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Effect of substituting IPV for tOPV on immunity to poliovirus in Bangladeshi infants: An open-label randomized controlled trial

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ABSTRACT

Background: The Polio Endgame strategy includes phased withdrawal of oral poliovirus vaccines (OPV) coordinated with introduction of inactivated poliovirus vaccine (IPV) to ensure population immunity. The impact of IPV introduction into a primary OPV series of immunizations in a developing country is uncertain.

Methods: Between May 2011 and November 2012, we enrolled 700 Bangladeshi infant-mother dyads from Dhaka slums into an open-label randomized controlled trial to test whether substituting an injected IPV dose for the standard Expanded Program on Immunization (EPI) fourth tOPV dose at infant age 39 weeks would reduce fecal shedding and enhance systemic immunity. The primary endpoint was mucosal immunity to poliovirus at age one year, measured by fecal excretion of any Sabin virus at five time points up to 25 days post-52 week tOPV challenge, analyzed by the intention to treat principle.

Findings: We randomized 350 families to the tOPV and IPV vaccination arms. Neither study arm resulted in superior intestinal protection at 52 weeks measured by the prevalence of infants shedding any of three poliovirus serotypes, but the IPV dose induced significantly higher seroprevalence and seroconversion rates. This result was identical for poliovirus detection by cell culture or RT-qPCR. The non-significant estimated culture-based shedding risk difference was −3% favoring IPV, and the two vaccination schedules were inferred to be equivalent within a 95% confidence margin of −10% to +4%. Results for shedding analyses stratified by poliovirus type were similar.

Conclusions: Neither of the vaccination regimens is superior to the other in enhancing intestinal immunity as measured by poliovirus shedding at 52 weeks of age and the IPV regimen provides similar intestinal immunity to the four tOPV series, although the IPV regimen strongly enhances humoral immunity. The IPV-modified regimen may be considered for vaccination programs without loss of intestinal protection.

1. Introduction

Global eradication of virulent poliovirus appears tantalizingly close as measured by the total incidence of polio cases per year, but challenges remain in the pursuit of this public health landmark. Oral poliovirus vaccine (OPV) provides excellent intestinal immunity and is highly effective at reducing viral fecal-oral transmission but contains live attenuated poliovirus, which can result in vaccine-associated paralytic poliomyelitis and reversion to a virulent circulating vaccine-derived strain. To eliminate that risk, a global switch to an inactivated poliovirus vaccine will be necessary. Injected inactivated poliovirus vaccine (IPV) induces excellent

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systemic immunity and does not revert to neurovirulence, but has a complex effect on intestinal immunity. IPV-only regimens are apparently inferior as measured by fecal transmission after challenge, but may depend on whether IPV is administered subsequent to doses of OPV [1–5]. Since a single coordinated global transition to solely administering inactivated vaccine is infeasible, and mass campaigns need repetition, primary vaccination schedules that blend doses of OPV with IPV will be necessary during the transition period. The optimal schedule to maintain individual immunity while minimizing transmission of active poliovirus is unknown and is likely to vary by region. Very recently in 2015, the Bangladesh government announced that it would incorporate an IPV dose into the existing four dose Expanded Program on Immunization (EPI) schedule and hence data on the effects of these substitutions will be critical for public health decision-makers.

We designed a randomized clinical trial to test the intestinal immune response and immunogenicity of a modified Bangladesh Expanded Program on Immunization (EPI) vaccination regimen with injected IPV substituted for the fourth trivalent OPV (tOPV) dose, administered at 39 weeks of age in this trial. Based on previous studies that suggested IPV can enhance intestinal immunity and reduce poliovirus transmission by fecal shedding, we hypothesized that infants receiving the IPV dose after OPV intestinal priming would enjoy enhanced intestinal and humoral immunity with lowered susceptibility to viral shedding.

2. Materials and methods

2.1. Study population and design

Seven hundred (700) eligible neonatal infant–mother dyads were enrolled into the Performance of Rotavirus and Oral Poliovirus Vaccines in Developing Countries (PROVIDE) study in Dhaka, Bangladesh, as participants in two separate concomitant randomized open-label clinical trials to study infant poliovirus and rotavirus vaccine interventions, structured in a 2×2 factorial design. The four study groups were: (1) dose 4 IPV + No Rotavirus; (2) dose 4 IPV + Rotavirus; (3) dose 4 tOPV + No Rotavirus; (4) dose 4 tOPV + Rotavirus. The study was funded by the Bill and Melinda Gates Foundation. The study design and baseline clinical characteristics of the PROVIDE study cohort have been reported [6]. The poliovirus outcomes were assumed to be independent of co-administered rotavirus vaccine in the second concurrent trial [7]. Pregnant mothers from the Mirpur slum area of Dhaka were screened for eligibility and upon live birth, enrolled by field research assistants into the cohort. Eligibility for enrollment required delivery of a live infant of maximum age 7 days, absent frank congenital abnormalities, birth defects, or irregular stool frequency or consistency. Oral tOPV (GlaxoSmithKline) doses were given at infant age 6, 10, and 14 weeks, and either oral tOPV or injected IPV (IMOVAX®, Sanofi Pasteur) at 39 weeks. The government EPI schedule for poliovirus vaccination at the time of the trial design in 2010 was 6, 10, 14, and 38 weeks. The fourth tOPV dose was administered at 39 weeks in this trial but was within the preferred vaccination window and was chosen to balance participant and clinic burden, safety, and science in the context of a larger program to study the impact of environmental enteropathy on vaccine under-performance. The rationale for this choice is described in more detail in the Supplementary Appendix. Participants received free primary care during the study.

The study protocol was reviewed and approved by the icddr,b Research Review and Ethical Review Committees, and by Institutional Review Boards at the Universities of Virginia and Vermont. All mothers signed an informed consent on behalf of their infant child before enrollment into the study and were free to withdraw at will. The study was conducted in compliance with the Declaration of Helsinki and the Belmont Report [8]. Good Clinical Practice standards were applied throughout with monitoring of study progress and adverse events by an independent medical monitor. Severe adverse events were recorded for the full protocol period until the final day 25 fecal excretion sample at 52 weeks of age; adverse events linked to vaccine administration were recorded for 48 h post-vaccination. Adverse events were reported to ethics boards or committees per local requirements.

2.2. Randomization and masking

Before enrollment, sequential study subject identification numbers (SIDs) were randomized to one of four 2×2 treatment groups using permuted block randomization with random block size (4 or 8). Sealed envelopes with treatment group assignment for each SID were produced by the Data Coordinating Center and sent to the Dhaka clinic. SIDs were assigned sequentially to each infant/mother pair at the enrollment visit. The envelope was opened at the infant's week six visit. Neither mothers nor clinic staff were masked. The laboratories that performed the outcomes assays to detect poliovirus in stool by cell culture (National Polio Laboratory) and serum neutralizing antibody (Centers for Disease Control and Prevention) were masked to specimen trial arm assignment.

2.3. Procedures

The poliovirus vaccine cell culture-based fecal excretion assays were performed at the National Polio Reference Laboratory, Institute of Public Health, Government of Bangladesh as per the WHO Polio Laboratory Manual [9,10]. Extraction of viral RNA from stool and fecal multiplex RT-qPCR detection has been previously described for the PROVIDE study [11,12]. Samples were tested for serum neutralizing antibodies (SNAb) at the Centers for Disease Control and Protection (Atlanta, Georgia, USA) using a standard microneutralization assay for antibodies to poliovirus types 1, 2, and 3 according to established protocols [13,14]. Each specimen was run in triplicate in the same assay run; neutralization titers were estimated by the Spearman-Kärber method[15] and reported as the reciprocal of the calculated 50% endpoint. A serum sample was considered seropositive if antibodies were present at $\geq$1:8 dilution, antibody titers <1:8 were seronegative.

2.4. Outcomes

The primary outcome was the presence of any of three Sabin poliovirus vaccine types determined by cell culture, in any of five fecal samples collected after tOPV challenge dose at week 52, sampled at day 0 (immediately pre-vaccination), and days 4, 11, 18, and 25 days post-vaccination. Secondary poliovirus shedding outcomes were similar but stratified to each Sabin poliovirus type. These outcomes were also assayed by direct fecal RT-qPCR detection as confirmation. A secondary RT-qPCR-based quantitative index of total viral shedding was tested by poliovirus type. Secondary measures of humoral immunity were seropositivity for neutralizing antibody by serotype one week post-intervention dose at 39 weeks, and seroconversion from 18 to 40 weeks. Week 18 SNAb titer was adjusted for residual maternal antibody, assuming 28 days half-life for maternally transmitted antibody measured at 6 weeks. Failure to seroconvert was defined as less than +2 change in $\log_2$ titer at week 40 if adjusted SNAb (week 18) $\leq$8.5 and >2.83; SNAb (week 40) <10.5 if adjusted SNAb (week 18) >8.5 and <10.5; and SNAb (week 40) $\leq$2.83 if adjusted SNAb (week 18) $\leq$2.83 (seronegative) [16]. Infants with $\log_2$ titer 10.5 at week 18 were excluded from seroconversion analyses.

2.5. Statistical analysis

The trial was designed to test superiority of the substituted IPV dose in reducing the prevalence of any poliovirus shedding in feces at week 52 compared to the standard fourth EPI tOPV dose, under a 2-sided test of proportions at alpha = 0.05. Absent existing region- and vaccination schedule-specific poliovirus excretion rates during study planning, the power was estimated from published studies [5,17–20]. Substitution of the 4th EPI tOPV dose for IPV was assumed to reduce any Sabin type shedding from 10 to 20% to 5%. Assuming 20% participant loss to follow-up and intention-to-treat analysis, 700 recruited infants gave >80% power to detect superiority at shedding rates 11–20% in the first arm versus 5% in the second at alpha = 0.05. Analyses of the primary outcome were performed according to the intention-to-treat (ITT) principle; occurrences of outcomes were compared with the use of tests of proportion and 95% confidence intervals by the Wilson method [21] for one group proportions or the Newcombe hybrid score for differences in proportions [22]. A per protocol (PP) sub-group analysis was pre-specified for the primary and secondary outcomes to assess possible retention bias. This group received the correct number doses of vaccine at the correct ages as described in the protocol. Post-hoc, after review of missing data patterns, a second analysis sub-group was defined, Received Protocol Dose (RPD). These infants received correct doses but one or more outside the preferred EPI schedule. Further details are given in Supplementary Appendix. During the post-week 52 tOPV challenge, some infants missed one or more fecal sampling visits, or did not produce a stool specimen. All primary analyses of fecal data assumed missing samples would have tested negative, resulting in an under-estimated prevalence of poliovirus shedding and a lower bound for true shedding proportions. Sensitivity of the trial analysis results to these assumptions was tested using an imputation model to predict missing shedding data points (Zhang D. et al., manuscript in preparation, details in Supplementary Appendix). The ITT, PP, and RPD results were compared with the corresponding complete data sets generated by imputation. A quantitative shedding index was calculated for each infant for each poliovirus type as described previously [12]. The difference in geometric means of the shedding index between the two arms was assessed by t-test. The incidence of adverse events was compared with the use of Fisher's exact test. All statistical analyses were conducted at the Data Coordinating Center using R version 3.1 [23]. This trial is registered with ClinicalTrials.gov, number NCT01289782.

3. Results

We screened 1048 mother-infant dyads for eligibility resulting in a target enrollment of 700 families, enrolled from May 22, 2011 to November 6, 2012, with 350 randomized to each trial arm. At enrollment the infants were on average 5 days old (range 0–7 days), predominantly born outside the home (75%), into a single-room dwelling family (73%), with only 50% of families having a toilet or septic tank, and attendant economic insecurity, Table 1. The infants were moderately-to-severely undernourished (mean WHO LAZ = −0.9; 10% stunted with LAZ <−2). Of these, 606 infants (86.6%) received the week 52 tOPV challenge dose (307 tOPV, 299 IPV arm) but a further two withdrew during the series (N = 604, CONSORT Diagram Fig. 1). Missed visits and specimens resulted in 493/606 (81.4%) infants with complete data for 5 fecal specimens. Some 481/700 (68.7%) of infants met the stricter PP group criteria of perfect vaccination protocol adherence while 598/700 (85.4%) received the correct doses in the RPD group (Supplementary Appendix). There was no evidence of differential missing data by trial arm measured by number of infants with complete week 52 shedding data (N = 244 vs 249, p = 0.6) or infants missing all five time points (N = 51 vs 43, p = 0.3).

There were 89 severe adverse events (SAEs) in the trial up to the last day 25 fecal sampling visit after week 52, five of which were deaths (mortality rate 5/700 = 0.7%), Supplementary Appendix Table S3. Most of the remaining SAEs were for diarrheal illness. Only a single SAE of hospitalization for diarrheal illness was coded as possibly attributable to vaccination, but this occurred at 24 weeks of age. The observed rates for mortality and morbidity were expected in this population. There were no differences in the SAE counts between arms (death: 2/350 vs 3/350, Fisher Exact p = 1.0; total SAEs: 46 vs 43, exact p = 0.8), or in the vaccination-related adverse event (AE) counts (total AEs: 9 vs 15, exact p = 0.3).

Substitution of the fourth EPI tOPV dose for IPV, administered at 39 weeks of age, did not result in superior intestinal protection from

Table 1
Clinical characteristics of the PROVIDE Poliovirus Trial infant participants at study enrollment*.

	IPV arm (N = 350)	tOPV arm (N = 350)	P-value
Age (days)	4.9 ± 1.7	5.0 ± 1.7	0.49
Female gender (%)	176 (50.3)	156 (44.6)	0.15
Weight (kg)	2.8 ± 0.4	2.8 ± 0.4	0.60
Length (cm)	48.7 ± 1.7	48.7 ± 1.8	0.80
Infant weight for age Z score (WAZ)	−1.3 ± 0.8	−1.3 ± 0.9	0.38
Infant length for age Z score (LAZ)	−0.9 ± 0.9	−0.9 ± 0.9	0.84
Infant stunted at enrollment (%)	29 (8.2)	38 (10.9)	0.30
Infant gestational age ≤36 weeks (%)†	66 (34.7)	57 (29.8)	0.36
Breastfed at birth (%)	330 (94.3)	332 (94.9)	0.86
Home birth (%)	89 (25.4)	92 (26.3)	0.86
Infant BCG administered at birth (%)	8 (2.3)	8 (2.3)	1.0
Maternal age at delivery (years)	24.7 ± 4.8	24.6 ± 4.5	0.62
Mother illiterate (%)	102 (29.1)	100 (28.6)	0.93
Mother homemaker (%)	304 (86.9)	297 (84.9)	0.51
Total household members	5.2 ± 2.2	5.2 ± 2.3	0.83
Number of children under 5 years of age in household	0.3 ± 0.5	0.3 ± 0.5	1.0
Total monthly income (Taka in thousands)§	12.6 ± 8.7	13.0 ± 10.0	0.56
Piped municipal water source (%)	344 (98.3)	334 (95.4)	0.05
Toilet, septic tank (%)	180 (51.4)	187 (53.4)	0.65
Dwelling size equals 1 room (%)	254 (72.6)	253 (72.3)	>0.99

* Continuous variables are summarized as mean ± SD, categorical variables as number (%). Infants in the IPV arm received an injected dose of IPV at 39 weeks of age while those in tOPV arm received the EPI standard oral trivalent vaccine dose.

† Infant gestational age was measured on a subset of 190 infants in the IPV arm and 191 in the tOPV arm to distinguish fetal growth restriction from prematurity using the Dubowitz–Ballard assessment scale (Ballard JL, Novak KK, Driver M. A simplified score for assessment of fetal maturation of newly born infants. J Pediatr 1979;95:769–74.).

§ 1 USD approximately equaled 80 Bangladesh Taka during the study period.

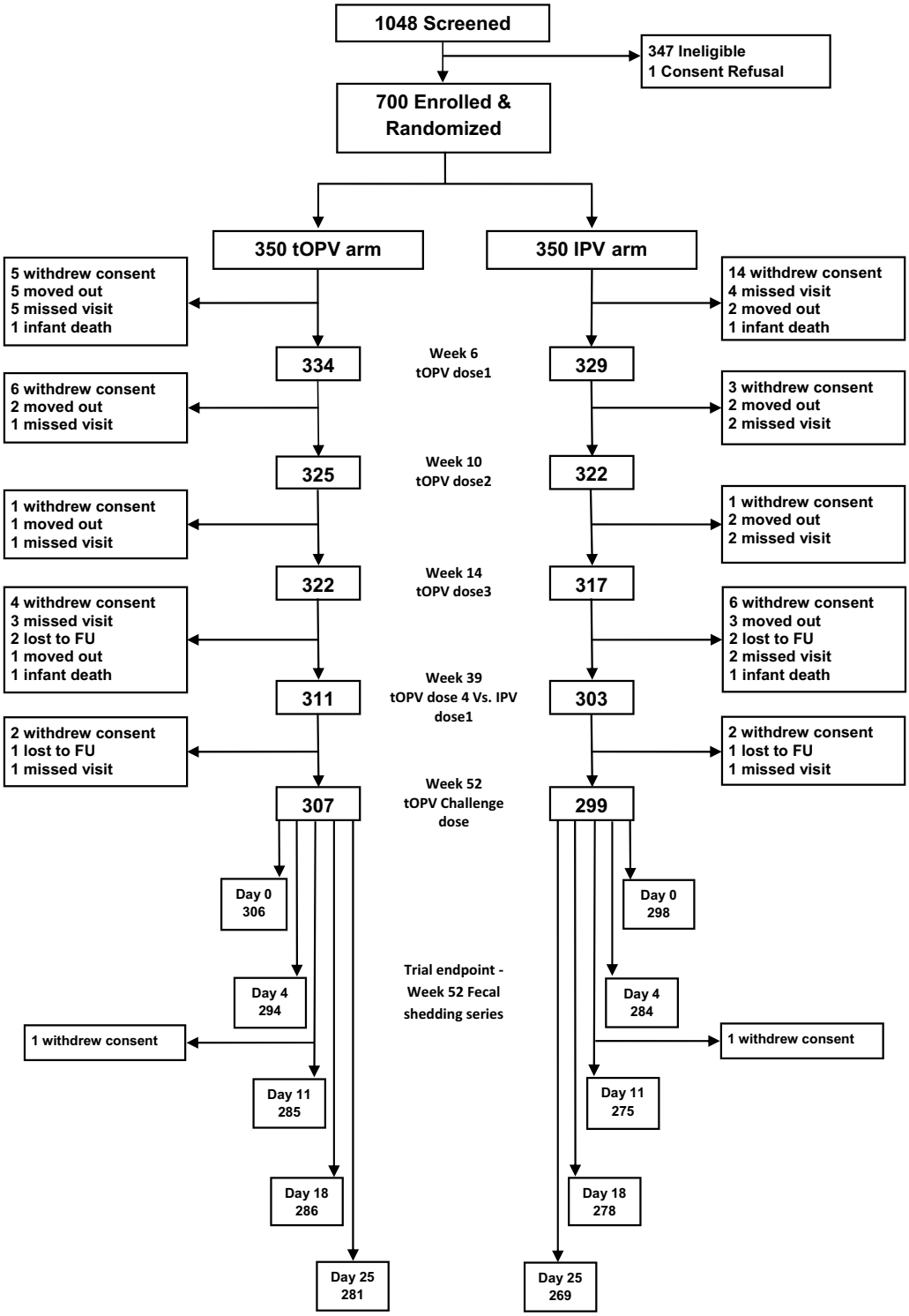

Fig. 1. The PROVIDE poliovirus trial CONSORT diagram.

shedding at 52 weeks of age for either vaccine regimen, measured by the prevalence of infants shedding any of 3 poliovirus serotypes at any of 5 time points (0, 4, 11, 18, or 25 days) after 52 week tOPV challenge, ITT analysis, Table 2 (2-sided test). The non-significant risk difference was −3% (favors IPV) with an inferred equivalence margin of −10%, +4%(95% CI) for cell culture assay and an all type total fecal shedding rate of 29.7%.

Secondary analyses of individual serotype fecal shedding similarly showed no significant differences for IPV compared to tOPV, Fig. 2. The PP and RPD sub-group analyses were also consistent with the ITT analysis for the primary and secondary shedding endpoints by serotype, hence the trial result and estimated effect sizes are insensitive to missing data or biases resulting from ITT analysis strategy. These results were not biased by missing data since inferences were the same for sub-group analyses using only infants with complete five time point data, or using imputation-augmented data, Table 2. Both complete shedding and imputation results were consistent in estimating total shedding prevalence at 35% with a

Table 2
Poliovirus trial primary endpoint results by trial arm.

	IPV N	tOPV N	IPV Prev (%)	tOPV Prev (%)	Risk Difference (95% CI)	Relative Risk (95% CI)	*P* value
Cell culture:							
Intention to treat	99/350	109/350	28.3	31.1	−2.9% (−9.6, 3.9)	0.91 (0.72, 1.14)	0.4
Received protocol dose	98/296	108/302	33.1	35.8	−2.7% (−10.2, 4.9)	0.93 (0.74, 1.15)	0.5
Per protocol	75/236	87/245	31.8	35.5	−3.7% (−12.1, 4.7)	0.89 (0.69, 1.15)	0.4
Cell culture: infants with complete shedding data[*]							
Intention to treat	86/244	89/249	35.2	35.7	−0.5% (−8.9, 7.9)	0.99 (0.78, 1.25)	0.9
Received protocol dose	85/242	88/245	35.1	35.9	−0.8% (−9.2, 7.7)	0.98 (0.77, 1.24)	0.6
Per protocol	66/198	72/201	33.3	35.8	−2.5% (−11.7, 6.8)	0.93 (0.71, 1.22)	0.9
Cell culture: imputed data[§]							
Intention to treat	N/A	N/A	34.8	36.7	−1.9% (−9.6, 5.8)	0.95 (0.84, 1.06)	0.6
Received protocol dose	N/A	N/A	34.5	36.9	−2.4% (−10.2, 5.4)	0.94 (0.85, 1.02)	0.6
Per protocol	N/A	N/A	33.3	36.4	−3.1% (−11.8, 5.6)	0.91 (0.82, 1.01)	0.5
Fecal RT-PCR:							
Intention to treat	145/350	141/350	41.4	40.3	1.1% (−6.1, 8.4)	1.03 (0.86,1.23)	0.8
Received protocol dose	144/296	140/302	48.6	46.4	2.3% (−5.7, 10.1)	1.05 (0.89, 1.24)	0.6
Per protocol	112/236	114/245	47.5	46.5	0.9% (−7.9, 9.8)	1.02 (0.84, 1.23)	0.8

The endpoint was the presence of any poliovirus Sabin type in any of the 5 fecal samples taken at 52 weeks of age, measured as the prevalence among infants in each arm (Prev%). Results are shown for the presence of poliovirus detected by cell line culture and direct RT-PCR assays.

[*] These results are for analysis of the subset of each study group who had complete five time point shedding data and therefore are less likely to be biased by assumptions about missing data.

[§] These results are for analysis of each study group with missing fecal data imputed. The prevalences and risk statistics are computed as means of 1000 imputed data sets, therefore discrete counts by arm are not applicable.

non-significant risk difference in the range −0.5% to −3% and a 95% CI in the approximate range of −10% to +8%. Repeating the same analyses using fecal RT-PCR-based detection resulted in identical conclusions, albeit at higher shedding rates, approx. 40% (ITT) or 47% (PP or RPD), Table 2. We tested whether the total amount of virus shed over the 25 day period differed between trial arms as measured by our shedding index. The mean log(shedding index) did not differ between the arms for infants who shed poliovirus

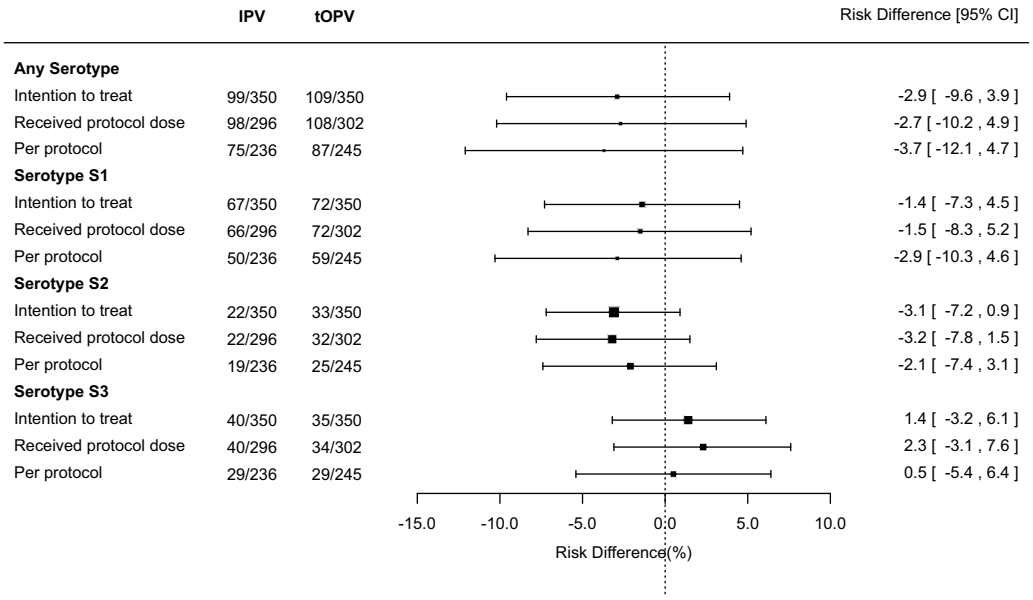

Fig. 2. Poliovirus fecal excretion results at 52 weeks of age. Numerator counts are the number of infants excreting the poliovirus serotype at any of the 5 time points post-tOPV challenge over the denominator total infants included in the analysis. For each serotype results are shown for intention-to-treat primary analysis, per protocol, and received protocol dose sub-group analyses. Any serotype refers to shedding of any of the 3 serotypes. Points designating the location of the estimated risk difference are sized proportional to the precision of the estimates. Two-sided bars delineate the 95% CI. Risk differences are shown as fractions. Negative (−) values imply less shedding in the IPV arm versus the tOPV arm.

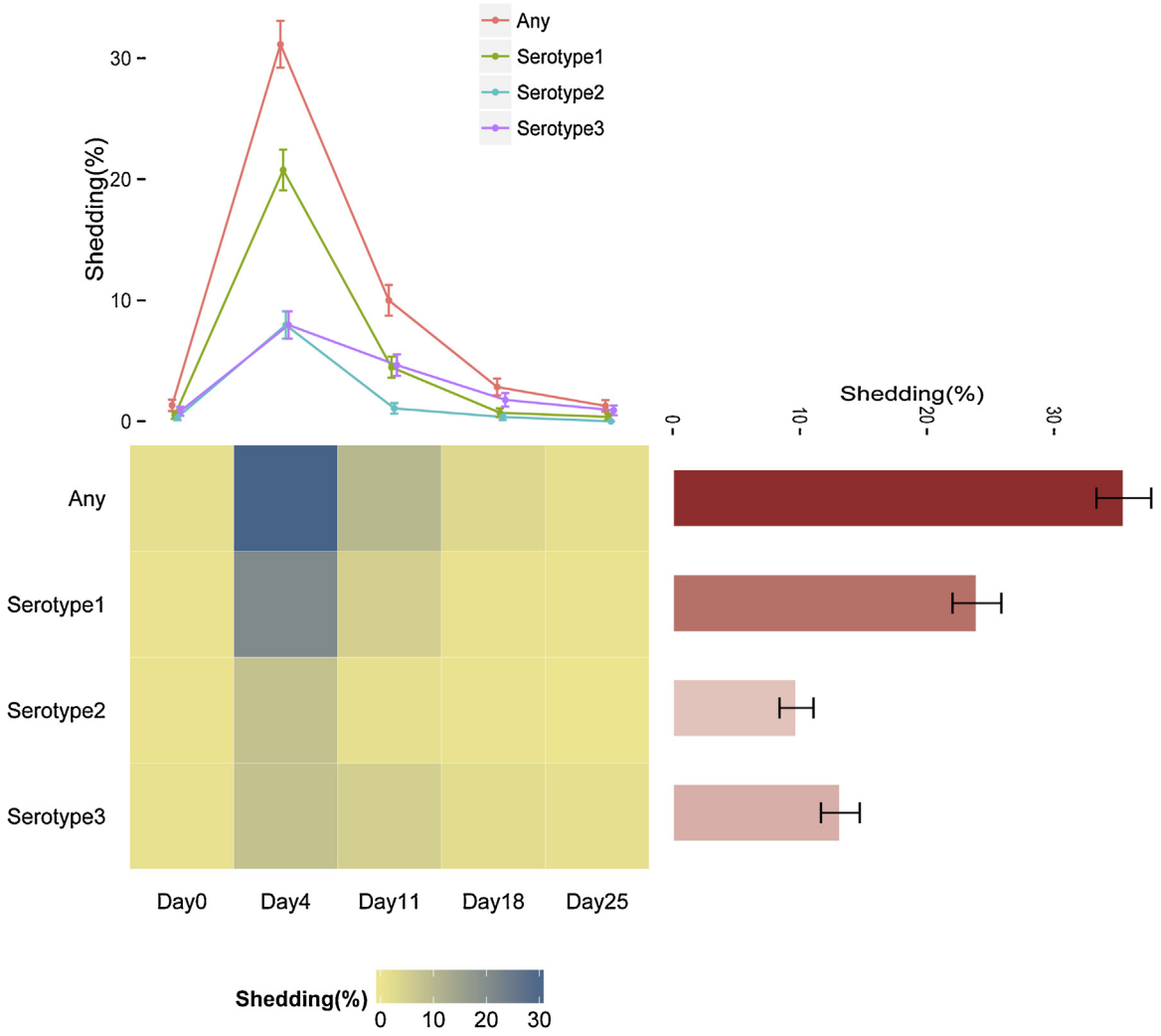

Fig. 3. Prevalence of poliovirus faecal excretion by time point and serotype. Results are shown for the combined data for all infants in both trial arms. The prevalence of shedding at each time point was estimated using all available non-missing data points. The total prevalence of shedding by serotype was estimated using only infants with complete five time data.

type 1 ($p = 0.5$), 2 ($p = 0.2$), or 3 ($p = 0.1$). Shedding infants predominantly shed on day 4 and 11, Fig. 3. The peak shedding rate was day 4(any: 180/578, 31%), then day 11(any: 56/560, 10%) for all three poliovirus types. Omission of the day 0, 18, and 25 assays would have missed only 9/208 (4.3%) infants shedding any poliovirus. Only eight infants shed on day 0 prior to tOPV challenge (8/208, 3.8%).

The IPV vaccination resulted in lower seronegativity rates for all three types compared to tOPV, Fig. 4. Seronegativity in the tOPV arm was 5.3% vs 0.3% in the IPV arm for type 1 (ITT, $p = 0.002$, Fisher exact) and 5.9% vs 1.4% for type 3 ($p = 0.01$, Fisher exact). Type 2 seronegativity also reduced from 1.0% to 0% ($p = 0.25$, Fisher Exact, statistical non-significance due to minute number of seronegative infants). Infants in the IPV arm enjoyed much higher rates of seroconversion of all three antibody types from week 18 to week 40 with 95–96% in the IPV arm, while the tOPV arm ranged from 49%(type 3) to 63%(type 1) and 71%(type 2) (all $p < 0.0001$). We tested the SNAb week 40 measures of seropositivity and seroconversion as predictive correlates of future week 52 shedding, Table 3. Infants who achieved seropositivity or had seroconverted between week 18 and week 40 were less likely to shed at week 52 than those that had not for all serotypes. Comparing the IPV to tOPV arm, while the absolute numbers varied, the percentages of infants who shed at week 52 were the same whether measured by their positive SNAb status as positivity or conversion, with percentages approximately 24%, 10%, and 13% for S1, S2, S3, respectively. For infants who had negative SNAb measures at week 40 (were seronegative or had not seroconverted), seronegativity was the better correlate of week 52 shedding, albeit with small absolute numbers in the case of IPV-vaccinated infants. In other words, for infants who were seropositive or had seroconverted, there was little difference in the predictive value of the two humoral measures. It is important to note that 53%, 59%, and 28% of infants achieved the maximum possible log2 titer of 10.5 at week 18 for types 1, 2, and 3, respectively, and were censored from the seroconversion analysis but included in the seropositivity analysis. Inferences were similar with all infants included that had at least one non-missing week 52 stool specimen, assuming missing specimens were negative.

4. Discussion

Global elimination of wild type poliovirus will require continued use of OPV to effectively disrupt transmission of live virus, while subsequent eradication of all circulating Sabin virus strains will necessitate a managed global transition to IPV. This transition will require interim regimens that blend both types of vaccine and requires rigorous data on specific pragmatic substitutions. With the impending withdrawal of OPV2 in April 2016, the Bangladesh EPI announced changes to their national immunization program

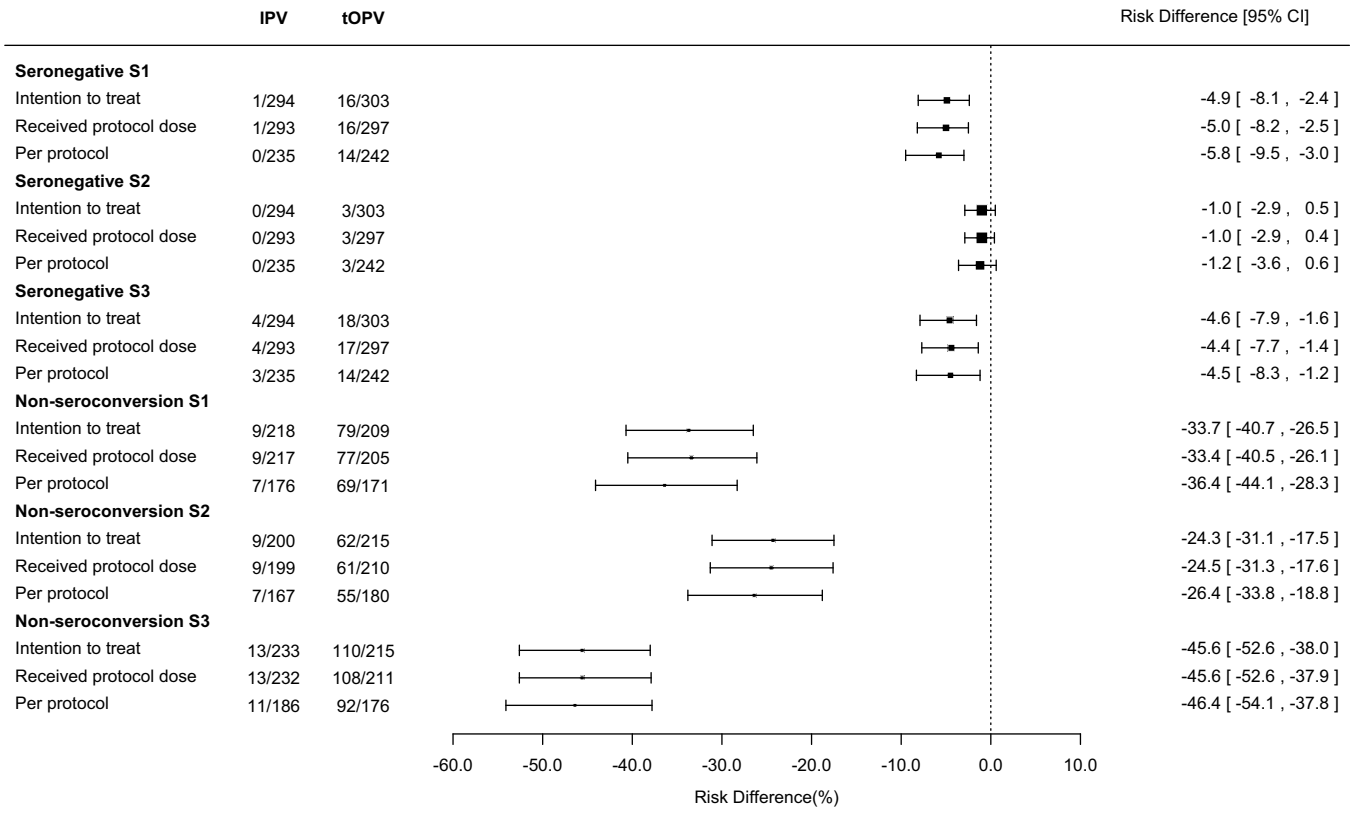

Fig. 4. Plot of serum neutralizing antibody (SNAb) secondary outcomes measured in serum from age week 40 infants after the trial IPV vs tOPV intervention at week 39. The outcomes are defined to focus on possible vaccine failure. Seronegative infants had no detectable SNAb at week 40. Seroconversion failure are infants who did not seroconvert between week 18 (post tOPV dose 2) and week 40, adjusted for residual maternal antibody, as described in the text. All of the differences are statistically significant in favor of IPV with the exception of seronegativity (SNA Failure) in type 2, due to the very low numbers of failures. Points designating the location of the estimated risk difference are sized proportional to the precision of the estimates. Risk differences are shown as fractions. Negative (−) values imply lower seronegativity or non-seroconversion in the IPV arm versus the tOPV arm.

Table 3
Comparison of infant week 40 serum neutralizing antibody (SNAb) humoral measures as correlates of future poliovirus shedding at 52 weeks of age, by virus type.

Study arm	Type	Week 40 serum neutralizing antibody measure*	Infants N shed/total (%) with +ve SNAb measure at Week 40	Infants N shed/total (%) with −ve SNAb measure at Week 40	*P*-value †
Both	S1	Seropositivity	110/474 (23%)	7/13 (54%)	0.02
	S2	Seropositivity	45/484 (9%)	2/3 (67%)	0.03
	S3	Seropositivity	57/467 (12%)	7/20 (35%)	0.01
	S1	Seroconversion	65/276 (24%)	24/76 (32%)	0.18
	S2	Seroconversion	27/279 (10%)	9/58 (16%)	0.24
	S3	Seroconversion	38/264 (14%)	18/101 (18%)	0.42
IPV	S1	Seropositivity	57/240 (24%)	1/1 (100%)	0.24
	S2	Seropositivity	20/241 (8%)	0/0 (N/A%)	N/A
	S3	Seropositivity	32/238 (13%)	1/3 (33%)	0.36
	S1	Seroconversion	41/169 (24%)	4/9 (44%)	0.23
	S2	Seroconversion	13/155 (8%)	2/8 (25%)	0.16
	S3	Seroconversion	28/181 (15%)	3/11 (27%)	0.39
tOPV	S1	Seropositivity	53/234 (23%)	6/12 (50%)	0.04
	S2	Seropositivity	25/243 (10%)	2/3 (67%)	0.03
	S3	Seropositivity	25/229 (11%)	6/17 (35%)	0.01
	S1	Seroconversion	24/107 (22%)	20/67 (30%)	0.29
	S2	Seroconversion	14/124 (11%)	7/50 (14%)	0.61
	S3	Seroconversion	10/83 (12%)	15/90 (17%)	0.52

* Seropositivity and Seroconversion at week 40 refer to the SNAb titer and change in SNAb titer from week 18 to week 40, respectively, as described in Procedures. The counts are restricted to those 493 infants with five complete week 52 shedding stool specimens. The % measures the predictive value of the week 40 SNAb measure (either +ve or −ve) to predict future shedding at week 52. A +ve SNAb measure refers to seropositivity or serconversion at week 40; the corresponding −ve measures are seronegative at week 40 and failed to seroconvert.

† *P*-value refers to exact test for difference in proportion of infants between the +ve and −ve week 40 SNAb groups, i.e. a difference in predictive value of this measure.

in March 2015, including addition of an IPV dose which will contain the sole administered type 2 vaccine in this new schedule. This work is timely in assessing the effects of the transition on shedding.

Prior work has suggested that IPV may be at least as effective as OPV in reducing viral excretion when administered to a child primed by prior OPV vaccination [1,2]. We found that neither of the schedules tested in this study was superior to other, and estimated that they were equivalent to within a shedding rate difference of −10% (favors IPV) to +4% (favors tOPV). This equivalence was reinforced by direct fecal RT-PCR assay and quantitative comparison of the total shedding index between the trial arms at 52 weeks. Furthermore, for the more neurovirulent and recent wild type 1 and 3 poliovirus strains, the IPV dose boosted the number of infants who were seropositive for type 1 by 5% compared to 4 OPV doses thereby reducing the fraction who were seronegative to about 0.3%; and by about 4.5% for type 3, reducing the seronegatives to 1%. Similarly, the IPV dose increased the percentage who seroconverted between week 18 and 40 by about 35% and 46% respectively for type 1 and 3. This suggests that IPV can be substituted into the fourth dose of the EPI program in Bangladesh without relative diminution of intestinal immunity while boosting immunogenicity.

Results for our modified EPI schedule extend previous work that tested combinations of OPV and IPV. An IPV dose has been shown to boost antibody response rates of seropositivity and seroconversion relative to OPV after prior OPV vaccinations [19,24–26] Furthermore, mixed IPV/OPV schedules seem to have similar intestinal immunity to equivalent dose OPV-only, and better than IPV alone [4,19,25]. Two very recent studies in India have shown that administering a single dose of IPV in older children post-scheduled vaccination improves both humoral and intestinal immunity and may help to close the waning immunity gap [27,28]. Jafari et al. found that IPV was superior to bOPV in reducing any shedding in three cohorts of children ages 6–11 months, and 5, and 10 years [27]. Unlike our results, the 6–11 month cohort, of most relevance to this study, also saw significant reduction of type 1 and type 3 shedding relative to controls and bOPV groups. The exact reason for the significant improvement in intestinal immunity using IPV compared to OPV in contrast to our study is unclear but may be related to local immunogenicity factors or previous dosing. Our results suggest that IPV can be substituted for tOPV without compromising intestinal immunity, but that IPV does not additionally improve intestinal immunity beyond OPV. Mass campaign interventions have been effective in regional elimination of poliovirus, but require repetition and can subtract from limited primary healthcare resources [29].

We found that either humoral measure, SNAb seropositivity or seroconversion at week 40, predicted future shedding at week 52 equally well. We also found that the future risk of shedding is the same after 3 tOPV doses + IPV versus the four tOPV dose arm for infants that were seropositive or had seroconverted at week 40. The best discriminating predictor of future shedding for the tOPV arm infants was seropositivity versus seronegativity. For the IPV arm, the small numbers of seronegative or non-seroconverted infants makes comparisons difficult, but neither humoral measure approached being a perfect correlate of future shedding risk.

Our trial has limitations. We recruited only from a single population with economic and sanitary deprivation. The tested vaccine responses could have been confounded by passive vaccination through community contact although the high excretion rate at week 52 suggests that this is likely to be a small effect if present. We investigated trivalent OPV and not selective bi- or monovalent vaccines that target less immunogenic serotypes. We did not measure oropharyngeal excretion although in this urban slum population lacking sanitation, the predominant mode of transmission is likely to be fecal-oral versus oral-oral and hence oropharyngeal immunity is a less important component in limiting viral transmission. This is supported by prior studies showing that oropharyngeal shedding did not substantially contribute to wild poliovirus transmission in older children in Bihar, India [30], and that type 1 oropharyngeal shedding was comparatively low compared to fecal shedding for both IPV and OPV schedules [31]. Finally, while the responses were measured up to one year of age, potential long-term changes or waning immunity over time were not addressed.

In summary, our study shows that incorporation of IPV into primary vaccination schedules is possible while maintaining existing intestinal immunity to limit fecal transmission, yet giving children extra seroprotection from IPV at an earlier age, reducing the need for supplementation later.

Contributions

WAP, BDK, RH, KZ conceived the trial and design, obtained funding, supervised the study. MA recruited participants, provided primary care, and managed clinical staff. JCM managed data coordinating center. MC, MT, EH, WCW, MSO performed or supervised laboratory assays and interpreted laboratory data. JCM, MC, UN, ERC, DD analyzed and interpreted data. DZ, JCM performed statistical analysis. JCM drafted the manuscript. All authors reviewed the manuscript.

Declaration of interests

We declare no competing interests.

US Government disclaimer

The findings and conclusions in this report are those of the author(s) and do not necessarily represent the views of the Centers for Disease Control and Prevention or other contributing agencies.

Conflicts of interest statement

None.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.vaccine.2015.11.046.

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