Appendix text

Methods

Laboratory methods - TAC

Stool samples stored at -80°C were used for the TaqMan® array card (TAC) assays for enteropathogens. Briefly, total nucleic acid (TNA) was extracted from 200 mg of stool using a modified extraction protocol with the QIAamp Stool DNA mini kit (Qiagen). The modification to the kit protocol included the performance of a bead beating step for 2-3 minutes, using glass beads of size 500 µm (Sigma) to increase the DNA yield from protozoan cysts and helminthic eggs. After extraction, 100µl of the extracted TNA was mixed with 100 µl of RNA storage solution (Ambion, CA) to keep the RNA stable for a prolonged period. Before extraction, each stool sample was spiked with extraction controls for both DNA and RNA targets, using 10^6 copies of phocine herpes virus (PhHV) and 10^7 MS2 bacteriophage per sample added to the lysis buffer, to monitor the PCR and extraction efficiency and inhibition. These positive controls were detected in all samples undergoing TAC analysis. Each batch of TNA extraction also included a "water control" where all the steps of extraction were followed on 200µl of nuclease free water. This was included to rule out cross-contamination during the TNA extraction.

For detection of multiple enteropathogen targets using a single assay, an enteropathogen TAC assay developed by the Division of Infectious Diseases and International Health, University of Virginia was used.[1] The enteropathogen TAC assay is a group of 384-well arrayed singleplex real-time PCR assays which can be used for semi-quantitative and quantitative detection of pathogens. The pathogen targets are given in appendix table 1. Each enteropathogen TAC card can test 8 samples for up to 48 targets, depending on the primers and probes coated on the 1 µl volume wells. About 40µl of the extracted TNA for each sample was mixed with 50 µl of Ag-Path ID RT-PCR buffer (Ambion, CA), 4 µl of enzyme mix (containing reverse transcriptase and taq polymerase enzymes) and 6 µl of DEPC treated water to make a final volume of 100 µl. This reaction mixture was loaded onto each port of the card and centrifuged at 4°C twice for 1 minute each. The card was then sealed using a staking device for the cards, the sample ports were excised and the card was run on a Quant Studio 12K Flex real-time PCR system (Applied Biosystems, CA, USA) with the following cycling conditions: 45°C for 20 min and 95°C for 10 min (reverse transcription followed by initial denaturation), followed by 45 cycles of 95°C for 15 secs and 60°C for 1 min (annealing and extension). Each lot of cards was first tested with a no template control to confirm no amplification and with pooled positive controls for quality control. A threshold cycle (Ct) value of 30 was used for pathogen detection.

Laboratory methods – environmental enteropathy biomarkers ELISA

Commercial ELISA assays were used for estimating levels of putative biomarkers of environmental enteropathy (EE) in stool (calprotectin, myeloperoxidase, neopterin and α-1 antitrypsin) and plasma (intestinal fatty acid binding protein (I-FABP), soluble CD14 and endotoxin-core IgG (EndoCAb)). Stool aliquots for the faecal biomarker ELISA assays were supplemented with a cocktail of protease inhibitors before being stored at -70°C. Blood samples collected in lithium heparin tubes were centrifuged at 2000 rpm for 15-20 minutes and the separated plasma aliquoted and stored at -70°C until the biomarker assays were carried out. Repeated freeze thaw cycles were avoided.

i) Faecal calprotectin

Faecal calprotectin levels were estimated using commercial ELISA kits (Hycult, Netherlands). The faecal calprotectin ELISA was performed on stool specimens which were diluted according to instructions provided in the kit. ELISA was performed with the diluted samples and the calprotectin standards provided in the kit according to instructions provided by the manufacturer. While calculating the final concentration of calprotectin in the faecal samples, the dilution factors were taken into consideration. The faecal calprotectin levels were finally expressed as µg/gram (µg/g) of faeces.

ii) Faecal myeloperoxidase
Faecal myeloperoxidase levels were estimated using commercial ELISA kits (Hycult, Netherlands). The assay procedures were modified for faecal samples and all faecal samples were diluted 1 in 500 using wash buffer before being tested with the myeoperoxidase ELISA kit. Apart from the sample dilutions, all other procedures and steps were as per the manufacturer's instructions. Faecal myeoperoxidase levels were expressed in units of ng/mL of undiluted faeces.

iii) Faecal neopterin
For determination of neopterin levels in faecal samples, a competitive ELISA (IBL International, Hamburg) was performed as per the instructions for the kit except that the faecal samples were diluted 1 in 500 in 0.9% saline before performing the ELISA. The final neopterin concentrations of the samples were calculated by multiplying the concentration obtained with the dilution factor for the samples. Faecal neopterin levels were expressed in units of nmol/L per gram of stool.

iv) Faecal α-1 antitrypsin
Faecal α-1 antitrypsin levels in diluted faecal samples were estimated using a sandwich ELISA (ImmuChrom, GmbH) as per manufacturer's instructions. Faecal α-1 antitrypsin levels were expressed in units of mg/g of faeces.

v) Plasma I-FABP
A commercial ELISA kit (Hycult, Netherlands) was used for the estimation of I-FABP levels in plasma. All plasma samples were brought to room temperature (18-25°C) before use. All the procedures and dilution factors were as per the manufacturer's instructions. In all assays, recommended dilutions of the standard solution (for IFABP) included in the kit were followed. Standard curves were generated using the OD readings obtained for the dilutions of the standard solution. The concentrations of the samples were derived from the standard curve and multiplied by the dilution factor to get the actual concentration of I-FABP in the samples. I-FABP concentration was expressed in pg/ml of plasma for all samples.

vi) Plasma soluble CD14
Soluble CD14 levels in plasma samples were determined by performing commercially available ELISA assays (Hycult, Netherlands). All plasma samples were diluted 1 in 80 using the dilution buffer in the kit (10µl plasma sample and 790 µl dilution buffer) before being used in the ELISA. Standard curves were generated using the OD readings corresponding to the dilutions of the standard solution included in the kit. The concentrations of the samples were derived from the standard curve and multiplied by the dilution factor to get the actual concentration of soluble CD14 in the samples. Soluble CD14 concentration was expressed in ng/ml of plasma for all samples.

vii) Plasma EndoCAb IgG
EndoCAb IgG ELISA was performed as per manufacturer's instructions using commercially available ELISA kits (Hycult, Netherlands). All plasma samples were diluted 1 in 200 in the dilution buffer before being tested. Standard curves were generated using the OD readings corresponding to the dilutions of the standard solution included in the kit. The concentrations of the samples were derived from the standard curve and multiplied by the dilution factor to get the actual concentration of EndoCab IgG. EndoCab IgG concentration was expressed in standard median units MU/ml of plasma for all samples.

Laboratory methods – poliovirus shedding PCR
For analysis of shedding a singleplex quantitative real-time PCR for Sabin poliovirus 3 was carried out, using RNA extracted using Vx reagents on a Qiaxtractor.[2] Before extraction, each sample was spiked with cultured mengovirus calibrated to yield a Ct value at 35-36 cycles. Complementary DNA was generated from the eluted RNA by reverse transcription using random primers. DNA amplification was

carried out in an ABI thermal cycler with detection using Taqman probe hybridization. Plasmids constructed by ligation of a poliovirus serotype 3 region of the VP1 PCR fragment in TOPO-TA 2.1 vector propagated in *Escherichia coli* DH5α cells were used as plasmid DNA standards for calibration of the assay. A Ct value of <40 corresponding to 3 plasmid copies per reaction was considered positive for poliovirus. Samples negative for poliovirus amplification were tested by mengovirus PCR to rule out inhibition.[3]

Appendix Table 1. TaqMan® array card targets

Target	Gene	Reference
Controls		
MS2	-	[4]
PhHV	-	[1]
Bacterial targets		
Bacterial 16S	16S	[5]
Aeromonas	Aerolysin	[1]
Bacteroides fragilis	EGBF	Modified from [6]
Campylobacter	Cpn60	Designed based on [7]
Campylobacter jejuni/coli	cadF	[8]
Clostridium difficile	tcdB	[1]
EAEC	aaiC	[9]
EAEC	aatA	[9]
EPEC	Eae	[1]
EPEC	bfpA	[1]
ETEC	LT	[10]
ETEC	ST	[1]
Helicobacter pylori	ureC	Designed based on [11]
Mycobacterium tuberculosis	IS6110	[12]
Salmonella	Ttr	[13]
Shigella/EIEC	ipaH	[14]
STEC	stx1	[1]
STEC	stx2	[10]
Vibrio cholerae	lyA	[1]
Eukaryotic targets		
Ancylostoma	Ribosomal gene	[15]
Ascaris	Ribosomal gene	Modified from [16]
Cryptosporidium	Ribosomal gene	[1]
Cryptosporidium typing	Lib13	[17]
Cyclospora	Ribosomal gene	[18]
Enterocytozoon bieneusi	Ribosomal gene	[19]
Entamoeba histolytica	18S	[20]
Encephalitozoon intestinalis	Ribosomal gene	[19]
Giardia	18S	[20]
Giardia typing	tpi	Modified from [21]
Isospora	Ribosomal gene	[22]
Necator	Ribosomal gene	[15]
Strongyloides	Dispersed repetitive sequence	[23]
Trichuris	Ribosomal gene	[1]
Viral targets		
Adenovirus serotypes 40/41	Fiber gene	[24]
Adenovirus	Hexon	[25]
Astrovirus	Capsid	[1]
Enterovirus	5′ UTR	Modified from [26]
Norovirus genogroup GI	ORF1-2	Modified from [27]
Norovirus genogroup GII	ORF1-2	[27]
Rotavirus	NSP3	[28]
Sapovirus	RdRp	[1]

Abbreviations: EAEC, enteroaggregative *Escherichia coli*; EIEC, enteroinvasive *E. coli*; EPEC, enteropathogenic *E. coli*; ETEC, enterotoxigenic *E. coli*; LT, heat-labile enterotoxin; PhHV, phocine herpesvirus; ST, heat-stable enterotoxin; STEC, Shiga toxin-producing *E. coli*.

Appendix Table 2. Comparison of serotype-3 poliovirus shedding at 7 days and seroconversion at 21 days after OPV.

	Seroconversion	
Shed poliovirus	**No**	**Yes**
No	121 (83.4%)	14 (9.5%)
Yes	24 (16.6%)	133 (90.5%)

data are n (column %)

Appendix Table 3. Univariate and multivariable logistic regression of association between baseline characteristics and seroconversion after OPV

Characteristic	*Univariate* Odds Ratio (95% confidence interval)	p-value	*Multivariable* Odds Ratio (95% confidence interval)	p-value
Age (months)	0.86 (0.78-0.95)	0.004	0.89 (0.79-0.99)	0.034
Sex (M vs. F)	0.91 (0.67-1.22)	0.515	-	-
Mother's education ~~(primary and below vs. secondary and above)~~	~~0.95 (0.62-1.46)~~	0.16682~~5~~	~~-~~	~~-~~
illiterate	ref.		-	-
primary	0.8 (0.36-1.79)		-	-
middle	0.83 (0.41-1.69)		-	-
secondary	0.92 (0.47-1.81)		-	-
graduate	1.35 (0.59-3.07)		-	-
House roof type ~~(other vs. concrete or better)~~	~~0.95 (0.69-1.32)~~	0.~~78~~171	~~-~~	~~-~~
concrete	ref.		-	-
tiled	0.77 (0.51-1.16)		-	-
thatched	0.83 (0.6-1.15)		-	-
Trivalent OPV doses	0.85 (0.73-0.98)	0.023	0.91 (0.78-1.06)	0.211
Diarrhea at enrolment	0.57 (0.18-1.76)	0.328	-	-
~~Breastfed~~ Currently breastfed (yes vs. no)	1.66 (0.94-2.96)	0.083	-	-
Height for age Z-score (HAZ)	1.08 (0.95-1.23)	0.238	-	-
Weight for age Z-score (WAZ)	1.11 (0.96-1.3)	0.166	-	-

Multivariable model included all variables significant in the univariate analyses

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Appendix Table 4. Prevalence and abundance of intestinal pathogens on the day of vaccination (day 14) by study arm. Abundance is measured by the PCR cycle threshold (Ct) value. This is the PCR cycle number when the target sequence is first amplified at a sufficient level to be detected and is therefore inversely related to abundance (copy number of target sequence in stool extract). Targets not identified in a sample were arbitrarily given a Ct value of 45 (below the limit of detection). Fisher's exact test was used to assess differences in prevalence. Wilcoxon's rank sum test was used to compare Ct values between samples where the targets were present in at least 1% of samples from day 14. Abbreviations: EAEC, enteroaggregative *Escherichia coli*; EPEC, enteropathogenic *E. coli*; ETEC, enterotoxigenic *E. coli*; STEC, shiga toxin-producing *E. coli*; *B. fragilis, Bacteroides fragilis*; *C. difficile, Clostridium difficile*; *H. pylori, Helicobacter pylori; M. tuberculosis, Mycobacterium tuberculosis; V. cholera, Vibrio cholera; E. bieneusi, Enterocytozoon bieneusi; E. histolytica, Entamoeba histolytica; E. intestinalis, Encephalitozoon intestinalis.*

Pathogen	Prevalence, n (%)			Abundance, mean Ct (SE)		
	Arm			Arm		
	Azithromycin	Placebo	p-value	Azithromycin	Placebo	p-value
EAEC	136 (39.2)	267 (74.8)	<0.0010	33.67 (0.51)	26.4 (0.42)	<0.0010
EPEC	100 (28.8)	157 (44.0)	0.004	36.59 (0.49)	32.67 (0.47)	<0.0010
ETEC	47 (13.5)	57 (16.0)	0.463	40.53 (0.42)	39.57 (0.44)	0.055
Campylobacter	25 (7.2)	87 (24.4)	<0.0010	41.38 (0.31)	37.57 (0.48)	<0.0010
STEC	1 (0.3)	9 (2.5)	0.021	44.23 (0.15)	43.55 (0.23)	0.115
Aeromonas	0 (0.0)	1 (0.3)	1.000			
B. fragilis	21 (6.1)	34 (9.5)	0.124	43.13 (0.29)	42.34 (0.35)	0.186
C. difficile	7 (2.0)	9 (2.5)	0.802	44.38 (0.16)	44.11 (0.19)	0.380
Shigella	3 (0.9)	11 (3.1)	0.056	44.66 (0.12)	44.08 (0.21)	0.077
H. pylori	0 (0.0)	1 (0.3)	1.000			
M. tuberculosis	0 (0.0)	0 (0.0)	1.000			
Salmonella	1 (0.3)	4 (1.1)	0.374			
V. cholerae	0 (0.0)	0 (0.0)	1.000			
Adenovirus	57 (16.4)	66 (18.5)	0.559	38.05 (0.43)	37.57 (0.42)	0.361
Astrovirus	5 (1.4)	5 (1.4)	1.000	43.85 (0.21)	44.19 (0.18)	0.210
Enterovirus	137 (39.5)	119 (33.3)	0.274	34.98 (0.43)	35.68 (0.41)	0.197
Norovirus (GI,GII)	32 (9.2)	29 (8.1)	0.690	41.89 (0.35)	42.27 (0.33)	0.284
Rotavirus	8 (2.3)	5 (1.4)	0.416	43.59 (0.22)	43.69 (0.18)	0.893
Sapovirus	10 (2.9)	8 (2.2)	0.640	43.89 (0.21)	43.89 (0.22)	0.983
Ancylostoma	0 (0.0)	0 (0.0)	1.000			
Ascaris	0 (0.0)	0 (0.0)	1.000			
Cryptosporidium	17 (4.9)	13 (3.6)	0.461	42.99 (0.27)	43.17 (0.27)	0.761
Cyclospora	2 (0.6)	1 (0.3)	0.620			
E. bieneusi	6 (1.7)	5 (1.4)	0.770	44.38 (0.17)	44.39 (0.17)	0.675
E. histolytica	0 (0.0)	0 (0.0)	1.000			
E. intestinalis	0 (0.0)	0 (0.0)	1.000			
Giardia	30 (8.6)	27 (7.6)	0.680	40.9 (0.41)	41.62 (0.37)	0.130
Isospora	0 (0.0)	0 (0.0)	1.000			
Necator	0 (0.0)	0 (0.0)	1.000			
Strongyloides	0 (0.0)	0 (0.0)	1.000			
Trichuris	0 (0.0)	0 (0.0)	1.000			

Appendix Table 5. Prevalence of intestinal pathogens before and after treatment with azithromycin or placebo. Differences in prevalence were assessed with McNemar's test for paired data. Abbreviations as for appendix table 4.

Pathogen	Azithromycin			Placebo		
	Prevalence, n (%)			Prevalence, n (%)		
	Study day			Study day		
	0	14	p-value	0	14	p-value
EAEC	260 (74.7)	136 (39.2)	<0.0010	254 (71.1)	267 (74.8)	0.208
EPEC	146 (42.0)	100 (28.8)	<0.0010	146 (40.9)	157 (44.0)	0.413
ETEC	71 (20.4)	47 (13.5)	0.024	54 (15.1)	57 (16.0)	0.828
Campylobacter	76 (21.8)	25 (7.2)	<0.0010	85 (23.8)	87 (24.4)	0.917
STEC	9 (2.6)	1 (0.3)	0.021	4 (1.1)	9 (2.5)	0.267
Aeromonas	1 (0.3)	0 (0.0)	1.000	1 (0.3)	1 (0.3)	1.000
B. fragilis	32 (9.2)	21 (6.1)	0.043	31 (8.7)	34 (9.5)	0.664
C. difficile	7 (2.0)	7 (2.0)	1.000	12 (3.4)	9 (2.5)	0.549
Shigella	7 (2.0)	3 (0.9)	0.289	7 (2.0)	11 (3.1)	0.388
H. pylori	0 (0.0)	0 (0.0)	1.000	1 (0.3)	1 (0.3)	1.000
M. tuberculosis	0 (0.0)	0 (0.0)	1.000	0 (0.0)	0 (0.0)	1.000
Salmonella	10 (2.9)	1 (0.3)	0.012	9 (2.5)	4 (1.1)	0.227
V. cholerae	0 (0.0)	0 (0.0)	1.000	3 (0.8)	0 (0.0)	0.250
Adenovirus	49 (14.1)	57 (16.4)	0.416	60 (16.8)	66 (18.5)	0.594
Astrovirus	6 (1.7)	5 (1.4)	1.000	4 (1.1)	5 (1.4)	1.000
Enterovirus	145 (41.7)	137 (39.5)	0.554	119 (33.3)	119 (33.3)	1.000
Norovirus (GI,GII)	24 (6.9)	32 (9.2)	0.291	30 (8.4)	29 (8.1)	1.000
Rotavirus	4 (1.1)	8 (2.3)	0.344	3 (0.8)	5 (1.4)	0.727
Sapovirus	12 (3.4)	10 (2.9)	0.824	5 (1.4)	8 (2.2)	0.549
Ancylostoma	0 (0.0)	0 (0.0)	1.000	1 (0.3)	0 (0.0)	1.000
Ascaris	0 (0.0)	0 (0.0)	1.000	0 (0.0)	0 (0.0)	1.000
Cryptosporidium	16 (4.6)	17 (4.9)	1.000	16 (4.5)	13 (3.6)	0.549
Cyclospora	0 (0.0)	2 (0.6)	0.500	0 (0.0)	1 (0.3)	1.000
E. bieneusi	4 (1.1)	6 (1.7)	0.625	9 (2.5)	5 (1.4)	0.289
E. histolytica	0 (0.0)	0 (0.0)	1.000	0 (0.0)	0 (0.0)	1.000
E. intestinalis	0 (0.0)	0 (0.0)	1.000	0 (0.0)	0 (0.0)	1.000
Giardia	29 (8.3)	30 (8.6)	1.000	24 (6.7)	27 (7.6)	0.508
Isospora	0 (0.0)	0 (0.0)	1.000	0 (0.0)	0 (0.0)	1.000
Necator	0 (0.0)	0 (0.0)	1.000	0 (0.0)	0 (0.0)	1.000
Strongyloides	0 (0.0)	0 (0.0)	1.000	0 (0.0)	0 (0.0)	1.000
Trichuris	0 (0.0)	0 (0.0)	1.000	0 (0.0)	0 (0.0)	1.000

Appendix Table 6. Abundance of intestinal pathogens before and after treatment with azithromycin or placebo. Wilcoxon's signed rank test (for paired data) was used to compare Ct values between samples from study days 0 and 14 where the targets were present in at least 1% of samples. Abbreviations as for appendix table 4.

Pathogen	Azithromycin			Placebo		
	Abundance, mean Ct (SE) Study day			Abundance, mean Ct (SE) Study day		
	0	14	p-value	0	14	p-value
EAEC	26.55 (0.44)	33.67 (0.51)	<0.0010	26.82 (0.44)	26.4 (0.42)	0.694
EPEC	33.21 (0.49)	36.59 (0.49)	<0.0010	33.92 (0.5)	32.67 (0.47)	0.102
ETEC	39.07 (0.47)	40.53 (0.42)	0.015	39.93 (0.43)	39.57 (0.44)	0.538
Campylobacter	37.56 (0.45)	41.38 (0.31)	<0.0010	37.18 (0.47)	37.57 (0.48)	0.321
STEC	43.67 (0.22)	44.23 (0.15)	0.040	43.95 (0.18)	43.55 (0.23)	0.185
B. fragilis	41.99 (0.37)	43.13 (0.29)	0.001	42.16 (0.36)	42.34 (0.35)	0.363
C. difficile	44.17 (0.18)	44.38 (0.16)	0.369	44.05 (0.2)	44.11 (0.19)	0.586
Shigella	44.16 (0.19)	44.66 (0.12)	0.002	43.93 (0.2)	44.08 (0.21)	0.194
Salmonella	43.81 (0.21)	44.66 (0.11)	<0.0010	44 (0.18)	44.21 (0.16)	0.379
Adenovirus	38.7 (0.41)	38.05 (0.43)	0.380	37.83 (0.42)	37.57 (0.42)	0.699
Astrovirus	43.88 (0.21)	43.85 (0.21)	0.971	44.02 (0.18)	44.19 (0.18)	0.469
Enterovirus	34.05 (0.41)	34.98 (0.43)	0.012	35.23 (0.42)	35.68 (0.41)	0.127
Norovirus (GI,GII)	42.56 (0.31)	41.89 (0.35)	0.074	42.23 (0.33)	42.27 (0.33)	0.721
Rotavirus	42.97 (0.22)	43.59 (0.22)	0.009	43.15 (0.2)	43.69 (0.18)	0.014
Sapovirus	43.31 (0.27)	43.89 (0.21)	0.017	43.64 (0.2)	43.89 (0.22)	0.118
Cryptosporidium	42.72 (0.29)	42.99 (0.27)	0.346	42.79 (0.3)	43.17 (0.27)	0.048
E. bieneusi	44.41 (0.15)	44.38 (0.17)	0.801	44.18 (0.19)	44.39 (0.17)	0.136
Giardia	41.28 (0.39)	40.9 (0.41)	0.345	41.48 (0.37)	41.62 (0.37)	0.575

Appendix Table 7. Prevalence and abundance of intestinal pathogens on the day of vaccination (day 14) by seroconversion status. Details as for appendix table 4. Adjusted p-values are based on a logistic regression that included infant's age in months as a potential confounder.

Pathogen	Prevalence, n (%) Seroconversion				Abundance, mean Ct (SE) Seroconversion			
	negative	positive	p-value	adjusted p-value	negative	positive	p-value	adjusted p-value
EAEC	204 (60.5)	199 (54.2)	0.382	0.083	29.36 (0.49)	30.56 (0.51)	0.204	0.155
EPEC	124 (36.8)	133 (36.2)	0.942	0.925	34.43 (0.5)	34.76 (0.49)	0.652	0.894
ETEC	46 (13.6)	58 (15.8)	0.529	0.335	40.29 (0.44)	39.82 (0.43)	0.412	0.419
Campylobacter	53 (15.7)	59 (16.1)	0.919	0.681	39.52 (0.42)	39.39 (0.41)	0.748	0.687
STEC	3 (0.9)	7 (1.9)	0.346	0.262	44.04 (0.18)	43.73 (0.2)	0.452	0.279
Aeromonas	0 (0.0)	1 (0.3)	1.000	0.980				
B. fragilis	25 (7.4)	30 (8.2)	0.780	0.439	42.85 (0.32)	42.61 (0.33)	0.878	0.338
C. difficile	10 (3.0)	6 (1.6)	0.314	0.243	44.16 (0.2)	44.32 (0.16)	0.984	0.446
Shigella	9 (2.7)	5 (1.4)	0.284	0.212	44.17 (0.2)	44.54 (0.14)	0.259	0.144
H. pylori	0 (0.0)	1 (0.3)	1.000	0.980				
M. tuberculosis	0 (0.0)	0 (0.0)	1.000	1.000				
Salmonella	2 (0.6)	3 (0.8)	1.000	0.811				
V. cholerae	0 (0.0)	0 (0.0)	1.000	1.000				
Adenovirus	70 (20.8)	53 (14.4)	0.078	0.034	37.3 (0.46)	38.28 (0.4)	0.125	0.126
Astrovirus	3 (0.9)	7 (1.9)	0.346	0.242	44.08 (0.19)	43.97 (0.2)	0.983	0.616
Enterovirus	149 (44.2)	107 (29.2)	0.005	<0.001	34.17 (0.44)	36.41 (0.4)	<0.0010~~00~~	<0.001
Norovirus (GI,GII)	34 (10.1)	27 (7.4)	0.285	0.156	41.74 (0.37)	42.39 (0.31)	0.174	0.127
Rotavirus	10 (3.0)	3 (0.8)	0.049	0.073	43.21 (0.24)	44.03 (0.15)	0.010	0.008
Sapovirus	7 (2.1)	11 (3.0)	0.484	0.321	43.94 (0.21)	43.85 (0.21)	0.848	0.632
Ancylostoma	0 (0.0)	0 (0.0)	1.000	1.000				
Ascaris	0 (0.0)	0 (0.0)	1.000	1.000				
Cryptosporidium	16 (4.7)	14 (3.8)	0.581	0.639	42.99 (0.29)	43.16 (0.25)	0.744	0.747
Cyclospora	1 (0.3)	2 (0.5)	1.000	0.572				
E. bieneusi	6 (1.8)	5 (1.4)	0.765	0.767	44.31 (0.19)	44.45 (0.15)	0.780	0.543
E. histolytica	0 (0.0)	0 (0.0)	1.000	1.000				
E. intestinalis	0 (0.0)	0 (0.0)	1.000	1.000				
Giardia	32 (9.5)	25 (6.8)	0.271	0.327	40.99 (0.42)	41.52 (0.37)	0.656	0.491
Isospora	0 (0.0)	0 (0.0)	1.000	1.000				
Necator	0 (0.0)	0 (0.0)	1.000	1.000				
Strongyloides	0 (0.0)	0 (0.0)	1.000	1.000				
Trichuris	0 (0.0)	0 (0.0)	1.000	1.000				

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Appendix Table 8. Prevalence and abundance of intestinal pathogens on the day of vaccination (day 14) according to poliovirus shedding in stool 7 days after OPV. Data available for the subset of infants with poliovirus PCR results and stool sample from day 14 who completed the study per protocol (n=291). Details as for appendix table 4.

Pathogen	Prevalence, n (%)			Abundance, mean Ct (SE)		
	Shedding negative	positive	p-value	Shedding negative	positive	p-value
EAEC	81 (60.0)	91 (58.3)	0.923	28.91 (0.78)	30.25 (0.74)	0.180
EPEC	53 (39.3)	64 (41.0)	0.913	34.12 (0.76)	33.28 (0.75)	0.543
ETEC	21 (15.6)	26 (16.7)	0.876	39.66 (0.73)	39.3 (0.66)	0.818
Campylobacter	23 (17.0)	20 (12.8)	0.416	39.21 (0.69)	39.95 (0.62)	0.250
STEC	0 (0.0)	3 (1.9)	0.252	44.07 (0.26)	44.2 (0.26)	0.347
Aeromonas	0 (0.0)	0 (0.0)	1.000			
B. fragilis	9 (6.7)	11 (7.1)	1.000	43 (0.49)	42.65 (0.5)	0.829
C. difficile	6 (4.4)	0 (0.0)	0.011	43.74 (0.39)	44.6 (0.18)	0.107
Shigella	4 (3.0)	3 (1.9)	0.709	44.12 (0.34)	44.37 (0.25)	0.734
H. pylori	0 (0.0)	0 (0.0)	1.000			
M. tuberculosis	0 (0.0)	0 (0.0)	1.000			
Salmonella	0 (0.0)	1 (0.6)	1.000			
V. cholerae	0 (0.0)	0 (0.0)	1.000			
Adenovirus	33 (24.4)	26 (16.7)	0.200	36.63 (0.73)	37.02 (0.62)	0.679
Astrovirus	0 (0.0)	3 (1.9)	0.252	44.31 (0.24)	43.65 (0.34)	0.153
Enterovirus	61 (45.2)	50 (32.1)	0.147	33.72 (0.67)	35.84 (0.61)	0.011
Norovirus (GI,GII)	6 (4.4)	10 (6.4)	0.609	42.75 (0.46)	42.69 (0.48)	0.685
Rotavirus	5 (3.7)	4 (2.6)	0.738	43.1 (0.39)	43.87 (0.29)	0.067
Sapovirus	3 (2.2)	3 (1.9)	1.000	43.95 (0.35)	43.77 (0.33)	0.862
Ancylostoma	0 (0.0)	0 (0.0)	1.000			
Ascaris	0 (0.0)	0 (0.0)	1.000			
Cryptosporidium	7 (5.2)	6 (3.8)	0.778	43.22 (0.42)	43.08 (0.4)	0.629
Cyclospora	1 (0.7)	1 (0.6)	1.000			
E. bieneusi	1 (0.7)	1 (0.6)	1.000			
E. histolytica	0 (0.0)	0 (0.0)	1.000			
E. intestinalis	0 (0.0)	0 (0.0)	1.000			
Giardia	18 (13.3)	5 (3.2)	0.004	40.61 (0.74)	42.58 (0.44)	0.236
Isospora	0 (0.0)	0 (0.0)	1.000			
Necator	0 (0.0)	0 (0.0)	1.000			
Strongyloides	0 (0.0)	0 (0.0)	1.000			
Trichuris	0 (0.0)	0 (0.0)	1.000			

Appendix Table 9. Change in faecal biomarkers of environmental enteropathy (EE) between day 0 and 14 according to study arm. Changes over time assessed using Wilcoxon signed rank test (for paired data).

	Azithromycin			Placebo		
Biomarker	day 0	day 14	p-value	day 0	day 14	p-value
Myeloperoxidase (ng/ml)	20404 (1408)	15780 (1277)	0.001	21715 (1587)	21251 (1407)	0.953
Calprotectin (µg/g)	1092 (61)	862 (65)	<0.001	1030 (53)	1094 (61)	0.538
Neopterin (nmol/l)	5912 (354)	6644 (396)	0.030	6659 (298)	6934 (423)	0.859
α-antitrypsin (mg/g)	0.98 (0.07)	1.03 (0.08)	0.904	1.08 (0.07)	1.27 (0.11)	0.116

data are mean (SE)

Biomarker	Azithromycin Absolute difference	p-value	Placebo Absolute difference	p-value
Myeloperoxidase (ng/ml)	-4624 (1749)	0.001	-464 (1639)	0.953
Calprotectin (µg/g)	-221 (64)	<0.001	64 (60)	0.538
Neopterin (nmol/l)	702 (335)	0.030	275 (423)	0.859
α1-antitrypsin (mg/g)	0.061 (0.091)	0.904	0.189 (0.110)	0.116

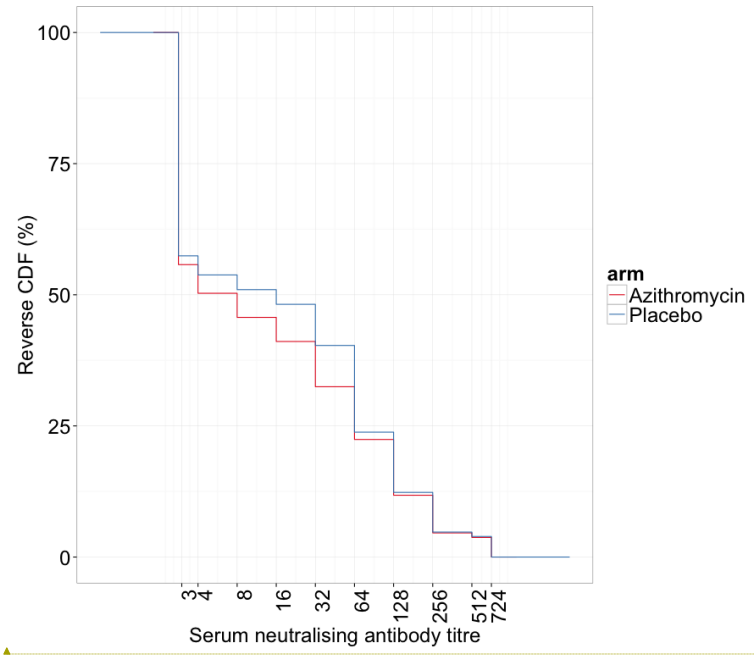
data are mean (SE)

Appendix Table 10. Correlation of biomarkers of EE and number of intestinal pathogens by group. Multivariable linear regression of the log-transformed biomarkers against the number of pathogens in each group was performed and coefficients (95% confidence intervals) and p-values are shown. Faecal biomarkers and the number of intestinal pathogens are compared for stool samples collected at enrolment. Plasma biomarkers are available on the day of vaccination only, and are compared to intestinal pathogens in stool collected at the same time in the placebo arm. Biomarkers were log-transformed after performing regression diagnostics to improve normality of the residuals and homoscedasticity.

Biomarker	**Correlation with number of pathogens**					
	bacteria	**p-value**	**viruses**	**p-value**	**eukaryotes**	**p-value**
Stool (day 0)						
Myeloperoxidase (ng/ml)	0.17 (0.05 - 0.28)	0.005	0.14 (-0.03 - 0.32)	0.108	0.03 (-0.31 - 0.37)	0.852
Calprotectin (µg/g)	0.19 (0.08 - 0.30)	0.001	0.11 (-0.07 - 0.28)	0.236	0.12 (-0.21 - 0.46)	0.467
Neopterin (nmol/l)	0.05 (-0.03 - 0.12)	0.206	-0.04 (-0.16 - 0.07)	0.470	-0.43 (-0.65 - -0.21)	<0.001
α1-antitrypsin (mg/g)	-0.04 (-0.12 - 0.05)	0.411	0.11 (-0.02 - 0.23)	0.104	0.09 (-0.16 - 0.33)	0.485
Plasma (day 14, placebo arm)						
Soluble CD14 (ng/ml)	0.00 (-0.09 - 0.10)	0.924	0.07 (-0.07 - 0.20)	0.353	0.19 (-0.17 - 0.54)	0.301
I-FABP (pg/ml)	0.02 (-0.07 - 0.11)	0.618	0.07 (-0.06 - 0.21)	0.285	-0.07 (-0.40 - 0.26)	0.680
EndoCAb IgG (GMU/ml)	0.08 (-0.06 - 0.22)	0.252	-0.13 (-0.34 - 0.07)	0.213	-0.11 (-0.63 - 0.41)	0.678

Appendix Table 11. Correlation matrix for biomarkers of EE measured on the day of vaccination by study arm. Spearman's rank correlation coefficient (p-value) are shown for significant correlations only ($p<0.05$). Correlations are shown in the upper triangular matrix.

	calprotectin	neopterin	α1-antitrypsin	soluble CD14	l-FABP	EndoCAb
Placebo						
myeloperoxidase	0.61 (<0.001)	0.21 (0.009)	0.30 (<0.001)			
calprotectin			0.17 (0.034)			
neopterin			0.25 (0.002)			
α1-antitrypsin						
soluble CD14						-0.17 (0.036)
l-FABP						
EndoCAb IgG						
Azithromycin						
myeloperoxidase	0.68 (<0.001)		0.33 (<0.001)			
calprotectin						
neopterin				-0.25 (0.003)		
α1-antitrypsin						
soluble CD14						
l-FABP						
EndoCAb IgG						



Appendix Figure 1 Reverse cumulative distribution of serum neutralizing antibody titres on day 35 according to study arm.

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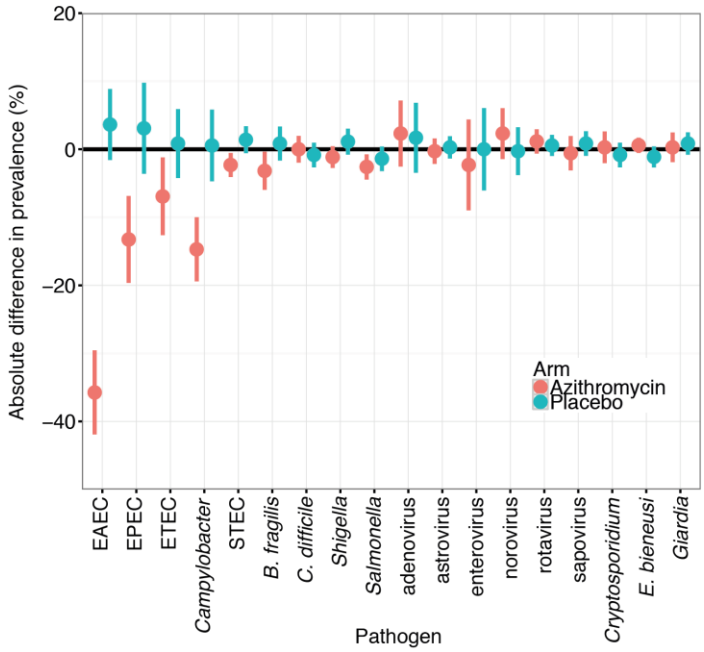
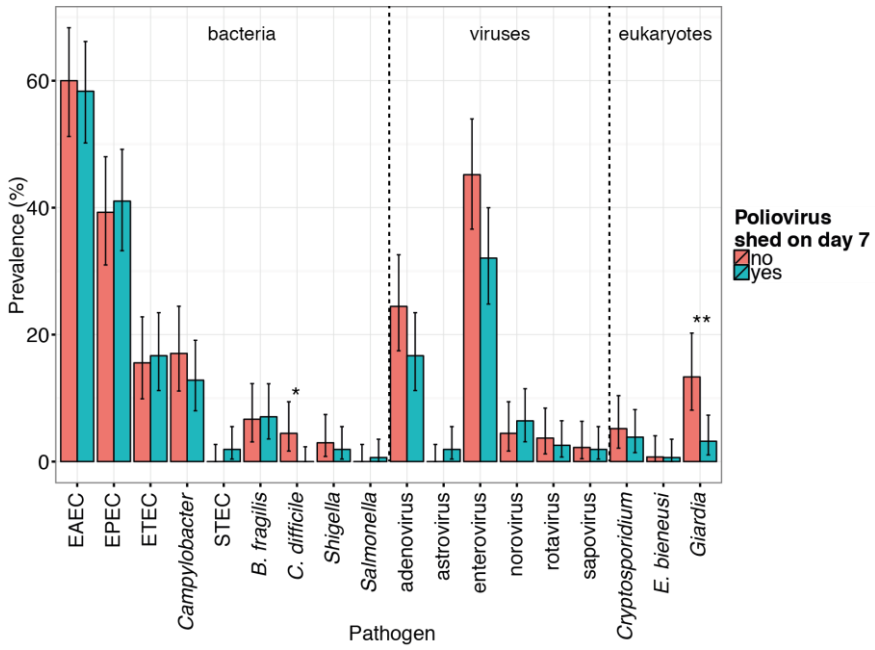


Appendix Figure ~~1~~ 2 Change in prevalence of intestinal pathogens detected by TAC between study day 0 and 14 according to study arm. The absolute difference in prevalence is presented together with Wald 95% confidence intervals for paired data. Only pathogens with a prevalence of at least 1% across all samples are shown. Abbreviations: EAEC, enteroaggregative *Escherichia coli*; EPEC, enteropathogenic *E. coli*; ETEC, enterotoxigenic *E. coli*; STEC, shiga toxin-producing *E. coli*; *B. fragilis*, *Bacteroides fragilis*; *C. difficile*, *Clostridium difficile*; *E. bieneusi, Enterocytozoon bieneusi.*



Appendix Figure ~~2~~ 3 Prevalence of intestinal pathogens at the time of vaccination with OPV according to whether they shed poliovirus 7 days after OPV. Results are shown for the subset where poliovirus shedding was assessed (n=299). Only pathogens with a prevalence of at least 1% across all samples are shown. Error bars indicate 95% confidence intervals. * p-value < 0.05; ** p-value < 0.01. All p-values are given in appendix table 8. Abbreviations: EAEC, enteroaggregative *Escherichia coli*; EPEC, enteropathogenic *E. coli*; ETEC, enterotoxigenic *E. coli*; STEC, shiga toxin-producing *E. coli*; *B. fragilis*, *Bacteroides fragilis*; *C. difficile*, *Clostridium difficile*; *E. bieneusi*, *Enterocytozoon bieneusi.*

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