et al*.[7] was used. Briefly, Vero cells at a concentration of 65,000 cells/ml were seeded into 6-well plates (Greiner Bio-One GmbH, Germany) at a volume of 3 ml/well. Cells were cultured in growth medium (1X Eagle's Minimum Essential Medium, 8% heat inactivated fetal bovine serum, 100 units penicillin/streptomycin, gentamycin 50 mg/ml and fungizone 1 mg/ml) at 37°C (with 5% CO_2) for 3–4 days. Culture medium was then removed from the cell monolayer by dumping. Test plasma were inactivated at 56°C for 30 min to remove complement factor, serially (two-fold) diluted from 1:10 to 1:20480 in BA-1 diluent (10X M199 Hanks' Salts without L-Glutamine, 5% Bovine Serum Albumin, 1M TRIS-HCL pH 7.5, L-Glutamine, sodium bicarbonate 7.5%, 100X penicillin/streptomycin, 1000X fungizone in sterile water), and mixed with approximately 200 Plaque Forming Units (PFU) of a reference YF-17D virus preparation. The plasma-virus mixture (0.1 ml) was added to the confluent monolayer of Vero cells in each well and incubated at 37°C (with 5% CO_2) for 1 hour. The first overlay medium (comprising Miller's 2X Yeast Extract-Lactalbumin hydrolysate medium, 10X Earle's Buffered Salts Solutions, 2% fetal bovine serum, 1000X fungizone, 1000X Gentamycin, and 2% low-melting agarose) was added, 3 ml per well, and allowed to solidify for 30 minutes at room temperature. The plates were incubated at 37°C with 5% CO_2 for 4 days. To stain cell layers, a Neutral Red dye (Sigma Aldrich) second overlay was added, 2 ml per well, and allowed to solidify for 30 minutes at room temperature. After this second overlay, plates were incubated at 37°C in 5% CO_2 for 2 days: plaques were counted first on day 1 and the final score documented on day 2 to establish 50% and 90% neutralization titres. Back titration plates were established to ensure infectivity of cell monolayer and standardization of virus to 200 PFU/0.1 ml. Neutralisation titres <1:10 were considered negative. Titres of 1:10 were interpreted as borderline. The PRNT antibody titres presented refer to the reciprocal of the last plasma dilution that reduced by 50% (PRNT50) or 90% (PRNT90) the number of virus plaque clusters infected by 100 PFU/0.1 ml of the reference 17D virus preparation.

For quality control, we used a high titre positive control (PC), with the last six titre dilution range, from 640 to 20480. So long as the PC titre was within the expected range and did not vary by greater than 4-fold, the assay passed quality control. Furthermore, we ran back-titrations of the virus inoculum (standardised to 200 PFU/0.1 ml) to determine the end-point specimen antibody titre at 50% or 90% neutralisation. The number of virus plaques infected at 50% neutralisation and at 90% neutralisation were expected to be within an approximate range of 25-100 and 5-20, respectively.

Detection of plasma IgG against *Salmonella* Typhi O-lipopolysaccharide (O:LPS)

Specific IgG to *S.* Typhi O-lipopolysaccharide (O:LPS) was measured by an in-house ELISA. Nunc Maxisorp 96-well plates (Thermo Fisher) were coated overnight at 4ºC with 50µl/well of 10 µg/ml of *S.* Typhi O:LPS (Sigma L2387) in bicarbonate (Na_2CO_3 + $NaHCO_3$) buffer (0.1M, pH 9.6). Plates were washed with phosphate-buffered saline (PBS 1X)-Tween 20 (0.05%) solution, blocked with 200µl of 5% skimmed milk diluted in PBS-Tween 20 for 1 hour at room temperature (RT), washed again and incubated for 2 hours at RT with 50µl of test plasma samples (diluted 1/320 with 1% skimmed milk in PBS-Tween 20) and two-fold serially diluted standard sera (top concentration 20 EU/ml). Standards were derived from a pooled sample generated from sera of known O:LPS-specific IgG titres, kindly provided by the Oxford Vaccine Centre Biobank. These sera had been collected from the highest responders to O-antigen following challenge with *S.* Typhi in a controlled human infection study.[8] Following test and standard sample incubation, plates were washed and O:LPS-specific IgG binding detected by incubating the plates for 1 hour at RT with goat anti-human IgG-horseradish peroxidase conjugate (Insight Biotechnology, UK), diluted 1/6000 in 1% skimmed milk–PBS-Tween 20. Plates were washed and developed by addition of 100µl of o-phenylenediamine (Sigma-Aldrich) and reactions stopped after 5 minutes with 30µl of 2M Sulphuric acid. Optical density (OD) values were measured at 490nm (reference wavelength 630nm) on a 96-well plate ELISA reader (BioTek ELx808, USA). Nominal ELISA units (EU/ml), representing O:LPS-specific IgG titres, were interpolated from standard curves using a five-parameter curve fit using Gen5 data collection and analysis software (BioTek Instruments Inc, Vermont, Winooski, USA).

Detection of plasma IgG against Human Papillomavirus type 16 (HPV-16) and HPV-18

Anti-HPV-16 and HPV-18 IgG concentrations were measured by ELISA, as previously described.[9-12] Nunc Maxisorp 96-well plates (Thermo Fisher) were coated with 100 µl of HPV-16 L1 virus-like particles (VLP) at a concentration of 2.7 µg/ml, or with HPV-18 L1-L2 VLP at a concentration of 2 µg/ml and incubated at 4°C overnight. Plates were washed three times with a 1X phosphate-buffered saline (PBS)-Tween 20 (0.25%) solution, and blocked for 90 minutes at room temperature (RT) with 4% skimmed milk diluted in a 1X PBS-0.25% Tween 20 solution. The plates were washed three times and and incubated (with gentle shaking) for 1 hour at RT with 100 µl of test plasma samples, assay controls and two-fold serially diluted standard sera. Pre-vaccination test plasma samples were diluted 1/50 (HPV-16 assay) or 1/200 (HPV-18 assay) with blocking buffer, while post-vaccination plasma samples were diluted 1/400 for both HPV-16 and HPV-18 assays. Standard sera were used at a top concentration of 1.28 EU/ml and 8.2425 EU/ml for HPV-16 and HPV-18 assays, respectively. Following test and standard sample incubation, plates were washed four times and further incubated for 1 hour at RT with peroxidase-labeled goat anti-human IgG (KPL, Gaithersburg,

Maryland). Plates were then developed with a tetramethylbenzidine substrate solution (KPL, Inc.) for 25 minutes in the dark at room temperature. Next, the reaction was stopped by adding 100 µl of 0.36N H_2SO_4 to each well. Optical density (OD) values were measured at 450 nm (reference wavelength 630 nm) on a 96-well plate ELISA reader (BioTek ELx808, USA). Nominal ELISA units (EU/ml), representing HPV-16 L1 VLP- and HPV-18 L1-L2 VLP-specific IgG titres, were interpolated from standard curves using a five-parameter curve fit using Gen5 data collection and analysis software (BioTek Instruments Inc, Vermont, Winooski, USA).

For quality control (QC), the acceptable R^2 for the standard curve was ≥ 0.990 and the average optical density (OD) range of the top standard was 2.0-4.0. The acceptable OD of the last (8^{th}) standard dilution was ≤0.25. The percentage difference in ODs between standard dilutions (i.e. n and n+1 dilution) was expected to be ≥ 0.3. Plates were repeated if they failed to meet these standard curve criteria. The calculated negative control cut-off was 4±3 EU/ml and 60±10 EU/ml for HPV-16 and HPV-18, respectively. Plates whose negative control concentration was above the cut-offs were repeated. The calculated positive control concentration was 450±10 EU/ml and 4500±10 EU/ml for HPV-16 and HPV-18 respectively. Background signal was measured by a blank whose OD was expected to be ≤0.05. Higher ODs indicated assay contamination and plates were repeated.

ELISA measurement of anti-diphtheria and anti-tetanus IgG

Nunc Maxisorp 96-well plates (Thermo Fisher) were coated with 50 µl of either 2 Lf units per ml of diphtheria toxoid (NIBSC product code 13/212) per ml or 0.56 Lf units per ml of tetanus toxoid (NIBSC product code 02/232) in Na_2CO_3/$NaHCO_3$ buffer (0.1M, pH 9.6) overnight at 4°C. Plates were washed with 0.05% Tween 20 in 1X phosphate-buffered saline (PBST) and blocked for 1 hour with 5% skimmed milk powder in PBST at 37 °C. The plates were washed four times and and incubated for 2 hours at 37 °C with 50 µl of test plasma samples, and serially diluted WHO International Standard anti-toxins for diphtheria (NIBSC 10/262) or tetanus (NIBSC 13/240). Samples and standards were prepared in PBST + 1% skimmed milk (assay buffer). Pre-vaccination test samples were added at a dilution of 1/150, while post-vaccination plasma samples were added at a dilution of 1/300 in assay buffer. The standards were used at a top concentration of 3 IU/ml and 0.125 IU/ml for the tetanus and diphtheria assays, respectively. Plates were washed four times and incubated for 1 hour at 37 °C with 50 µl of polyclonal rabbit anti-human IgG HRP-conjugate (Agilent Dako, CA, USA) diluted 1/3000 in assay buffer. After another washing step, plates developed by adding 100 µl/well of o-phenylenediamine (Sigma-Aldrich) and reactions stopped after 5 minutes with 25 µl/well of 2M sulphuric acid. Optical density (OD) values were measured at 490nm (reference wavelength 630nm) on a 96-well plate ELISA reader (BioTek ELx808, USA). Tetanus and diphtheria toxoid-specific IgG

concentrations (IU/ml) were interpolated from standard curves using a five-parameter curve fit using Gen5 data collection and analysis software (BioTek Instruments Inc, Vermont, Winooski, USA).

Detection of *Plasmodium falciparum* DNA by PCR

Prior to the PCR, DNA extraction was performed using the QIAamp DNA Blood Mini Kit (Catalogue number 51106, QIAGEN). Briefly, whole blood pellet samples stored at -80^{0}C were retrieved and thawed at room temperature. Reagents in the extraction kit were reconstituted as per the manufacturer's instructions. Volumes of retrieved samples were checked and Phosphate Buffered Saline (PBS) was added to samples with inadequate volumes. These were vortexed thoroughly for 2 to 3 minutes. The protease enzyme (20 µl) was pipetted to the bottom of a 2 ml Eppendorf tube, and the whole blood pellet (200 µl) was transferred to the tube. The AL lysis buffer (200 µl), after thorough mixing, was added and pulse vortexed for 20 seconds. The samples were then placed in a heating block set to 56^{0}C for 15 minutes and thereafter centrifuged at 6000g for 1 minute to ensure no sample was trapped in the Eppendorf tube lid. 200 µl of absolute molecular grade ethanol was added to the sample, pulse vortexed, and then centrifuged. 500 µl of the sample was transferred to a spin column and centrifuged at 6000g for 1 minute, discarding the flow-through thereafter. This process was repeated for the remainder of the sample. The DNA was then purified using consecutive washes with AW1 (500ul, 250ul) and AW2 (500ul) buffers. The QIAGEN AE buffer (150 µl) was used to elute the DNA. The samples were then stored at -20^{0}C until used in the PCR.

The real-time PCR was performed with the ABI 7500 Fast Real-time machine and data processed using 7500 Fast Systems software version 1.5.1. The PCR reaction was performed with a final volume of 25 µl containing 2 µl of DNA 1 µl of PhHV DNA (as an internal control, detailed below) and 22 µl of PCR master mix made of HotStarTaq Master Mix (Catalogue Number 203446), primers, and probes. The primers and probes used in this study are listed below.

PFal-F 5`-CCG ACT AGG TGT TGG ATG AAA GTG TTA A-3`
Plas-171R 5`-AAC CCA AAG ACT TTG ATT TCT CAT AA-3
Pfal114-XS_YY 5`-CTT TCG AGG TGA CTT TTA GAT-3`-BHQ1

Phocine herpes virus (PhHV) DNA, extracted from the Phocine herpes virus (kindly provided by Dr. Martin Schutten, Erasmus Medical Center, Rotterdam, the Netherlands), was included in the PCR master mix, thus distributed to all reaction wells as an internal control to check for PCR inhibition. The PhHV forward primer PhHV-267s (5'-GGGCGAATCACAGATTGAATC-3'), reverse primer PhHV-337as (5'-GCGGTTCCAAACGTACCAA-3') and probe PhHV-305tq (Cy5-5'-TTTTTATGTGTCCGCCACCATCTGGATC-3'-BHQ2) were used for Phocin herpes virus DNA detection. A pool of DNA extracted from *P. falciparum* positive samples was used to set serially diluted standards

tested alongside the samples on every plate run. The amplification conditions were 15 minutes at 95°C, 50 cycles of 15 seconds at 95°C, 30s at 60°C, and 30s at 72°C.

Literature search key words

(BCG or "bacille calmette-guerin" or Cholera OR dengue or diphtheria or "hepatitis A" or "hepatitis B" or HBV or "hepatitis E" or "Haemophilus influenza" or influenza or "human papilloma virus" or HPV or "Japanese encephalitis" or measles or meningococcus or meningococcal or mumps or pertussis or pneumococcus or pneumococcal or poliomyelitis or polio or rabies or rotavirus or rubella or tetanus or "tick-borne encephalitis" or tuberculosis or typhoid or varicella or "yellow fever")

AND

("soil transmitted helminths" or hookworm* or Ancylostom* or uncinariasis or Necator or whipworm* or Trichuris or roundworm* or Ascaris or Schistosoma or bilharzia or Echinococcus or hydatid or Hymenolepis or Rodentolepis or Fasciola or "liver fluke*" or filariasis or elephantiasis or Wuchereria or Brugia or Mansonella or "Diptalonema streptocerca" or Onchocerca or Dracunculiasis or "Guinea worm*" or Trichinella or Trichina or Toxocara or helminth* or worms or worm or nematode*)

AND

((vaccin* or immun* or antibod*) NEAR/3 (reaction* or response*)) OR (produc* NEAR/3 (antibod* or cytokine*))

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