Randomized, controlled trial of trivalent oral poliovirus vaccine (Sabin) starting at birth in Ghana*

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To evaluate the efficacy of the schedule currently recommended for immunization with trivalent oral poliovirus vaccine (TOPV) (i.e., at birth, 6 weeks, 10 weeks, and 14 weeks after birth), we randomly assigned 452 infants into test (231 infants) and control (221 infants) groups. The test group received TOPV as currently recommended, and the dose at birth was omitted for the control group. At 10, 14, and 18 weeks of age, the levels of poliovirus neutralizing antibodies as well as seroconversion rates were consistently higher for the test group than for the control group. The final seroconversion rates against poliovirus types 1, 2, and 3 were 83.5%, 91% and 83%, respectively, for the test group and 75%, 83.2%, and 79.1%, respectively, for the control group. The TOPV immunization schedule starting at birth therefore produced better results. Seroconversion rates as well as antibody levels were highest in infants with low maternal antibodies.

Introduction

The much documented inadequate seroresponse to trivalent oral poliovirus (Sabin) vaccine (TOPV) in some tropical countries (*1–6*) has made it necessary to find other ways of achieving better immunization results (*7–12*).[a] The presence of interfering enteroviruses in the intestinal tract of vaccinees, breast-feeding, high levels of maternal antibodies, and the use of low potency vaccines have been linked to the inadequate seroresponse to TOPV in tropical countries (*3–7*). In 1986, Don De-Xiang et al. demonstrated that immunization at birth with TOPV can produce almost 100% seroconversion rates (*11*). As a result of this and other studies, the WHO Expanded Programme on Immunization adopted a revised schedule, with the first dose of TOPV being given at birth, followed by three subsequent doses at 6 weeks, 10 weeks, and 14 weeks after birth. In a preliminary study in Ghana, nearly 100% seroconversion (for all serotypes) was achieved using this new schedule to immunize infants with TOPV (Osei-Kwasi, M et al., unpublished data, 1987); the sample size used was, however, small. The present study was undertaken to assess the seroresponse of newborns to immunization with TOPV, by comparing the levels of poliovirus neutralizing antibodies in infants vaccinated at birth, 6 weeks, 10 weeks, and 14 weeks of age with the levels in infants immunized with TOPV at 6 weeks, 10 weeks, and 14 weeks after birth.

Materials and methods

In a single-blind, randomized controlled trial, 452 newborns were enlisted into the study from October 1990 to July 1991 in Ashaiman, a peri-urban township near the port of Tema, Ghana, through the maternity unit of the health post, a private maternity home, as well as the Tema General Hospital. After the aims and objectives of the study had been explained, only infants whose parents gave their consent were enlisted. For inclusion a birth weight of ≥2.5 kg was required. The newborns were allocated to a test group (231 infants) and to a control group (221 infants) using computer-generated random numbers.

The study group of infants was immunized with one dose of TOPV at birth, 6 weeks, 10 weeks, and 14 weeks of age. The schedule for the control group of infants was at 6 weeks, 10 weeks and 14 weeks after birth, i.e., omitting the dose at birth.

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[a] **Assaad F.** Reassessment of inactivated poliomyelitis vaccine in national immunization programmes. Paper presented at: *International Symposium on Reassessment of Inactivated Poliomyelitis Vaccine, Bilthoven, 24–27 June 1980.*

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The TOPV (Institut Mérieux, France; lot D1417) used in the study was obtained from the Epidemiology Division of the Ministry of Health. The vaccine was stored at Noguchi Memorial Institute for Medical Research (NMIMR). The potency of 20 vials of the vaccine that had been left in storage and of the residual vaccine in 20 vials used in the field was tested. The results of all the tests showed that the log $TCID_{50}$ per dose was ≥6.0 (type 1), ≥5.0 (type 2) and ≥5.5 (type 3). These titres, which were in conformity with the manufacturer's formulation of 10:1:3 per dose, satisfied WHO requirements.[b]

Blood collection

Prior to the administration of each dose of vaccine, a 0.2-ml sample of blood was obtained from each infant by heel-prick and capillary action. Each blood sample was added to 0.7 ml of transport medium in well-labelled polystyrene tubes with caps. These were then transported in cool boxes to NMIMR, where each sample was separated using low-speed centrifugation (3000 rpm) to give a serum with an initial dilution of 1:8. The sera were appropriately labelled and stored at –20 °C until tested.

Test procedures

The sera of infants were titrated in accordance with WHO-recommended procedures for the titration of human sera for poliovirus neutralizing antibodies.[b] Before being tested, each serum sample was inactivated at 56 °C for 30 minutes. A dilution series in twofold steps of 1:8 to 1:1024 was tested for each of the inactivated serum samples. Aliquots (25 µl) of the diluted sera were pipetted in duplicate into 96-well microplates. A virus suspension containing approximately 100 $TCID_{50}$ per 50 µl was then added to the sera (volume ratio, 1:1). This procedure was performed for all three poliovirus serotypes. The plates were then incubated for 2 hours at 36 °C, after which 100 µl of Hep-2 cell suspension (concentration, 10^4 cells per ml) was added to each well. The plates were subsequently incubated at 36 °C under 5% carbon dioxide for 7 days and the observed cytopathic effects recorded. Finally, the serum antibody titres were calculated using the method described by Reed & Muench.[b]

All infants who were seronegative at the end of the study were given an extra dose of TOPV.

All the data were analysed using Student's *t*-test and the *z*-test.

Results

The cumulative dropouts in the test and control groups were 31 (13.4%) and 25 (11.3%), respectively. For the serological analysis 200 complete sets of blood samples were obtained for the test group and 196 for the control group.

There were no adverse reactions in any of the infants during the period of immunization and 4 weeks after the last dose had been administered. Twenty-four cases of diarrhoea (watery stools >3 times within 24 hours) were reported, but these cleared up 1–3 days after being treated with oral rehydration salts (ORS).

Seropositivity among the study infants

For the serological analysis any serum with poliovirus neutralizing antibodies measurable at a dilution of 1:8 or above was taken as seropositive. Of 200 infants in the test group, 82.5% (165), 80.5% (161), and 76.0% (152), respectively, had pre-existing maternal antibodies to poliovirus serotypes 1, 2, and 3. The corresponding proportions for the 196 infants in the control group were 89.3% (175), 88.8% (174), and 73.0% (143). The differences between the two groups were not statistically significant. At the end of the study (i.e., after 18 weeks), 90.5% (181), 93% (186), and 83.5% (167), respectively, of the 200 infants in the test group had measurable poliovirus neutralizing antibodies against the serotypes 1, 2, and 3, respectively (Table 1). For the infants in the control group the corresponding proportions were 84.2% (165), 88.8% (174), and 82.7% (162).

Seropositivity rates for all serotypes were higher in the test group at 10, 14 and 18 weeks than in the control group; however, the differences were not statistically significant.

Seroconversion rates

Table 2 shows the seroconversion rates for infants in the test and control groups. To calculate these rates, we deducted the residual maternal antibody levels from the antibody levels measured for each infant at the various immunization periods; this was based on the half-life estimation of 28 days for maternally derived poliovirus neutralizing antibodies. A vaccinee was considered to have seroconverted at a given time when, after the deduction of the calculated level of residual maternal antibodies (rmAbs) from the measured neutralizing antibodies (mnAbs), the level remaining was greater than or equal to four times that of the residual level of maternal antibodies.

[b] Titration of human sera for neutralizing antibodies to poliovirus. In: *Laboratory methods for the titration of live virus vaccines using cell culture techniques.* Unpublished WHO document BLG/EPI/89.1.

Table 1: **Poliovirus seropositivity rates among the study infants, by serotype and age**

Age	No. in test group (n = 200):			No. in control group (n = 196):		
	Type 1	Type 2	Type 3	Type 1	Type 2	Type 3
At birth	165 (82.5)[a]	161 (80.5)	152 (76.0)	175 (89.3)	174 (88.8)	143 (73.0)
6 weeks	167 (83.5)	182 (91.0)	152 (76.0)	165 (84.2)	161 (82.1)	122 (62.2)
10 weeks	173 (86.5)	191 (95.5)	169 (84.5)	160 (81.6)	180 (91.8)	144 (73.5)
14 weeks	177 (88.5)	191 (95.5)	165 (82.5)	162 (82.7)	176 (89.8)	148 (75.5)
18 weeks	181 (90.5)	186 (93.0)	167 (83.5)	165 (84.2)	174 (88.8)	162 (82.7)

[a] Figures in parentheses are percentages.

At 10, 14, and 18 weeks, seroconversion rates were consistently higher in the test group than the control group. By the end of the study, the seroconversion rates for the test group for poliovirus serotypes 1, 2, and 3, respectively, were 83.5%, 91% and 83%; the corresponding rates for the control group were 75%, 83.2%, and 79.1%. The differences between the test and control groups were statistically significant for poliovirus serotype 1 ($P = 0.0184$) and serotype 2 ($P = 0.009963$).

Comparisons of the seroconversion rates at 14 weeks in the test group with those at 18 weeks in the control group (when both groups had received 3 doses of TOPV), however, indicated a significantly higher rate only for serotype 2 ($P = 0.0373$).

Geometric mean titres

The geometric mean titres (GMTs) of poliovirus neutralizing antibodies at the various stages of immunization for all three serotypes (Table 3) were calculated using the following relationship:

$$GMT = \text{antilog}\ \{(\log X_1 + \log X_2 + \log X_3 ...)/n\}$$

where n is the total number of vaccinees (200 for test group, 196 for control group).

At weeks 10, 14 and 18, infants in the test group had consistently higher levels of antibodies against all three poliovirus serotypes than those in the control group. With the exception of type 3, the GMTs to type 1 and type 2 were significantly higher at 14 weeks in the test group than at 18 weeks in the control group, when both groups had received three doses of TOPV. GMTs were highest for poliovirus type 2 at all periods sampled, in both the test and control groups.

Maternal antibodies and seroconversion rates

Table 4 shows the distribution of maternally derived poliovirus neutralizing antibody levels at birth and their correlation with seroconversion rates after immunization with TOPV, for both the test and control groups. The highest seroconversion rates were among infants whose maternally transferred antibodies were <1:64. Infants whose pre-existing antibodies were >1:128 at birth had the lowest seroconversion rates.

Discussion

Administration of vaccines to infants early in life has as its prime objective the induction of active immunity before the loss of protective maternal antibodies. In tropical countries, early colonization of the gut of young infants by other enteroviruses could interfere with TOPV, making its early administration even more important (*6, 7, 13*). In the study we compared the seroresponse to TOPV given in four doses starting at birth with that given in three doses starting at

Table 2: **Seroconversion rates to trivalent oral poliovirus vaccine (TOPV) among the study infants, by serotype and age**[a]

Age (weeks)	No. in test group (n = 200):			No. in control group (n = 196):		
	Type 1	Type 2	Type 3	Type 1	Type 2	Type 3
6	67 (33.5)[b]	119 (56.5)	70 (35.0)	— —	— —	— —
10	118 (59.0)	158 (79.0)	133 (66.5)	81 (41.5)	131 (67.2)	101 (51.8)
14	146 (73.0)	180 (90.0)	155 (77.5)	125 (64.1)	156 (80.0)	131 (67.0)
18	167 (83.5)	182 (91.0)	166 (83.0)	147 (75.0)	163 (83.2)	155 (79.1)

[a] *Tests of significance.* Test group at 14th week versus control group at 18th week: type 1, $P = 0.4$; type 2, $P = 0.04$; type 3, $P = 0.3$. Test group at 18th week versus control group at 18th week: type 1, $P = 0.02$; type 2, $P = 0.01$; type 3, $P = 0.2$.
[b] Figures in parentheses are percentages.

Table 3: **Geometric mean titres (GMTs) of poliovirus neutralizing antibodies among the study infants, by serotype and age**[a]

Age	GMTs for test group (n = 200):			GMTs for control group (n = 196):		
	Type 1	Type 2	Type 3	Type 1	Type 2	Type 3
At birth	18.1	16.8	11.8	21.2	22.3	9.7
6 weeks	19.2	47.8	14.7	14.3	13.9	7.4
10 weeks	35.5	119.4	40.2	21.7	56.7	16.7
14 weeks	62.5	191.3	56.7	41.6	97.5	33.0
18 weeks	89.3	196.0	74.8	56.7	115.5	55.5

[a] *Tests of significance:* Test group at 14th week versus control group at 18th week (after 3 doses of TOPV): type 1, P = 0.00895; type 2, $P<10^{-6}$; type 3, $P>0.20000$ (not significant). Test group at 18th week versus control group at 18th week: type 1, $P<0.0001$; type 2, $P<0.0001$; type 3, $P<0.0001$.

6 weeks of age. The results indicate that the schedule starting at birth is quantitatively (as assessed by seroconversion rates) and qualitatively (as determined by the GMTs of the vaccinee's antibodies) better than the schedule that began at 6 weeks of age. For example, at 6 weeks of age the GMTs of antibodies in the test group (infants who received their first dose of TOPV at birth) were greater than the GMTs of their maternally derived antibodies. However, in the control group (infants who received their first dose of TOPV at 6 weeks of age), the GMTs of maternal antibodies had declined for all three serotypes. Moreover, at 6 weeks of age, 35.5%, 56.5%, and 35% of infants in the test group had seroconverted against poliovirus serotypes 1, 2, and 3, respectively, after having received a single dose of TOPV at birth. Administration of TOPV at a very early age therefore primes a substantial proportion of infants to give a seroresponse, followed by good secondary responses with subsequent doses.

By week 18 the test group had higher seroconversion rates than the control group for all serotypes. Also GMTs were significantly higher for all serotypes in the test than in the control group ($P<0.01$). Furthermore, comparison of the GMTs in the test group at 14 weeks and the control group at 18 weeks (when both groups had received 3 doses of TOPV) indicated that higher antibody titres were achieved by starting the TOPV series at birth. The differences were highly significant for serotypes 1 and 2 ($P<0.01$); for serotype 3, although the difference was not statistically significant, the GMT of the test group at 14 weeks was still higher than that of the control group at 18 weeks. These results support the findings of other workers (*3, 11*), which have suggested that early immunization with TOPV provides a better immune response than that produced by starting later in life.

We also investigated the effect of pre-existing maternally transferred, poliovirus-specific antibodies on seroresponses to TOPV. Infants with lower levels of maternal antibodies at birth had the best seroconversion rates in both groups. These findings are in agreement with previous reports (*3, 4, 6*) and also, more recently, with results from the Gambia (*MRC Laboratories Annual Report*, 1991).

Further analysis of our data revealed that in both the test and control groups the type 2 Sabin vaccine

Table 4: **Maternal antibody levels and seroconversion rates to poliovirus serotypes among the study infants at the 18th week**

Titre of maternally transferred antibodies	Type 1:		Type 2:		Type 3:	
	No. in test group	No. in control group	No. in test group	No. in control group	No. in test group	No. in control group
8	34/35 (97.1)[a]	18/21 (85.7)	37/39 (94.9)	19/22 (86.4)	36/48 (75.0)	43/53 (81.2)
8	27/29 (93.1)	32/37 (86.5)	30/31 (96.8)	27/32 (84.4)	37/41 (90.2)	26/35 (74.3)
16	34/38 (89.5)	33/36 (88.9)	30/30 (100)	28/32 (87.5)	29/34 (85.3)	42/49 (85.7)
32	30/34 (88.2)	38/44 (86.4)	38/41 (92.7)	46/51 (90.2)	35/39 (90.5)	25/28 (89.3)
64	22/33 (66.6)	14/28 (50.0)	24/27 (88.8)	25/30 (83.3)	19/21 (90.5)	12/19 (63.2)
128	15/21 (71.4)	8/20 (40.0)	17/23 (73.9)	11/15 (73.3)	6/9 (66.6)	6/9 (66.6)
256	5/8 (62.0)	3/9 (33.0)	6/8 (75.0)	7/12 (58.3)	4/6 (66.6)	1/2 (50.0)
512	0/2 (0)	1/1 (100)	0/1 (0)	0/2 (0)	0/2 (0)	0/1 (0)

[a] Figures in parentheses are percentages.

strain in the formulation used is a better immunogen than the types 1 and 3.

Our findings also confirm that the rates of seroconversion produced using the TOPV schedule recommended by WHO (i.e., 3 or 4 doses) are still less than optimum. Immunization with four doses of TOPV, starting at birth, led to significantly higher rates of seroconversion and GMTs than immunization with three doses starting at 6 weeks of age. Nevertheless, 16.5%, 9%, and 17%, respectively, of the 200 infants in the test group for whom data were complete remained unprotected against poliovirus serotypes 1, 2, and 3.

We can infer from the results of this study and those of others that TOPV administered as currently recommended by WHO (first dose at birth or shortly thereafter followed by subsequent doses at 6, 10, and 14 weeks of age) is inadequate for interrupting the circulation of wild polioviruses in some developing countries in tropical and subtropical areas. This has been borne out by recent reports of outbreaks of paralytic poliomyelitis in a number of countries with high TOPV coverage rates administered through the routine delivery system (*14–17*).

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Résumé

Essai contrôlé randomisé du vaccin antipoliomyélitique buccal trivalent (Sabin) administré dès la naissance au Ghana

Afin d'évaluer l'efficacité du calendrier actuel de vaccination par le vaccin antipoliomyélitique buccal trivalent (VPO trivalent), nous avons effectué un essai de vaccination en simple aveugle sur 452 nouveau-nés répartis par tirage au sort dans le groupe traité ou dans le groupe témoin. Les nourrissons du groupe traité recevaient le vaccin à la naissance puis à l'âge de 6 semaines, 10 semaines et 14 semaines. Pour les nourrissons du groupe témoin, le calendrier était le même, mais sans la dose néonatale. Le vaccin utilisé était du type 10:1:3 par dose (pour les poliovirus types 1, 2 et 3 respectivement). Avant chaque administration de vaccin, et 4 semaines après, un prélèvement de sang était réalisé sur chaque nourrisson pour la sérologie des anticorps neutralisants à l'égard du poliovirus. On a ainsi recueilli 200 séries complètes de prélèvements de sang pour le groupe traité et 196 séries pour le groupe témoin. Tout prélèvement ayant un taux mesurable d'anticorps neutralisants à l'égard du poliovirus à une dilution ≥1:8 a été considéré comme séropositif.

Les taux d'anticorps neutralisants à l'égard des trois sérotypes de poliovirus (exprimés par leur titre moyen géométrique) à 10, 14 et 18 semaines ainsi que les taux de séroconversion étaient plus élevés chez les nourrissons du groupe traité que chez ceux du groupe témoin. A 18 semaines, les taux de séroconversion étaient plus élevés dans le groupe traité (83,5%, 91% et 83% contre les poliovirus de types 1, 2 et 3) que dans le groupe témoin (75%, 83,2% et 79,1% respectivement). Les différences entre les deux groupes en ce qui concerne les sérotypes 1 et 2 étaient statistiquement significatives ($p<0,05$). Le vaccin Sabin de type 2 était le meilleur immunogène dans les deux groupes. Les taux de séroconversion et les titres d'anticorps les plus élevés ont été trouvés chez les nourrissons dont le titre d'anticorps maternels était faible. Bien que l'administration de VPO trivalent dès la naissance donne de meilleurs résultats, le nombre de nourrissons encore non protégés après avoir reçu 4 doses de vaccin reste préoccupant.

References

1. **John TJ, Jayabal P.** Oral poliomyelitis vaccination in the tropics: the poor seroconversion rates. *American journal of epidemiology*, 1972, **96**: 263–269.
2. **Pangi NS et al.** Efficacy of oral poliomyelitis in infancy. *Indian journal of pediatrics*, 1977, **14**: 523–528.
3. **Halsey N, Galazka A.** The efficacy of DPT and oral poliomyelitis immunization schedules initiated at birth. *Bulletin of the World Health Organization*, 1985, **63**: 1151–1169.
4. **Chaudry DS et al.** Poliomyelitis vaccination of infants: preimmunization status and seroconversion. *Bulletin of the World Health Organization*, 1973, **48**: 195–198.
5. **Plotkin SA et al.** Oral poliovirus vaccination in newborn African infants: the inhibitory effects of breastfeeding. *American journal of diseases of children*, 1966, **111**: 27–30.

6. **Dömök I et al.** Factors affecting the efficacy of type 1 live oral poliovirus in warm climates: efficacy of type 1 Sabin vaccine administered together with antihuman gamma-globulin horse serum to breast-fed and artificially fed infants in Uganda. *Bulletin of the World Health Organization*, 1974, **51**: 333–347.
7. **Sabin AB et al.** Live orally given poliovirus vaccine: effects of rapid mass immunization on a population under conditions of massive enteric infection with other viruses. *Journal of the American Medical Association*, 1960, **173**: 1521–1526.
8. **John TJ.** Antibody response of infants in the tropics to five doses of oral polio vaccine. *British medical journal*, 1972, **1**: 812.
9. **Ehrengut Y et al.** Seroconversion rates following oral immunization against poliomyelitis in Mali/Africa. *Monatsschrift für Kinderheilkunde*, 1984, **132**: 29–31.
10. **Melnick JL.** Combined uses of live and killed vaccines to control poliomyelitis in the tropical areas. *Developments in biological standardization*, 1981, **47**: 265–273.
11. **De-Xiang D et al.** Vaccination of neonates against poliomyelitis with the trivalent oral poliomyelitis (Sabin) vaccine. *Bulletin of the World Health Organization*, 1986, **66**: 853–860.
12. **Osei-Kwasi M et al.** Two double-dose immunization trials with TOPV in Ghana. *Ghana medical journal*, 1990, **24**: 9–12.
13. **Kok PW et al.** Serological and virological assessment of oral and inactivated poliovirus vaccines in a rural population in Kenya. *Bulletin of the World Health Organization*, 1992, **70**: 93–103.
14. **Patriarca PA et al.** Randomized trial of alternative formulations of oral poliovaccine in Brazil. *Lancet*, 1988, **1**: 429–433.
15. **Kim-Farley RJ et al.** Outbreak of paralytic poliomyelitis, Taiwan. *Lancet*, 1984, **2**: 1322–1324.
16. **Sutter RW et al.** Outbreak of paralytic poliomyelitis in Oman: evidence of widespread transmission among fully immunized children. *Lancet*, 1991, **1**: 715–720.
17. **Patriarca PA et al.** Factors affecting the immunogenicity of oral polio vaccine in developing countries: review. *Reviews of infectious diseases*, 1991, **13**: 926–939.