SUPPLEMENTARY MATERIALS

MATERIALS AND METHODS

Participants

A two-step process was established to identify and select eligible study subjects in Moradabad District, Uttar Pradesh State, India. First a census was conducted in the study areas to identify families with infants and children up to 15 years of age. Secondly, Surveillance Medical Officers (SMOs) of WHO India-National Polio Surveillance Project (NPSP) visited households of eligible children to identify potential study subjects. As stool needed to be collected a day prior to the subject's visit to the health facility, the SMOs initiated home visits a day before (day minus 1) the actual visit to the health facility. The day after (day 0), the SMO re-visited the houses and facilitated transportation of the subject to the assigned study site.

Procedures

At the study site, a physician conducted a short interview and a physical examination. After obtaining informed consent from the parents (and assent from children aged 10 years), the study participant was assigned a subject identification number. Then a baseline blood sample was collected by venepuncture. Thereafter, the child was randomly allocated to a study group. For this, the parent or child selected a sealed envelope that included the study group allocation from a basket of envelopes. The child was administered either IPV, bOPV, or no vaccine as per the randomization. Adverse events were monitored at the study site for 30 minutes after vaccine administration.

The SMO made reminder household visits to the participating families, examined the subjects and provided stool kits a day before day 28. On day 28, SMOs re-visited the home, and facilitated transportation to the study site for blood collection and administration of bOPV challenge dose.

After day 28, serial stool samples were collected at households under the direct supervision of SMOs at 3 (day 31), 7 (day 35), and 14 (day 42) days after bOPV challenge.

The inclusion criteria were: Healthy children aged 6-11 months, 5 years or 10 years, who resided within a relatively short and easily accessible distance (<30 km) to the study sites, and did not plan to travel during the study period and consented to participate in the study. The exclusion criteria were: Children with known thrombocytopenia or bleeding disorders, children acutely ill or with signs of acute infection (e.g. fever ≥ 101^{o}F) at the time of enrolment, residence >30 km from study site, families expecting to be absent during the study period, a diagnosis or suspicion of immunodeficiency disorder (either in the participant or a family member) and families refusing participation.

Subjects could withdraw at any point. Any subject who did not turn up for the day 28 follow up and challenge dose was also withdrawn from the study. Apart from collecting the reasons for withdrawal, no additional follow-up was conducted. If children missed a scheduled blood or stool sample collection, they were excluded from final analysis.

Panacea Biotec Ltd (New Delhi, India) provided single-dose vials with IPV containing 40, 8, 32 D-antigen units of poliovirus types 1, 2, and 3, respectively, per 0.5 mL dose; and bivalent types 1+3 OPV (bOPV) containing > 10^{6} cell culture infectious dose 50% ($CCID_{50}$) of Sabin-strain poliovirus type 1 and >$10^{5.8}$ $CCID_{50}$ of Sabin-strain poliovirus type 3 per 0.1 mL (two drops). All vaccines were shipped by the manufacturer and stored in appropriate cold-chain conditions. Refrigerator temperature was recorded twice daily and vaccine vial monitors were checked before vaccine was given.

Blood specimens (1 mL) were collected at days 0, 28, and 56 by venepuncture and serum was separated within 3 hours of collection, and stored at 4-8^{o}C until shipment the next day to the Enterovirus Research Center (ERC), Mumbai, India. The sera were then tested for neutralizing antibody against poliovirus types 1, 2, and 3, with a modified micro-neutralization assay [20]. We assigned unobserved titers values of <1:8 (if they were below the starting dilution) or $\geq$1:1448 (if

they were above the final dilution). We defined seroconversion as a change from a negative (<1:8) to a positive (≥1:8) titer after vaccine administration, and boosting as a 4-fold rise in antibody titer for infants and children with a baseline titer of 1:8-1:362.

Stool specimens were shipped the day after collection in gel packs to the ERC, Mumbai, India, where these samples were processed for poliovirus isolation following WHO guidelines [21]. Samples positive for poliovirus were titrated to estimate the amount of virus present (log_{10} median cell culture infectious doses [$CCID_{50}$] per gram).

Statistical analysis

The primary outcome measure of the study was the reduction in the prevalence of poliovirus excretion in any stool sample collected after challenge with bOPV, and the effect of the intervention was expressed as a change in the percentage with poliovirus excretion in stool samples. To detect a 20% absolute reduction in poliovirus excretion with 90% power in the IPV or bOPV vaccinated children compared with the control group we estimated that a sample size of at least 801 children was needed to complete the 2-month study (89 per study group and age group; two-tailed test with 5% significance level). To account for an estimated drop-out of 15-20%, 110 children were enrolled in each group, for a total sample of 990.

Demographic and clinical data were double-entered and merged with laboratory data. We did the statistical analysis with R (R Foundation) and STATA (version 11.2) [22, 23]. Fisher's exact (two-tailed) tests were used to compare the prevalence of excretion and seroprevalence, seroconversion rates, and the proportion of excretion by antibody titer quartile. Wilcoxon rank-sum tests were used to compare excretion titer values. 95% confidence intervals were calculated using the Clopper-Pearson method for binomial proportions. Confidence intervals for median titer of shedding were calculated using bootstrapping with 1000 replications. The median duration of excretion was estimated based on the prevalence of poliovirus detected on days 3, 7, and 14 after challenge by fitting a hidden

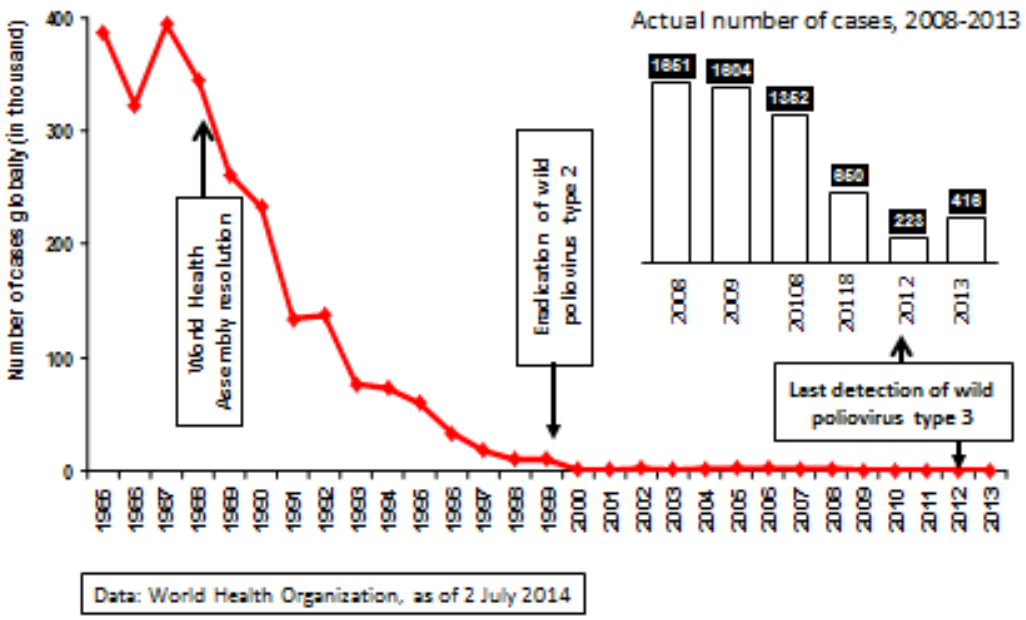
Markov model (HMM). In this model we estimated the initial prevalence of shedding in each study group 3 days after challenge and assumed that children in each group stopped shedding at a constant rate γ_i day^{-1} (where *i* indexes the study group) such that the duration of shedding followed an exponential distribution. Culture of poliovirus was assumed to be 100% specific and we estimated sensitivity during the fit of the HMM (75.4% (69.2-80.7%) for serotype 1 and 76.1% (70.0-81.3%) for serotype 3). We assumed that no children stopped shedding before day 3 after challenge and therefore present the median duration of shedding in study group *i* as $3 + \ln(2)/\gamma_i$ days. Models for each poliovirus serotype were fit to the shedding data using the msm package in the R statistical programming language [22, 24].

Supplemental figure legends:

Figure S1: Poliomyelitis case, worldwide, 1985-2013.

Figure S2: CONSORT flow diagram of study participants.

Figure S1


Number of cases globally (in thousand)
400
300
200
100
0
World Health Assembly resolution
Eradication of wild poliovirus type 2
Actual number of cases, 2008-2013
1651
1604
1352
650
223
416
2008
2009
2010
2011
2012
2013
Last detection of wild poliovirus type 3
1966
1966
1967
1968
1969
1960
1961
1962
1963
1964
1965
1966
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2004
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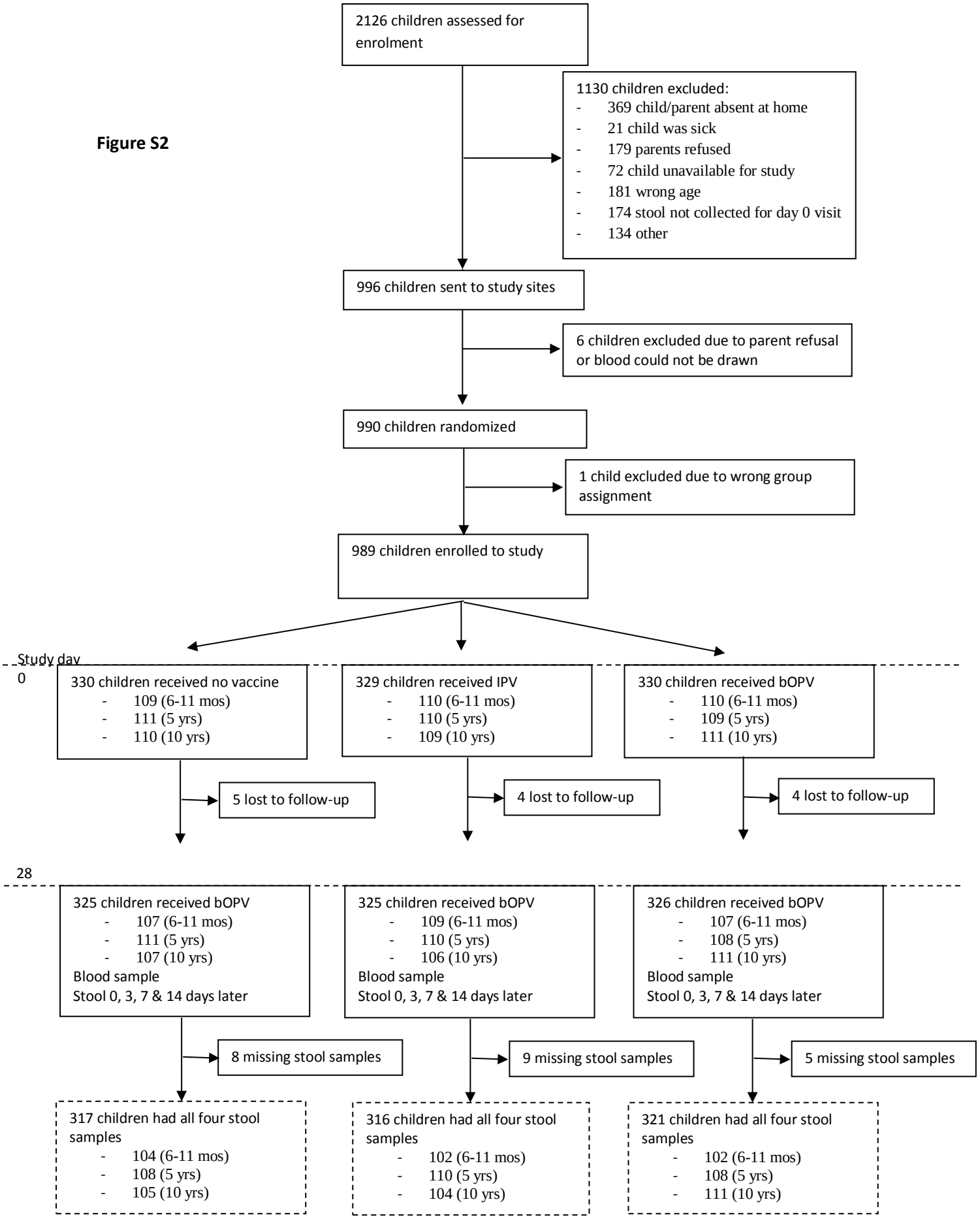
2008
2009
2010
2011
2012
2013
Data: World Health Organization, as of 2 July 2014

Figure S2


2126 children assessed for enrolment
1130 children excluded:
- 369 child/parent absent at home
- 21 child was sick
- 179 parents refused
- 72 child unavailable for study
- 181 wrong age
- 174 stool not collected for day 0 visit
- 134 other
996 children sent to study sites
6 children excluded due to parent refusal or blood could not be drawn
990 children randomized
1 child excluded due to wrong group assignment
989 children enrolled to study
Study day
0
330 children received no vaccine
- 109 (6-11 mos)
- 111 (5 yrs)
- 110 (10 yrs)
329 children received IPV
- 110 (6-11 mos)
- 110 (5 yrs)
- 109 (10 yrs)
330 children received bOPV
- 110 (6-11 mos)
- 109 (5 yrs)
- 111 (10 yrs)
5 lost to follow-up
4 lost to follow-up
4 lost to follow-up
28
325 children received bOPV
- 107 (6-11 mos)
- 111 (5 yrs)
- 107 (10 yrs)
Blood sample
Stool 0, 3, 7 & 14 days later
325 children received bOPV
- 109 (6-11 mos)
- 110 (5 yrs)
- 106 (10 yrs)
Blood sample
Stool 0, 3, 7 & 14 days later
326 children received bOPV
- 107 (6-11 mos)
- 108 (5 yrs)
- 111 (10 yrs)
Blood sample
Stool 0, 3, 7 & 14 days later
8 missing stool samples
9 missing stool samples
5 missing stool samples
317 children had all four stool samples
- 104 (6-11 mos)
- 108 (5 yrs)
- 105 (10 yrs)
316 children had all four stool samples
- 102 (6-11 mos)
- 110 (5 yrs)
- 104 (10 yrs)
321 children had all four stool samples
- 102 (6-11 mos)
- 108 (5 yrs)
- 111 (10 yrs)

Supplementary Table S1: Excretion of poliovirus following challenge with bOPV by visit

			Days post challenge					
			0	3	7	14	Ever excretion[&]	
			n (proportion excreting)	n (proportion excreting)	n (proportion excreting)	n (proportion excreting)	n (proportion excreting)	P-value
Type 1	6-11 mos	Control	0/104 (0.0)	14/104 (13.5)	4/104 (3.8)	0/104 (0.0)	15/104 (14.4)	Ref
		IPV	0/102 (0.0)	6/102 (5.9)	6/102 (5.9)	0/102 (0.0)	9/102 (8.8)	0.278
		bOPV	0/102 (0.0)	19/102 (18.6)	12/102 (11.8)	3/102 (2.9)	25/102 (24.5)	0.079
	5 yrs	Control	0/108 (0.0)	21/108 (19.4)	11/108 (10.2)	7/108 (6.5)	26/108 (24.1)	Ref
		IPV	1/110 (0.9)	8/110 (7.3)	5/110 (4.5)	2/110 (1.8)	10/110 (9.1)	0.003
		bOPV	0/108 (0.0)	19/108 (17.6)	14/108 (13.0)	3/108 (2.8)	23/108 (21.3)	0.745
	10 yrs	Control	0/105 (0.0)	46/105 (43.8)	41/105 (39.0)	12/105 (11.4)	55/105 (52.4)	Ref
		IPV	0/104 (0.0)	13/104 (12.5)	7/104 (6.7)	2/104 (1.9)	14/104 (13.5)	<0.001
		bOPV	2/111 (1.8)	24/111 (21.6)	21/111 (18.9)	9/111 (8.1)	28/111 (25.2)	<0.001
	Overall	Control	0/317 (0.0)	81/317 (25.6)	56/317 (17.7)	19/317 (6.0)	96/317 (30.3)	Ref
		IPV	1/316 (0.3)	27/316 (8.5)	18/316 (5.7)	4/316 (1.3)	33/316 (10.4)	<0.001
		bOPV	2/321 (0.6)	62/321 (19.3)	47/321 (14.6)	15/321 (4.7)	76/321 (23.7)	0.062
Type 3	6-11 mos	Control	0/104 (0.0)	14/104 (13.5)	6/104 (5.8)	3/104 (2.9)	14/104 (13.5)	Ref
		IPV	0/102 (0.0)	4/102 (3.9)	3/102 (2.9)	0/102 (0.0)	4/102 (3.9)	0.024
		bOPV	2/102 (2.0)	13/102 (12.7)	10/102 (9.8)	1/102 (1.0)	16/102 (15.7)	0.696
	5 yrs	Control	0/108 (0.0)	20/108 (18.5)	20/108 (18.5)	12/108 (11.1)	27/108 (25.0)	Ref
		IPV	0/110 (0.0)	10/110 (9.1)	6/110 (5.5)	3/110 (2.7)	13/110 (11.8)	0.014
		bOPV	2/108 (1.9)	17/108 (15.7)	17/108 (15.7)	9/108 (8.3)	25/108 (23.1)	0.874
	10 yrs	Control	0/105 (0.0)	40/105 (38.1)	47/105 (44.8)	17/105 (16.2)	54/105 (51.4)	Ref
		IPV	0/104 (0.0)	10/104 (9.6)	8/104 (7.7)	7/104 (6.7)	13/104 (12.5)	<0.001
		bOPV	11/111 (9.9)	28/111 (25.2)	24/111 (21.6)	14/111 (12.6)	34/111 (30.6)	0.002
	Overall	Control	0/317 (0.0)	74/317 (23.3)	73/317 (23.0)	32/317 (10.1)	95/317 (30.0)	Ref
		IPV	0/316 (0.0)	24/316 (7.6)	17/316 (5.4)	10/316 (3.2)	30/316 (9.5)	<0.001
		bOPV	15/321 (4.7)	58/321 (18.1)	51/321 (15.9)	24/321 (7.5)	75/321 (23.4)	0.061

[&]Excretion in any of the stool samples collected after bOPV challenge. P-value calculated using Fisher's exact (two-tailed) test.

Supplementary Table S2: Length of poliovirus excretion following challenge with bOPV			
			Length of excretion (days) (95% CI)[&]
Type 1	6-11 mos	Control	5.2 (4.2-7.1)
		IPV	6.7 (4.7-10.9)
		bOPV	6.8 (5.4-9.0)
	5 yrs	Control	8.0 (6.0-11.1)
		IPV	6.3 (4.6-9.7)
		bOPV	7.2 (5.6-9.9)
	10 yrs	Control	10.6 (8.0-14.4)
		IPV	6.9 (5.1-10.2)
		bOPV	9.8 (7.0-14.7)
	Overall	Control	8.7 (7.3-10.5)
		IPV	7.6 (6.0-10.1)
		bOPV	9.1 (7.4-11.4)
Type 3	6-11 mos	Control	7.6 (5.3-11.8)
		IPV	6.9 (4.2-15.3)
		bOPV	7.0 (5.2-10.2)
	5 yrs	Control	14.4 (8.9-25.2)
		IPV	7.6 (5.4-11.9)
		bOPV	10.3 (7.2-15.6)
	10 yrs	Control	15.1 (10.1-23.8)
		IPV	25.9 (7.8-112.7)
		bOPV	16.3 (9.7-29.6)
	Overall	Control	13.1 (10.1-17.4)
		IPV	10.4 (7.4-15.4)
		bOPV	11.6 (9.0-15.4)

[&]Confidence intervals for the duration of poliovirus excretion were calculated assuming normality on the log scale with the variance based on the curvature of the likelihood surface at the maximum likelihood estimate (the Hessian matrix).

Supplementary Table S3: Poliovirus titer in specimens collected at days 0 (priori to), and 3, 7, and 14 following challenge with bOPV.

			Days post challenge											
			0			3			7			14		
			n	Median titer (95% CI)	P-value	n	Median titer (95% CI)	P-value	n	Median titer (95% CI)	P-value	n	Median titer (95% CI)	P-value
Type 1	6-11 mos	Control	0	0.0 (NA)	Ref	14	3.8 (2.2-5.4)	Ref	3	5.6 (5.1-6.2)	Ref	0	0.0 (NA)	Ref
		IPV	0	0.0 (NA)	NA	6	5.7 (3.8-7.5)	0.213	5	3.0 (0.7-5.3)	0.177	0	0.0 (NA)	NA
		bOPV	0	0.0 (NA)	NA	18	2.9 (2.3-3.5)	0.360	11	5.6 (4.8-6.3)	0.482	3	4.3 (2.5-6.0)	NA
	5 yrs	Control	0	0.0 (NA)	Ref	17	1.5 (0.3-2.7)	Ref	11	3.2 (2.0-4.4)	Ref	7	4.2 (3.7-4.7)	Ref
		IPV	1	3.6 (NA)	NA	8	2.9 (1.7-4.1)	0.517	5	2.6 (0.9-4.4)	0.650	2	3.6 (3.2-4.1)	0.143
		bOPV	0	0.0 (NA)	NA	18	2.6 (1.8-3.4)	0.876	14	3.5 (2.9-4.1)	0.547	3	3.9 (2.8-4.9)	0.210
	10 yrs	Control	0	0.0 (NA)	Ref	44	3.4 (2.9-4.0)	Ref	40	4.1 (3.4-4.8)	Ref	12	3.2 (2.3-4.0)	Ref
		IPV	0	0.0 (NA)	NA	11	2.6 (1.7-3.5)	0.086	7	5.0 (3.4-6.6)	0.256	2	3.1 (2.7-3.4)	0.854
		bOPV	2	2.6 (1.1-4.1)	NA	22	2.8 (2.1-3.4)	0.458	19	3.6 (3.0-4.1)	0.259	9	3.8 (2.9-4.8)	0.643
	Overall	Control	0	0.0 (NA)	Ref	75	3.3 (2.8-3.8)	Ref	54	4.1 (3.5-4.7)	Ref	19	3.8 (3.0-4.6)	Ref
		IPV	1	3.6 (NA)	NA	25	2.8 (2.0-3.6)	0.800	17	3.8 (2.1-5.4)	0.872	4	3.3 (2.8-3.8)	0.516
		bOPV	2	2.6 (1.1-4.1)	NA	58	2.7 (2.5-2.8)	0.203	44	3.7 (3.2-4.2)	0.875	15	3.9 (3.1-4.7)	0.890
Type 3	6-11 mos	Control	0	0.0 (NA)	Ref	14	2.8 (1.9-3.8)	Ref	6	3.4 (1.4-5.4)	Ref	2	4.3 (3.1-5.5)	Ref
		IPV	0	0.0 (NA)	NA	4	2.2 (0.8-3.7)	0.337	3	4.8 (2.2-7.4)	0.796	0	0.0 (NA)	NA
		bOPV	2	5.8 (5.6-5.9)	NA	12	4.7 (3.9-5.6)	0.064	9	5.4 (4.2-6.7)	0.345	1	5.5 (NA)	0.221
	5 yrs	Control	0	0.0 (NA)	Ref	17	2.9 (1.7-4.0)	Ref	20	3.4 (2.6-4.2)	Ref	12	3.3 (2.5-4.1)	Ref
		IPV	0	0.0 (NA)	NA	10	2.0 (1.0-3.1)	0.202	6	3.3 (2.2-4.4)	0.927	3	4.0 (1.8-6.2)	0.885
		bOPV	2	3.2 (3.1-3.2)	NA	16	2.7 (1.5-3.9)	0.655	17	3.6 (3.0-4.1)	0.532	9	3.7 (2.4-5.0)	0.568
	10 yrs	Control	0	0.0 (NA)	Ref	39	3.6 (3.2-4.0)	Ref	47	4.4 (3.7-5.1)	Ref	16	3.5 (2.9-4.1)	Ref
		IPV	0	0.0 (NA)	NA	8	2.6 (2.1-3.2)	0.007	8	3.2 (2.3-4.2)	0.041	7	3.4 (2.1-4.8)	0.973
		bOPV	11	2.8 (2.4-3.3)	NA	27	2.9 (2.6-3.1)	0.023	23	3.1 (2.7-3.4)	0.001	14	3.2 (2.7-3.7)	0.479
	Overall	Control	0	0.0 (NA)	Ref	70	3.5 (2.9-4.0)	Ref	73	4.1 (3.6-4.5)	Ref	30	3.4 (3.0-3.9)	Ref
		IPV	0	0.0 (NA)	NA	22	2.6 (1.8-3.4)	0.001	17	3.4 (2.6-4.2)	0.143	10	3.6 (2.6, 4.6)	1.000
		bOPV	15	3.1 (2.7-3.6)	NA	55	3.0 (2.7-3.3)	0.397	49	3.5 (3.1-3.9)	0.159	24	3.3 (2.7-3.8)	0.979

Note: only subjects shedding virus were included in this analysis. P-value calculated using the Wilcoxon rank-sum test. Bootstrapping used to calculate 95% confidence intervals.