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Supplementary appendix

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

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Supplementary Methods

Table S1: Key timepoints for study activities by study arm and age of the participant

		Age of participant				
	Study group	6 weeks	10 weeks *	14 weeks	18 weeks	22 weeks
RotaTeq arm	Group A1	Blood collection; RotaTeq dose 1	RotaTeq dose 2	Blood collection; RotaTeq dose 3; IPV	Blood and saliva collection	Blood collection; IPV
	Group B1	Blood collection; RotaTeq dose 1	RotaTeq dose 2	Blood collection; RotaTeq dose 3; IPV	Blood and saliva collection	Blood collection; fIPV
	Group C1	Blood collection; RotaTeq dose 1; IPV	RotaTeq dose 2	Blood collection; RotaTeq dose 3	Blood and saliva collection	Blood collection; fIPV
	Group D1	Blood collection; RotaTeq dose 1; fIPV	RotaTeq dose 2	Blood collection; RotaTeq dose 3; fIPV	Blood and saliva collection	Blood collection; fIPV
Rotarix arm	Group A2	Blood collection; Rotarix dose 1	Rotarix dose 2	Blood collection; IPV	Saliva collection	Blood collection; IPV
	Group B2	Blood collection; Rotarix dose 1	Rotarix dose 2	Blood collection; IPV	Saliva collection	Blood collection; fIPV
	Group C2	Blood collection; Rotarix dose 1; IPV	Rotarix dose 2	Blood collection	Saliva collection	Blood collection; fIPV
	Group D2	Blood collection; Rotarix dose1; fIPV	Rotarix dose 2	Blood collection; fIPV	Saliva collection	Blood collection; fIPV

IPV=inactivated poliovirus vaccine. fIPV=fractional inactivated poliovirus vaccine.

* Saliva samples were also collected at age ten weeks as back-up for samples at age 18 weeks.

EIA to detect rotavirus specific IgA and IgG antibodies in serum samples

Rotavirus IgA and Rotavirus IgG were performed using a custom EIA. [2] Briefly, 96-well microplate wells coated with rabbit hyperimmune serum to rhesus rotavirus were incubated with clarified lysates of either RotaTeq G1 or Rotarix strains-infected MA104 cell cultures, according to the vaccine arm. Serial dilutions of test sera (1:20–1:10,240) were added, followed by biotin-conjugated goat anti-human IgA or IgG antibodies (KPL, USA). Extravidin (Sigma, USA) was added to the wells, and then the reactions were developed with 3,30,5,50-tetramethylbenzidine (Sigma, USA) and stopped with 1N hydrogen chloride. Absorbance was subsequently read at 450 nm. Rotavirus IgA and Rotavirus IgG titres in serum were calculated as the reciprocal of the highest dilution that gave a mean OD greater than the cutoff value (3 standard deviations above the mean OD of the negative control serum wells). The CDC laboratory staff testing one type of sample (e.g., serum, saliva) were blinded to the results of testing the other sample type.

EIA to determine secretor, Lewis and salivary ABO blood group phenotype in saliva samples

Saliva samples were also collected at age ten weeks, as back-up for samples at age 18 weeks. Once saliva was collected, the swab was placed in a Swab Storage Tube (SST) by inserting the saturated end of the swab and folding the dry end into the SST. Saliva was heat-treated at 100° C for 10 minutes, centrifuged at 21,000 g for 1 minute, and stored at -20° C until use. Samples diluted 1:100 in PBS were coated into EIA plates. HRP-conjugated Ulex europaeus agglutinin (UEA-I) (Sigma, USA) was added and developed using TMB substrate (Sigma, USA) to establish secretor status. [3] To establish the ABO and Lewis phenotype, monoclonal antibodies to blood group A (Santa Cruz Biotechnology, USA), blood group B (Santa Cruz Biotechnology, USA), Lewis a, Lewis b, Lewis x (Santa Cruz Biotechnology, USA), and Lewis y (Sigma, USA) were added at 1 µg/mL. [4] Bound monoclonal antibodies were detected using anti-mouse IgG/IgM-AP conjugate (Sigma, USA) followed by color development with SIGMAFAST™ p-Nitrophenyl phosphate (Sigma, USA). For each infant, both secretor and Lewis, and salivary ABO blood group phenotype test results were used to classify infants as positive or negative for the secretor ($Ulex^{+}$ or $Ulex^{-}$, respectively), Lewis (Le^{a+}, Le^{b+}, Le^{x+}, Le^{y+} or $Le^{a-/b-/x-/y-}$, respectively) and A or B blood group antigens.

For samples that were negative across all the phenotyping assays (Ulex-, Lea-/b-/x-/y-, A-, B-), secretor genotyping was performed. Secretor genotype was determined by pyrosequencing and defined by a single nucleotide polymorphism at position 428G>A in the fut2 gene. [5]

Change in rotavirus IgA titres between the timepoints

For infant's rotavirus IgA seropositive in the earlier sample, the change was classified as rotavirus IgA waning by the time of the later sample if there was either 1) four-fold or greater decrease in the titre, or 2) the participant was now rotavirus IgA seronegative (IgA titre <40) in the later sample. The change was classified as rotavirus IgA boosting by the time of the later sample if 1) for infants who were rotavirus IgA seropositive in the earlier sample, there was a four-fold or greater increase in rotavirus IgA titre, or 2) for infants who were rotavirus IgA seronegative in the earlier sample, the participant was now rotavirus IgA seropositive in the later sample. If no additional rotavirus vaccine doses had been administered between the sample timepoints, any boosting was presumed to be from an intervening wild-type rotavirus infection.

Statistical analysis (Continued)

Potential causes for lack of seroresponse: rotavirus IgG titres s at six age weeks were categorized in tertiles of similar sample sizes (low [80 to 1,280], medium [2,560], and high [5,120 to 10,240] rotavirus IgG titres). The degree of exposure to the rotavirus season between the collection of samples at ages 6 and 14 weeks was categorized based on national surveillance data from hospitals in Bangladesh: low exposure (calendar period between the collection of the two samples included only October or November 2016), moderate exposure (period included December 2016 but not later), or high exposure (period included January or February 2017). [6] Feeding practice at the time of rotavirus vaccines doses was defined as a dichotomous variable (exclusive breastfeeding at the time of each of the two or three vaccine doses vs. partial breastfeeding at the time of at least one of the doses of the vaccine.

Z length for age (LAZ) and Z weight for age (WAZ) scores at each study visit were calculated using the R Anthro software package (version 0.9.4) according to the child-growth standard curves from WHO's Multicentre Growth Reference Study. [7] Wasting or stunting was deemed to be present if participants' measurements were more than two SD below the mean of the reference population. We generated two dichotomous variables for LAZ (lowest quartile (LAZ ≤ -1.5) / second, third and fourth quartiles) and WAZ (lowest quartile (WAZ ≤ -1.0)/ second, third and fourth quartiles) at the blood draws.

Regression models were used to estimate the relationship between potential predictors and rotavirus vaccine immunogenicity among infants who were rotavirus IgA seronegative at age six weeks. The regression analyses were conducted separately for each arm. Multivariable log-binomial regression models were used to estimate the risk ratios (RRs) with 95% CIs of rotavirus IgA seroconversion. Multivariable linear regression models were used to estimate the beta with 95% CIs of rotavirus IgA titres. We initially included variables with a p value < 0.20 from the univariable analyses in the multivariable models. Then, the hierarchical backward elimination approach was used to select the covariates in the final model, retaining only variables significant at p value < 0.05. Interaction between variables was also assessed in the models.

Supplementary Results

Table S2: Rotavirus IgA GMTs of those infants who were seropositive at the indicated timepoint

	RotaTeq arm		Rotarix arm	
Schedule	**n**	**Rotavirus IgA GMT (95% CI)**	**n**	**Rotavirus IgA GMT (95% CI)**
Age 6 weeks (before first dose)	67	90 (67-120) [a]	75	93 (74-116) [e]
Age 14 weeks (4 weeks after second dose)	303	305 (252-370) [b]	382	331 (284-384) [f]
Age 18 weeks (4 weeks after third dose)	414	488 (410-581) [c]	..	..
Age 22 weeks 8 weeks after third dose [RotaTeq]; 12 weeks after second dose [Rotarix]	412	549 (456-662) [d]	307	319 (265-384) [g]
p values		[b] vs [a]: <0.0001 [c] vs [b]: 0.01 [d] vs [c]: 0.34		[f] vs [e]: <0.0001 [g] vs [f]: p=0.77

Data are the number of rotavirus IgA seropositives and GMT (95% CIs). GMT=geometrical mean titre

Table S3: Classification of infants in RotaTeq and Rotarix arms by secretor phenotype, salivary ABO blood group (among secretors), and Lewis antigen phenotype

	RotaTeq arm	Rotarix arm	Total
Secretor phenotype			
Non-secretor	170/526 (32%)	156/538 (29%)	326/1064 (31%)
Secretor	356/526 (68%)	382/538 (71%)	738/1064 (69%)
Not determined *	5	11	16
Salivary ABO blood group among secretors			
Blood group O	122/356 (34%)	153/382 (40%)	275/738 (37%)
Blood group A	78/356 (22%)	84/382 (22%)	162/738 (22%)
Blood group B	128/356 (36%)	128/382 (34%)	256/738 (35%)
Blood group AB	28/356 (8%)	17/382 (4%)	45/738 (6%)
Lewis phenotype			
Negative	15/531 (3%)	27/549 (5%)	42/1080 (4%)
Positive	516/531 (97%)	522/549 (95%)	1038/1080 (96%)

Data are n/N (%). * Negative for secretor and Lewis status by enzyme-linked immunosorbent assay (phenotyping analysis) but determined to be secretor-positive by genotyping.

Table S4: **Rotavirus IgA GMTs and GMT ratios by secretor phenotype**

	Rotavirus IgA GMT at age 6 weeks	GMT ratio (95%CI)	p value	Rotavirus IgA GMT at age 14 weeks	GMT ratio (95%CI)	p value	Rotavirus IgA GMT at age 18 weeks	GMT ratio (95%CI)	p value	Rotavirus IgA GMT at age 22 weeks	GMT ratio (95%CI)	p value
RotaTeq arm												
Secretor phenotype												
Non-secretor	4 (3-5)	··	··	34 (24-48)	··	··	97 (67-139)	··	··	123 (86-175)	··	··
Secretor	4 (3-4)	0.97 (0.70-1.35)	0.86	67 (51-89)	1.97 (1.23-3.14)	0.01	275 (216-351)	2.84 (1.48-3.65)	<0.0001	286 (220-371)	2.33 (1.48-3.65)	0.01
Rotarix arm												
Secretor phenotype												
Non-secretor	3 (2-4)	··	··	51 (34-75)	··	··	NA	NA	NA	16 (11-25)	··	··
Secretor	3 (2-3)	0.94 (0.68-1.31)	0.73	102 (79-132)	2.03 (1.26-3.26)	0.01	NA	NA	NA	51 (38-68)	3.12 (1.83-5.30)	< 0.0001

Data are GMT (95% CI), unless otherwise specified. GMT=geometric mean titre. NA=not applicable.

Table S5: Rotavirus IgA GMTs of those infants who were rotavirus IgA seropositive at the indicated timepoints in RotaTeq and Rotarix arms by secretor phenotype

	Non-secretor		Secretor		
RotaTeq arm	**n**	**Rotavirus IgA GMT (95% CI)**	**n**	**Rotavirus IgA GMT (95% CI)**	**p value**
Age 6 weeks (before first dose)	20	70 (47-104)	47	100 (68-146)	0.26
Age 14 weeks (4 weeks after second dose)	88	192 (142-260)	213	368 (290-467)	0.01
Age 18 weeks (4 weeks after third dose)	117	322 (237-438)	294	563 (457-695)	0.01
Age 22 weeks (8 weeks after third dose)	120	345 (246-483)	289	652 (523-814)	0.01
Rotarix arm					
Age 6 weeks (before first dose)	21	83 (51-134)	53	99 (76-128)	0.49
Age 14 weeks (4 weeks after second dose)	98	255 (195-333)	276	364 (303-436)	0.04
Age 22 weeks (12 weeks after second dose)	66	226 (155-330)	235	357 (287-443)	0.05

Data are GMT (95% CIs). Five (1%) of 531 saliva samples in the RotaTeq arm and 11 (2%) of 549 in the Rotarix arm were not successfully phenotyped for histo-blood group antigens by enzyme-linked immunosorbent assay. GMT=geometric mean titre.

Table S6: Change in rotavirus IgA titres at the individual participant level between ages 14 and 18 weeks, and between ages 18 and 22 weeks in the RotaTeq arm, and between ages 14 and 22 weeks in the Rotarix arm

		n	Change in rotavirus IgA titre between the timepoints		
			Waning	No change	Boosting
RotaTeq (change in rotavirus IgA titre between 14 and 18 weeks [third dose given at age 14 weeks])	Of those without rotavirus IgA seroconversion in sample at age 14 weeks (after second dose)	263	2 (1%)	119 (45%)	142 (54%)
	Of those with rotavirus IgA seroconversion in sample at age 14 weeks (after second dose)	268	37 (14%)	132 (49%)	99 (37%)
	Total	531	39 (7%)	251 (47%)	241 (45%)
RotaTeq (change in rotavirus IgA titre between 18 and 22 weeks [no intervening doses])	Of those without rotavirus IgA seroconversion in sample at age 18 weeks (after third dose)	141	4 (3%)	87 (62%)	50 (35%)
	Of those with rotavirus IgA seroconversion in sample at age 18 weeks (after third dose)	390	103 (26%)	215 (55%)	72 (18%)
	Total (1)	531	107 (20%)	302 (57%)	122 (23%)
Rotarix (change in rotavirus IgA titre between 14 and 22 weeks [no intervening doses])	Of those without rotavirus IgA seroconversion in sample at age 14 weeks (after second dose)	195	12 (6%)	135 (69%)	48 (25%)
	Of those with rotavirus IgA seroconversion in samples at age 14 weeks (after second dose)	354	210 (59%)	101 (29%)	43 (12%)
	Total (2)	549	222(40%)	236 (43%)	91 (17%)
	P value (1 vs 2)	..	< 0.0001	..	0.01

Data are n or n (%), unless otherwise specified.
Information about the categories in the second column:
For top row for each category (i.e., those without seroconversion in the sample at the referenced first time point) the starting cohort are those in either of these three categories:
1. seropositive at age 6 weeks and the referenced first timepoint listed, but without a four-fold increase in titre based on those two samples.
2. seropositive at age 6 weeks but seronegative at the referenced first timepoint
3. seronegative at age 6 weeks and the referenced first timepoint.
For the second row for each category (i.e., those with seroconversion in the sample at the referenced first timepoint) the starting cohort are those in either of these two categories:
1. seropositive at age 6 weeks and the referenced first timepoint listed and have a four-fold increase based on those two samples
2. seronegative at age 6 weeks and seropositive in the referenced first timepoint.
Waning or boosting was defined by the participant's rotavirus IgA titre change between timepoints in the column headings:
For infants who were rotavirus IgA seropositive in the earlier sample, the change was classified as rotavirus IgA waning by the time of the later sample if there was either a four-fold or greater decrease in the titre or the participant was now rotavirus IgA seronegative (IgA titre <40) in the later sample. For infants who were rotavirus IgA seropositive in the earlier sample, the change was classified as rotavirus IgA waning by the time of the later sample if there was either 1) four-fold or greater decrease in the titre, or 2) the participant was now rotavirus IgA seronegative (IgA titre <40) in the later sample. The change was classified as rotavirus IgA boosting by the time of the later sample if 1) for infants who were rotavirus IgA seropositive in the earlier sample, there was a four-fold or greater increase in rotavirus IgA titre, or 2) for infants who were rotavirus IgA seronegative in the earlier sample, the participant was now rotavirus IgA seropositive in the later sample.

Rotavirus IgA seroconversion with fIPV and IPV administration: There were no differences in rotavirus IgA seroconversion in the RotaTeq arm among the groups that received concomitant fIPV administration with RotaTeq doses one and three, and the two groups that received IPV concomitantly with either RotaTeq doses one or three (table S6). Similarly, in the Rotarix arm, the group with concomitant fIPV administration with Rotarix dose one did not have significantly different rotavirus IgA seroconversion or GMT than those with IPV concomitantly with Rotarix dose one or than the group that did not receive IPV with Rotarix dose one (table S7).

Table S7: Rotavirus IgA seroconversion 4 weeks after the full series* of RotaTeq or Rotarix by IPV administration

RotaTeq arm	n	Rotavirus IgA seroconversion, n (%)	p value
fIPV concomitant with RotaTeq dose 1 and dose 3 (Group D)	132	94 (71%)	ref
IPV concomitant with RotaTeq dose 1 only (Group C)	134	104 (78%)	0.23
IPV concomitant with RotaTeq dose 3 only (Groups A+B)	265	192 (72%)	0.80
Rotarix arm			
fIPV concomitant with Rotarix dose 1 only (Group D)	139	83 (60%)	ref
IPV concomitant with Rotarix dose 1 only (Group C)	134	85 (63%)	0.53
No IPV received (Groups A+B)	276	186 (67%)	0.12

Data are n or n (%), unless otherwise specified. * Seroconversion 4 weeks after third dose [RotaTeq], or 4 weeks after second dose 2 [Rotarix]. IPV=inactivated poliovirus vaccine. fIPV=fractional inactivated poliovirus vaccine. ref=reference.

Table S8. Multivariable regression for rotavirus IgA titres at ages 14 (a), 18 (b), and 22 (c) weeks in infants who were rotavirus IgA seronegative at age 6 weeks in the RotaTeq arm
(a)

		Rotavirus IgA titres at age 14 weeks			
	N	**GMT (95% CI)**	**Univariable p value**	**Multivariable beta (95% CI)**	**Multivariable p value**
Rotavirus IgG titres at age 6 weeks (tertiles)					
80-1280 (ref)	169	79 (54-116)	..	..	..
2560	158	53 (35-80)	..	..	..
5120-10 240	133	22 (15-34)	<0.0001	0.6 (0.4-0.7)	<0.0001
Secretor phenotype					
Non-secretor (ref)	150	31 (21-46)	..	..	..
Secretor	309	59 (43-79)	0.01	1.3 (1.1-3.1)	0.01
Rotavirus season exposure between ages 6 and 14 weeks					
Low (October to November 2016; ref)	135	32 (21-50)	..	..	..
Moderate (December 2016)	179	36 (25-51)	..	..	..
High (January to February 2017)	150	91 (58-142)	0.01	1.5 (1.3-2.4)	0.01
Feeding practices					
Partial breastfeeding at time of ≥1 dose (ref)	345	55 (41-72)	..	..	..
Exclusive breastfeeding at time of each dose	119	33 (20-52)	0.05	0.8 (0.3-1.0)	0.03

Log-transformed rotavirus IgA titres measured at ages 14 (a), 18 (b), and 22 (c) weeks served as the dependent variable. Variables with a p value of <0.05 during multivariable analyses are shown. Univariable regression results of not retained variables are provided in table S9. Four (1%) of 464 serum samples were missing for the rotavirus IgG titres at age 6 weeks variable. Five (1%) of 464 saliva samples were missing for the secretor phenotype variable. This analysis was done among all individuals who were rotavirus IgA seronegative at age 6 weeks (N=464). GMT=geometric mean titre. ref=reference.

(b)

	Rotavirus IgA titres at age 18 weeks				
	N	**GMT (95%CI)**	**Univariable p value**	**Multivariable beta (95%CI)**	**Multivariable p value**
Rotavirus IgG titres at age 6 weeks (tertiles)					
80-1280 (ref)	169	307 (219-431)	..	..	..
2560	158	202 (138-297)	..	..	..
5120-10 240	133	76 (50-115)	<0.0001	0.6 (0.4-0.7)	<0.0001
Secretor phenotype			..	..	..
Non-secretor (ref)	150	88 (60-130)	..	..	..
Secretor	309	249 (191-324)	<0.0001	1.6 (1.7-4.2)	<0.0001
Feeding practices					
Partial breastfeeding at time of ≥1 dose (ref)	358	224 (176-285)	..	..	..
Exclusive breastfeeding at time of each dose	106	81 (49-135)	0.01	0.7 (0.3-0.7)	0.01

(c)

	Rotavirus IgA titres at age 22 weeks				
	N	**GMT (95%CI)**	**Univariable p value**	**Multivariable beta (95%CI)**	**Multivariable p value**
Rotavirus IgG titres at age 6 weeks (tertiles)					
80-1280 (ref)	169	343 (245-481)	..	..	..
2560	158	210 (140-314)	..	..	..
5120-10 240	133	77 (50-119)	<0.0001	0.6 (0.4-0.6)	<0.0001
Secretor phenotype					
Non-secretor (ref)	150	114 (78-166)	..	..	..
Secretor	309	240 (180-319)	0.01	1.4 (1.3-3.2)	0.01
Feeding practices					
Partial breastfeeding at time of ≥1 dose (ref)	358	224 (174-289)	..	..	..
Exclusive breastfeeding at time of each dose	106	105 (62-175)	0.01	0.8 (0.3-0.9)	0.01

Log-transformed rotavirus IgA titres measured at ages 14 (a), 18 (b), and 22 (c) weeks served as the dependent variable. Variables with a p value of <0.05 during multivariable analyses are shown. Univariable regression results of not retained variables are provided in table S9. Four (1%) of 464 serum samples were missing for the rotavirus IgG titres at age 6 weeks variable. Five (1%) of 464 saliva samples were missing for the secretor phenotype variable. This analysis was done among all individuals who were rotavirus IgA seronegative at age 6 weeks (N=464). GMT=geometric mean titre. ref=reference.

Table S9. Univariable regression of variables not retained in multivariable regression for rotavirus IgA seroconversion at ages 14 (a), 18 (b), and 22 (c) weeks in infants who were rotavirus IgA seronegative at age 6 weeks in the RotaTeq arm

(a)

	Rotavirus IgA seroconversion at age 14 weeks		
	N	**n (%)**	**Univariable p value**
Sex			
Female (ref)	244	125 (51%)	..
Male	220	123 (56%)	0.35
Secretor phenotype			
Non-secretor (ref)	150	73 (49%)	..
Secretor	309	173 (56%)	0.16
LAZ-score lowest quartile (<-1.5) at the age of blood draw			
No (ref)	332	175 (53%)	..
Yes	132	73 (55%)	0.68
WLZ-score lowest quartile (<-1.0) at the age of blood draw			
No (ref)	329	178 (54%)	..
Yes	135	70 (52%)	0.68
IPV administration group			
(Group D) (ref)	121	59 (49%)	..
(Group C)	114	65 (57%)	0.21
(Groups A+B)	229	124 (54%)	0.34
Mother's education			
No formal school or primary (ref)	245	132 (54%)	..
Middle, high school, or university	219	116 (57%)	0.85

Rotavirus IgA seroconversion at ages 14 (a), 18 (b), and 22 (c) weeks served as the dependent variable. Five (1%) of 464 saliva samples were missing for the secretor phenotype variable. This analysis was done among all individuals who were rotavirus IgA seronegative at age 6 weeks (N=464). LAZ= Length for age Z score. WLZ= Weight for length Z score. ref=reference.

(b)

		Rotavirus IgA seroconversion at age 18 weeks	
		n/N (%)	**Univariable p value**
LAZ-score lowest quartile (<-1.5) at the age of blood draw			
No (ref)	341	267 (78%)	..
Yes	123	86 (70%)	0.07
WLZ-score lowest quartile (<-1.0) at the age of blood draw			
No (ref)	328	251 (77%)	..
Yes	136	102 (75%)	0.72
IPV administration group			
(Group D) (ref)	121	87 (72%)	..
(Group C)	114	92 (81%)	0.12
(Groups A+B)	229	174 (76%)	0.40
Mother's education			
No formal school or primary (ref)	245	186 (76%)	..
Middle, high school, or university	219	167 (76%)	1.00

Rotavirus IgA seroconversion at ages 14 (a), 18 (b), and 22 (c) weeks served as the dependent variable.
This analysis was done among all individuals who were rotavirus IgA seronegative at age 6 weeks (N=464).
LAZ= Length for age Z score. WLZ= Weight for length Z score. ref=reference.

(c)

		Rotavirus IgA seroconversion at age 22 weeks	
		n/N (%)	Univariable p value
Sex			
Female (ref)	244	184 (75%)	..
Male	220	168 (76%)	0.83
Secretor phenotype			
Non-secretor (ref)	150	104 (69%)	..
Secretor	309	245 (79%)	0.03
LAZ-score lowest quartile (<-1.5) at the age of blood draw			
No (ref)	342	265 (77%)	..
Yes	122	87 (71%)	0.18
WLZ-score lowest quartile (<-1.0) at the age of blood draw			
No (ref)	334	254 (76%)	..
Yes	130	98 (75%)	0.9
IPV administration group			
(Group D) (ref)	121	89 (74%)	..
(Group C)	114	90 (79%)	0.33
(Groups A+B)	229	173 (76%)	0.68
Mother's education			
No formal school or primary (ref)	245	185 (76%)	..
Secretor phenotype	219	167 (76%)	0.91

Rotavirus IgA seroconversion at ages 14 (a), 18 (b), and 22 (c) weeks served as the dependent variable.
Five (1%) of 464 saliva samples were missing for the secretor phenotype variable.
This analysis was done among all individuals who were rotavirus IgA seronegative at age 6 weeks (N=464).
LAZ= Length for age Z score. WLZ= Weight for length Z score. ref=reference.

Table S10. Univariable regression of variables not retained in multivariable regression for rotavirus IgA titres at ages 14 (a), 18 (b), and 22 (c) weeks in infants who were rotavirus IgA seronegative at age six weeks in RotaTeq arm
(a)

		Rotavirus IgA titres at age 14 weeks	
	N	**GMT (95%CI)**	**Univariable p value**
Sex			
Female (ref)	244	39 (28-55)	..
Male	220	57 (41-80)	0.12
LAZ-score lowest quartile (<-1.5) at the age of blood draw			
No (ref)	332	47 (35-62)	..
Yes	132	47 (31-73)	0.95
WLZ-score lowest quartile (<-1.0) at the age of blood draw			
No (ref)	329	43 (33-57)	..
Yes	135	57 (35-92)	0.31
IPV administration group			
(Group D) (ref)	121	47 (30-73)	..
(Group C)	114	55 (32-94)	..
(Groups A+B)	229	43 (31-61)	0.63
Mother's education			
No formal school or primary (ref)	245	49 (35-70)	..
Middle, high school, or university	219	44 (32-61)	0.65

Log-transformed rotavirus IgA titres measured at ages 14 (a), 18 (b), and 22 (c) weeks served as the dependent variable.
Variables with a p value of ≥ 0.05 during multivariable analyses are shown.
This analysis was done among all individuals who were rotavirus IgA seronegative at age 6 weeks (N=464).
LAZ= Length for age Z score. WLZ= Weight for length Z score. GMT=geometric mean titre. ref=reference.

(b)

	Rotavirus IgA titres at age 18 weeks		
	N	**GMT (95%CI)**	**Univariable p value**
Sex			
Female (ref)	244	169 (121-236)	..
Male	220	186 (140-248)	0.67
LAZ-score lowest quartile (<-1.5) at the age of blood draw			
No (ref)	341	183 (142-237)	..
Yes	123	161 (103-253)	0.62
WLZ-score lowest quartile (<-1.0) at the age of blood draw			
No (ref)	328	170 (132-221)	..
Yes	136	194 (126-299)	0.60
IPV administration group			
(Group D) (ref)	121	154 (98-242)	..
(Group C)	114	212 (137-326)	..
(Groups A+B)	229	174 (127-239)	0.89
Mother's education			
No formal school or primary (ref)	245	185 (136-251)	..
Middle, high school, or university	219	169 (122-234)	0.69

Log-transformed rotavirus IgA titres measured at ages 14 (a), 18 (b), and 22 (c) weeks served as the dependent variable. Variables with a p value of ≥ 0.05 during multivariable analyses are shown.
LAZ= Length for age Z score. WLZ= Weight for length Z score. GMT=geometric mean titre. ref=reference.

(c)

	Rotavirus IgA titres at age 22 weeks		
	N	GMT (95%CI)	Univariable p value
Sex			
Female (ref)	244	191 (139-264)	..
Male	220	184 (132-256)	0.87
LAZ-score Lowest Quartile (<-1.5) at the age of blood draw			
No (ref)	342	196 (151-255)	..
Yes	122	166 (103-269)	0.53
WLZ-score Lowest Quartile (<-1.0) at the age of blood draw			
No (ref)	334	183 (139-239)	..
Yes	130	202 (130-315)	0.69
IPV group			
(Group D) (ref)	121	205 (131-322)	..
(Group C)	114	185 (117-291)	..
(Groups A+B)	229	181 (19-253)	0.69
Mother's education			
No formal school or primary (ref)	245	178 (130-245)	..
Middle, high school, or university	219	199 (142-278)	0.64

Log-transformed rotavirus IgA titres measured at ages 14 (a), 18 (b), and 22 (c) weeks served as the dependent variable. Variables with a p value of ≥ 0.05 during multivariable analyses are shown.
LAZ: Length for age Z score. WLZ: Weight for length Z score. GMT=geometric mean titre. ref=reference.

Table S11. Multivariable regression for rotavirus IgA titres at ages 14 (a) and 22 (b) weeks in infants who were rotavirus IgA seronegative at age six weeks in Rotarix arm

(a)

		Rotavirus IgA titres at age 14 weeks			
	N	**GMT (95%CI)**	**Univariable p value**	**Multivariable beta (95%CI)**	**Multivariable p value**
Rotavirus IgG titres at age 6 weeks (tertiles)					
80-1280 (ref)	173	103 (72-147)	..	..	..
2560	132	86 (53-140)	..	..	..
5120-10 240	169	44 (29-66)	0.02	0.7 (0.5-0.8)	0.01
Secretor phenotype					
Non-secretor (ref)	135	46 (29-71)	..	..	..
Secretor	329	88 (66-116)	0.01	1.4 (1.3-3.5)	0.01
Rotavirus season exposure between ages 6 and 14 weeks					
Low (October to November 2016; ref)	133	41 (25-66)	..	..	..
Moderate (December 2016)	182	73 (51-105)	..	..	..
High (January to February 2017)	159	116 (77-175)	0.01	1.5 (1.3-2.4)	0.01

Log-transformed Rotavirus IgA titres measured at ages 14 (a) and 22 (b) weeks served as the dependent variable. Variables with a p value of <0.05 during multivariable analyses are shown. Univariable regression results of not retained variables are provided in table S12. Ten (2%) of 474 saliva samples were missing for the secretor phenotype variable. This analysis was done among all individuals who were rotavirus IgA seronegative at age 6 weeks (N=474). GMT=geometric mean titre. ref=reference.

(b)

		Rotavirus IgA titres at age 22 weeks			
	N	**GMT (95%CI)**	**Univariable p value**	**Multivariable beta (95%CI)**	**Multivariable p value**
Rotavirus IgG titres at age 6 weeks (tertiles)					
80-1280 (ref)	173	39 (26-60)	..	..	..
2560	132	43 (25-72)	..	..	..
5120-10 240	169	16 (10-25)	0.01	0.7 (0.5-0.8)	0.01
Secretor phenotype					
Non-secretor (ref)	135	14 (9-23)	..	..	..
Secretor	329	39 (28-54)	0.01	1.5 (1.7-5.4)	0.01

Log-transformed Rotavirus IgA titres measured at ages 14 (a) and 22 (b) weeks served as the dependent variable. Variables with a p value of <0.05 during multivariable analyses are shown. Univariable regression results of not retained variables are provided in table S12. Ten (2%) of 474 saliva samples were missing for the secretor phenotype variable. This analysis was done among all individuals who were rotavirus IgA seronegative at age 6 weeks (N=474). GMT=geometric mean titre. ref=reference.

Table S12: Univariable regression of variables not retained in multivariable regression for rotavirus IgA seroconversion at 14 (a), and 22 (b) age weeks in infants who were rotavirus IgA seronegative at age six weeks in Rotarix arm (a)

	Rotavirus IgA seroconversion at age 14 weeks		
	N	**n (%)**	**Univariable p value**
Sex			
Female (ref)	231	149 (65%)	..
Male	243	168 (69%)	0.33
Feeding practices			
Partial breastfeeding at time of ≥1 dose (ref)	346	234 (68%)	..
Exclusive breastfeeding at time of each dose	128	83 (65%)	0.58
LAZ-score lowest quartile (<-1.5) at the age of blood draw			
No (ref)	348	235 (68%)	..
Yes	126	82 (65%)	0.66
WLZ-score lowest quartile (<-1.0) at the age of blood draw			
No (ref)	354	239 (68%)	..
Yes	120	78 (65%)	0.65
IPV group			
(Group D) (ref)	123	78 (63%)	..
(Group C)	118	78 (66%)	0.66
(Groups A+B)	233	161 (69%)	0.28
Mother's education			
No formal school or primary (ref)	266	177 (67%)	..
Middle, high school, or university	208	140 (67%)	0.92

Rotavirus IgA seroconversion at ages 14 (a) and 22 (b) weeks served as the dependent variable.
Variables with a p value of ≥ 0.05 during multivariable analyses are shown. This analysis was done among all individuals who were rotavirus IgA seronegative at age 6 weeks (N=474). LAZ: Length for age Z score.
WLZ: Weight for length Z score. GMT=geometric mean titre. ref=reference.

(b)

	Rotavirus IgA seroconversion at age 22 weeks		
	N	**n (%)**	**Univariable p value**
Sex			
Female (ref)	231	124 (54%)	..
Male	243	125 (51%)	0.65
Feeding practices			
Partial breastfeeding at time of ≥1 dose (ref)	346	191 (55%)	..
Exclusive breastfeeding at time of each dose	128	58 (45%)	0.06
LAZ-score lowest quartile (<-1.5) at the age of blood draw			
No (ref)	359	188 (52%)	..
Yes	115	61 (53%)	0.91
WLZ-score lowest quartile (<-1.0) at the age of blood draw			
No (ref)	349	187 (54%)	..
Yes	125	62 (50%)	0.47
IPV group			
(Group D) (ref)	123	61 (50%)	..
(Group C)	118	55 (47%)	0.64
(Groups A+B)	233	133 (57%)	0.18
Mother's education			
No formal school or primary (ref)	266	143 (54%)	..
Middle, high school, or university	208	106 (51%)	0.58

Rotavirus IgA seroconversion measured at ages 14 (a) and 22 (b) weeks served as the dependent variable. Variables with a p value of ≥ 0.05 during multivariable analyses are shown. This analysis was done among all individuals who were rotavirus IgA seronegative at age 6 weeks (N=474). LAZ: Length for age Z score. WLZ: Weight for length Z score. GMT=geometric mean titre. ref=reference.

Table S13. Univariable regression of variables not retained in multivariable regression for rotavirus IgA titres at ages 14 (a) and 22 (b) weeks in infants who were rotavirus IgA seronegative at age six weeks in Rotarix arm
(a)

	Rotavirus IgA titres at age 14 weeks		
	N	**GMT (95%CI)**	**Univariable p value**
Sex			
Female (ref)	231	60 (43-85)	··
Male	243	87 (63-121)	0.13
Feeding practices			
Partial breastfeeding at time of ≥1 dose (ref)	346	77 (58-102)	··
Exclusive breastfeeding at time of each dose	128	63 (42-97)	0.47
LAZ-score lowest quartile (<-1.5) at the age of blood draw			
No (ref)	348	82 (62-107)	··
Yes	126	54 (34-84)	0.12
WLZ-score lowest quartile (<-1.0) at the age of blood draw			
No (ref)	354	76 (58-100)	
Yes	120	64 (40-102)	0.52
IPV group			
(Group D) (ref)	123	65 (41-102)	··
(Group C)	118	63 (40-100)	··
(Groups A+B)	233	84 (59-118)	0.29
Mother's education			
No formal school or primary (ref)	266	72 (53-98)	··
Middle, high school, or university	208	75 (52-107)	0.86

Log-transformed rotavirus IgA titres at ages 14 (a) and 22 (b) weeks served as the dependent variable. Variables with a p value of ≥ 0.05 during multivariable analyses are shown. This analysis was done among all individuals who were rotavirus IgA seronegative at age 6 weeks (N=474). LAZ: Length for age Z score. WLZ: Weight for length Z score. GMT=geometric mean titre. ref=reference.

(b)

	Rotavirus IgA titres at age 22 weeks		
	N	**GMT (95%CI)**	**Univariable p value**
Sex			
Female (ref)	231	27 (19-39)	..
Male	243	31 (21-45)	0.62
Feeding practices			
Partial breastfeeding at time of ≥1 dose (ref)	346	33 (25-45)	..
Exclusive breastfeeding at time of each dose	128	19 (12-32)	0.07
LAZ-score lowest quartile (<-1.5) at the age of blood draw			
No (ref)	359	31 (23-42)	..
Yes	115	23 (14-38)	0.33
WLZ-score lowest quartile (<-1.0) at the age of blood draw			
No (ref)	349	31 (23-42)	..
Yes	125	24 (15-40)	0.44
IPV group			
(Group D) (ref)	123	23 (14-39)	..
(Group C)	118	20 (12-34)	..
(Groups A+B)	233	39 (27-56)	0.04
Mother's education			
No formal school or primary (ref)	266	30 (21-42)	..
Middle, high school, or university	208	28 (19-41)	0.84

Log-transformed rotavirus IgA titres at ages 14 (a) and 22 (b) weeks served as the dependent variable. Variables with a p value of ≥ 0.05 during multivariable analyses are shown. This analysis was done among all individuals who were rotavirus IgA seronegative at age 6 weeks (N=474). LAZ: Length for age Z score. WLZ: Weight for length Z score. GMT=geometric mean titre. ref=reference.

Supplementary References

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