Serological and virological assessment of oral and inactivated poliovirus vaccines in a rural population in Kenya

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A study was carried out in a rural community in Kenya to compare the humoral and intestinal immunity provided by three doses of oral poliovirus vaccine (OPV) and two or three doses of enhanced-potency inactivated poliovirus vaccine (IPV). The immunization series was started at 8–12 weeks of age and the interval between doses was 2 months. In children with low levels of maternal antibodies (i.e., those most at risk), the first dose of either vaccine stimulated antibody response. Children with high levels of maternal antibodies responded to the first dose of OPV, but not to that of IPV. Subsequent doses led to increases in the mean antibody titres with both vaccines. After three doses of OPV, the proportion of children with antibody titres of ≥1:8 was 92% for type 1 virus, 98% for type 2, and 90% for type 3. After two doses of IPV the proportion of children with antibody titres of ≥1:8 was 94%, 88%, and 97% for type 1, type 2, and type 3, respectively; after three doses of IPV, 100% of children had antibodies ≥1:8 for types 1 and 3, and 98% for type 2. Intestinal immunity was tested with a challenge dose of type 1 OPV, but the dose used was too small to detect a significant difference between the vaccines.

Introduction

Poliomyelitis continues to be a serious health problem in developing countries, where WHO estimates 120,000 cases of paralytic poliomyelitis still occur annually.a

As a result of the widespread use of lameness surveys in the 1970s and 1980s, paralytic poliomyelitis is recognized in developing countries to be a public health problem of a similar magnitude to the situation that prevailed in Europe and the USA before the introduction of immunization against poliomyelitis (1). The results of such surveys have drastically altered the perception, resulting from gross underreporting, that poliomyelitis is an insignificant problem and have led many countries to implement poliomyelitis immunization programmes.b–e

Poliomyelitis has been successfully controlled in industrialized countries, where either oral poliovirus vaccine (OPV) or inactivated poliovirus vaccine (IPV) has been used. The advantages and disadvantages of these two vaccines have been discussed in excellent reviews (3,4). Low cost, ease of administration and acceptance, expected long-lasting immunity, rapid production of intestinal immunity that can

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interrupt transmission of wild virus, even in epidemic situations, and the spread of OPV virus to unvaccinated persons, which can induce immunity in these persons, were the basis for the decision to use OPV in many countries (5).

In 1988, the Forty-first World Health Assembly committed WHO and all Member States to the goal of global eradication of poliomyelitis by the year 2000 (6). Global plans draw heavily on the experience gained in the Region of the Americas, which began its polio eradication efforts in 1985 (7,8). Much has been learned in the Americas, where maintenance of high levels of coverage with OPV and the institution of intensive surveillance mechanisms that ensure investigation of every suspected or probable case of paralytic poliomyelitis have resulted in a markedly reduced transmission of wild polioviruses. The Region of the Americas is now poised to eradicate the disease.

There is good evidence that the application of OPV in developing countries, through routine services (as in Botswana (9), Cameroon (10), Singapore (11), Sri Lanka (12), and Thailand (13)) or through mass campaigns (as in Cuba (14) and Brazil (15)), has resulted in a significant decrease in the number of polio cases. While the serological response to OPV has been excellent in a number of countries (16–18), it has sometimes been less than optimal (19) and reports of poliomyelitis epidemics of type 1 in the Gambia (20) and Oman (21) and of type 3 in Brazil (22) in spite of extensive OPV use have raised concerns about the efficacy of the vaccine.

In recent years, interest has been shown in evaluating the enhanced-potency IPV preparation (23–29). In view of this, and the need to immunize infants at an early age, we undertook a study of the efficacy of OPV and IPV in stimulating humoral and intestinal immunity in children who lived in a rural community in Kenya.

Materials and methods

Study population

The study population comprised children born in 1982 and 1983 in two distinct but neighbouring geographical areas in Machakos District, in the Eastern Province of Kenya. In one area, 100 children received three doses of OPV, while in the other, 50 children received two doses of IPV and 50 children received three doses of IPV.

Vaccines

The trivalent OPV that was used contained 1 million, 100 000, and 300 000 TCID₅₀ of poliovirus types 1, 2 and 3, respectively, per dose of two drops. Type 1 monovalent OPV (3000 TCID₅₀ per two-drop dose) was used as a challenge dose to follow the excretion pattern of poliovirus type 1 in stools and throat washings. Potency testing of these vaccines after completion of the study showed that the total virus titre for the trivalent OPV was 10₆.₇ TCID₅₀ per dose and that for the type 1 monovalent OPV was 3000–7000 TCID₅₀ per dose.

The enhanced potency IPV contained 40, 8, and 32 D-antigen units of poliovirus type 1, 2 and 3, respectively, per 0.5 ml dose. This vaccine was combined with diphtheria, pertussis and tetanus (DPT) as a quadruple vaccine.

Immunization schedule

The immunization schedule is shown in Fig. 1. Immunization against poliomyelitis started at 2–3 months of age and the interval between doses was 2 months. Breast-feeding was not discouraged during immunization. Children who failed to attend the clinic were followed up at home. Children immunized outside their study area were excluded from the study.

The following immunizations were also administered: BCG at birth or first contact; three doses of DPT given at the same time as the polio vaccine; and measles vaccine at 9 months of age or thereafter (Fig. 1).

Challenge with type 1 monovalent OPV

A challenge dose of type 1 monovalent OPV was fed to all children in the study 2 months after the last immunization dose. In addition, a separate group of 24 children aged 2–4 months were fed the challenge dose 1 week prior to their first polio immunization.

Blood, faeces, and nasopharyngeal washing samples

Capillary blood samples were collected using 200 µl calibrated pipettes on the day each dose of polio vaccine was administered and 2 months after the last dose (Fig. 1). Serum was separated in the field and kept cool until transport to the laboratory. Faecal

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¹ Shin, H.K. Seroprevalence of antibody to poliovirus type 1, 2 and 3 following three doses of standard TOPV in Korea. Unpublished WHO document EPI/GAG/89/WP.7.5.

² UNICEF/Sclavo, Italy.

³ Smith Kline–RIT, Belgium.

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Fig. 1. Study timetable for immunization and collection of blood, faeces, and nasopharyngeal washing samples, Kenya, 1982–83. DPT = diphtheria–pertussis–tetanus; OPV = oral poliovirus vaccine; IPV = inactivated poliovirus vaccine.

B = blood sample
F = faeces sample
NW = nasopharyngeal washing

Determination of antibodies, isolation and typing of enteroviruses

The determination of poliovirus antibodies was performed at the Rijksinstituut voor de Volksgezondheid en Milieuhygiene (RIVM), Netherlands, using the standard microtitre neutralization test and Vero cells (30). Serum was tested for poliovirus type 1 against 61 TCID_{50} of Mahoney strain, for type 2 against 147 TCID_{50} of MEF.1 strain, and for type 3 against 142 TCID_{50} of Saukett strain. The titres are given as the dilution that produced complete virus neutralization. Geometric mean titres are expressed as the reciprocal of the dilution. Titres with a dilution >1:8192 were taken to be equal to 1:8192 and those with a dilution lower than 1:4 were taken to be 1:4. Serum titres of ≥1:8 were considered to be positive.

Initial virus isolation was performed at the Medical Research Centre, Nairobi. Further isolation and intratypic serodifferentiation of polioviruses as well as typing of other viruses was carried out at RIVM, using published methods (31). The type of immunization was not known to the laboratories involved in the polio antibody titration. Titration of the challenge virus was performed by Smith Kline-RIT in Belgium, and that of the trivalent OPV by the National Institute of Biological Standards and Control in England.

Results

Serum antibody titres

Follow-up rates. Serum antibody results were available from 144 of the children (follow-up rate, 72%). In the OPV group 60 out of 100, in the IPV two-dose group 41 out of 50, and in the IPV three-dose group 43 out of 50 completed the trial, corresponding to follow-up rates of 60%, 82%, and 86%. Apprehension about the frequent bloodtaking, pro-
Fig. 2. Geometric means of the poliovirus neutralizing antibody titres before and after immunization with oral poliovirus vaccine (OPV) or inactivated poliovirus vaccine (IPV), Kenya, 1982–83.

The three groups were comparable in terms of their mean age, sex, and baseline antibody levels.

Response to OPV. The geometric mean titres of polio neutralizing antibodies are shown in Fig. 2 and Tables 1, 2, and 3. At the average age of 2 months most children had antibodies, presumably of maternal origin (Fig. 2, Table 1). The mean baseline antibody level before immunization for all children was 1:18 for type 1, 1:14 for type 2, and 1:8 for type 3 (Table 1). Administration of OPV increased the antibody level after each consecutive dose: the first dose resulting in a 3- to 5-fold increase in titres of neutralizing antibodies against all three types of polioviruses (Fig. 2, Table 1). After three doses of OPV, the proportion of children with antibody titres ≥1:8 was 92% for type 1, 98% for type 2, and 90% for type 3 (Table 4).

Response to IPV. The first dose of IPV appeared not to have an impact on the neutralizing antibody titres when measured 2 months after immunization (Table 1). After the second dose of IPV, the mean

Table 1: Geometric mean titres of neutralizing poliovirus antibodies before and after immunization with one dose of oral poliovirus vaccine (OPV; 60 children) or inactivated poliovirus vaccine (IPV; 84 children), Kenya, 1982–83

<table>
<thead>
<tr>
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</tr>
<tr>
<td>Type 3</td>
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Table 2: Geometric mean titres of neutralizing poliovirus antibodies after immunization with two doses of oral poliovirus vaccine (OPV; 60 children) or inactivated poliovirus vaccine (IPV; 84 children), Kenya, 1982–83

<table>
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<tbody>
<tr>
<td></td>
<td>OPV</td>
</tr>
<tr>
<td>Type 1</td>
<td>111.4</td>
</tr>
<tr>
<td>Type 2</td>
<td>147.0</td>
</tr>
<tr>
<td>Type 3</td>
<td>78.9</td>
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</table>
Table 3: Geometric mean titres of neutralizing poliovirus antibodies after immunization with three doses of oral poliovirus vaccine (OPV; 60 children) or Inactivated poliovirus vaccine (IPV; 43 children), Kenya, 1982–83

<table>
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<th>Virus type</th>
<th>OPV</th>
<th>IPV</th>
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<tr>
<td>Type 1</td>
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<td>274.4</td>
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<tr>
<td>Type 3</td>
<td>114.1</td>
<td>630.3</td>
</tr>
</tbody>
</table>

antibody response (1:224 for type 1 and 1:273 for type 3) was 2- to 3.5-times higher than that after two doses of OPV (Table 2). For type 2 the mean antibody titre was half that observed after two doses of OPV. After the third dose, the mean antibody response to IPV was greater than that to OPV (Fig. 2). The proportion of IPV recipients with titres ≥1:8 after three doses was 100% for types 1 and 2, and 98% for type 3 (Table 4).

The level of antibodies attained after each dose of IPV was related to the presence of maternal antibodies before immunization (Fig. 3). Children with low levels of maternal antibody (titre ≤1:16) generally showed a better response than those with high levels of maternal antibody (titre >1:16) (P<0.05). Children with high levels of maternal antibody showed a decrease in mean antibody titre 2 months after the first dose, and, with the exception of that to type 3, their response following subsequent doses tended to be less than that of children with low levels of maternal antibody (Fig. 3). The same occurred after OPV immunization, but for children with high levels of maternal antibody, the drop in mean antibody titre after the first dose was less pronounced than in the IPV group.

**Virus excretion in stools**

**Isolation of poliovirus.** Poliovirus was isolated from only 36 (3.5%) of the 1018 stool samples examined from the study children. Six of these isolates were wild strains; three were of type 1 strains: one of type 2; and two of type 3 (Table 5). Five wild strains were isolated from the IPV study area and one wild strain from the OPV area. It should be noted that three wild polioviruses were isolated from two children immunized with one dose of IPV and from one child during the challenge period who had been immunized with three doses of IPV. At the time of virus isolation, children had high antibody titres against the isolated type of poliovirus: 2048 (type 1), 1024 (type 2), and 1024 (type 3) (Table 5). In the OPV group, no wild virus was isolated after the first dose of vaccine.

A total of 18 vaccine-related strains were isolated prior to or at the time of administration of a challenge dose: nine of type 1; two of type 2; and seven of type 3. Of these strains, 17 came from children in the OPV group and only one from a child in the IPV study group. A total of 12 vaccine-related strains of type 1 were isolated after administration of a challenge dose. Presumably these were of challenge-virus origin.

**Excretion of challenge virus.** Of 144 study children, only 12 excreted challenge virus. One week after being fed the challenge vaccine virus, it was shed by two (3.3%) of the 60 children in the OPV group and six (7.1%) of the 84 children in the IPV group. Of the latter children, four were in the two-dose IPV group and two in the three-dose IPV group. The difference in excretion rates was not significant (P > 0.05). In the following 3 weeks of observation, only four more isolates of type 1 vaccine virus were made, all from the IPV group. There was no difference in serum antibody level between children who excreted poliovirus and those who did not. No poliovirus was obtained from nasopharyngeal washings.

For a separate group of 24 nonimmunized children aged 2–4 months who were administered a challenge dose, only two (8.3%) excreted challenge virus.

**Excretion of nonpolio enteroviruses.** Of the 1111 stool samples that were examined, 516 (46%) were positive for nonpolio enteroviruses (NPEV) as follows: echoviruses (75%), nontypable enteroviruses (13%), coxsackievirus A (4.7%), and coxsackievirus B (4.5%). The rate of excretion of NPEV increased with age (Fig. 4), and its seasonal variation
Fig. 3. Antibody response of children with high (>1:16) (bold line) or low (≤1:16) (thin line) levels of maternal antibodies to immunization with oral poliovirus vaccine (OPV) or inactivated poliovirus vaccine (IPV), Kenya, 1982–83.
(14–74%) was pronounced, being highest in January–March, a dry period between two rainy seasons. The seasonality could not be differentiated from the effect of age-specific diarrhoea morbidity and possible virus excretion (32).

No relation was found between the occurrence of diarrhoea and virus excretion. In fact, diarrhoea was more frequent among virus-culture-negative children (14.5%) than among those who were virus-culture positive (10%) (P < 0.05). In the OPV group, the presence of nonpolio enterovirus in the stools (65%), the occurrence of diarrhoea (47%), or the presence of both these possibilities (29%) had no effect on the antibody level attained after three doses of OPV (P > 0.1). This was also observed for the IPV group.

**Discussion**

The aim of immunization against poliomyelitis is to provide immunity against the disease as early in life as possible, before the first contact with the wild virus. The passive immunity acquired from the mother apparently provides sufficient protection during the first few months of life, and, at least in Kenya, the occurrence of poliomyelitis before 4 months of age is exceptional. Poliomyelitis immunization has to start well before this age to ensure a continuous increase in active immunity, which can replace the passive immunity as this declines.

In developing countries, a child’s gut is colonized very early in life by microbial flora and it is important that OPV be administered at a time such that possible interference with these flora can be avoided. This was the basis for WHO’s recommendation that a “zero” dose of OPV should be adminis-
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Fig. 5. Geometric mean titres (GMT) of neutralizing poliovirus antibodies in children immunized with oral poliovirus vaccine (OPV) or inactivated poliovirus vaccine (IPV) at 2, 4, and 12 or 18 months of age in the USA, 1984 and 1990. a) See ref. 25; b) see ref. 28.

TABLE 1

<table>
<thead>
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<th>Type</th>
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<th>GMT (dilution)</th>
</tr>
</thead>
<tbody>
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* = OPV
x = IPV

In the present study, three doses of either OPV or enhanced-potency IPV stimulated an immune response in Kenyan infants when the series was started at 8–12 weeks of age. The first dose of OPV stimulated a clear increase in polio antibody, which was modified only slightly by the presence of maternal antibody. In contrast, the response to the first dose of IPV seemed to be strongly dependent on the level of maternal antibody, with a low level allowing a good response (Fig. 3). Similar results have been obtained in the USA (Fig. 5), where two primary doses of OPV and enhanced potency IPV were administered to children at 2 and 4 months of age, with the third dose being given when they were 12–18 months of age (25, 28). Although in general it appears that the first dose of IPV administered at 8–12 weeks of age has little effect on enhancing immunity, for infants with low levels of maternal polio antibodies the vaccine produced an increase in antibody titres for all three types of poliovirus (Fig. 3). This has also been reported by other workers (23).

It appears from our study that children with low levels of maternal antibodies (presumably those most at risk of infection with wild poliovirus) do show an accentuated and rapid antibody response to the first dose of polio vaccine. This was most pronounced for the OPV group, among whom the response after the first dose was higher for those with lower levels of passive antibody than for children with higher levels of passive antibody (Fig. 3). This indicates that OPV is most suitable when a rapid increase in immunity is indicated.

After the second dose, the mean antibody response to type 1 and type 3, but not to type 2 poliovirus, was greater for IPV than OPV, while the pro-
portion of infants with sufficient antibody (titre \( \geq 1:8 \)) was greater for the IPV group than for the OPV group, for all three virus types. A similar result has been reported by McBean et al. in the USA (25). In a study in Brazil, the antibody response was greater for IPV than for OPV for all three types of poliovirus (24), while in the USA the antibody response was greater with OPV for types 1 and 2 and greater with IPV for type 3 (28).

In the present study the third dose of OPV led to a slight increase in geometric mean poliovirus antibody titres for types 1 and 3, while there was no increase for type 2. However, the third dose did increase the proportion of children with detectable antibodies for all three types and the proportion of those with high levels of antibody (Table 4).

Breast-feeding, diarrhoea, or the presence of nonpolio enterovirus in the gut at the time of immunization did not prevent the attainment of significant levels of poliovirus antibody. If it is assumed that the presence of serum neutralizing antibodies at a titre of \( \geq 1:8 \) indicates protection against poliomyelitis, three doses of OPV or two doses of IPV provide a similar level of humoral immunity in children.

A second objective of this study was to assess gut immunity. This was tested by feeding a moderate dose (3000–7000 TCID\(_{50}\)) of a type 1 vaccine strain and isolating the challenge virus from the stools of immunized children. An early study performed in England showed that a small infective dose of 50–500 TCID\(_{50}\) of poliovirus type 1 was able to infect about half the children vaccinated with IPV (containing 75 D-antigen units of type 1 poliovirus), while a higher infective dose (50 000–500 000 TCID\(_{50}\)) was needed to initiate infection in children immunized with OPV (38). A recent study conducted in the USA showed that after challenge with a low dose of type 1 vaccine virus (500–800 TCID\(_{50}\)) only 18% of children previously immunized with three doses of OPV excreted the challenge virus, whereas 46% of children previously immunized with three doses of enhanced-potency IPV (containing 40 D-antigen units of type 1 poliovirus) excreted the challenge virus. A 10-fold increase in the challenge dose to about 600 000 TCID\(_{50}\) increased the proportion of children who excreted the challenge virus to 31% in the OPV group and 82% in the IPV group (29).

In our study only six wild polioviruses were isolated, five of them from the IPV study area. Two strains were isolated from children who had been immunized once with IPV, and one strain from a child immunized three times with IPV. It seems that the presence of high poliovirus antibody levels in all these children did not prevent the infection with and replication of the wild poliovirus. Since no wild virus was isolated from children after immunization with OPV, it seems that even one dose of OPV can prevent the circulation of wild poliovirus.

Of 18 vaccine-related strains of poliovirus, 17 were isolated from the OPV study area. This suggests that OPV virus contamination in the IPV study area was minimal.

A small dose of the challenge virus was unable to differentiate between the two vaccines in terms of their ability to induce intestinal immunity. This was probably due to the high carrier rate of nonpolio enteroviruses in the study population, which increased with age. However, the challenge virus tended to survive better in the IPV than in the OPV group; in the challenge period, 10 type 1 vaccine-related viruses were isolated from children immunized with IPV and only two strains from children immunized with OPV. These numbers are too small to be significant. A high dose of challenge virus (about 1 million TCID\(_{50}\)) should be used in future studies of intestinal immunity in developing countries.

Data on the clinical efficacy of IPV have been reported from Senegal, where an epidemic of type 1 poliomyelitis occurred in 1986. The clinical efficacy of one dose of enhanced-potency IPV in preventing paralytic poliomyelitis was 36% (95% confidence interval (CI), 0–67%). For two doses the point estimate of efficacy was 89% (95% CI, 62–97%) (26).

Immunization of 64% of Gambian children with three or more doses of OPV did not prevent an outbreak of poliomyelitis in Gambia in 1986. Among 1–2-year-olds the estimated efficacy of three or more doses of OPV was 81% (95% CI, 66–90%) and among 1–7-year-olds, 72% (95% CI, 57–82%) (20).

Recent outbreaks of poliomyelitis indicate that there are lessons still to be learned about intestinal immunity and poliovirus vaccines. Despite immunization coverage of more than 90%, an outbreak of type 1 poliovirus occurred in Israel in 1988 (39). Fifteen cases caused by type 1 poliovirus were reported mainly in young adults who had been immunized a considerable time previously with OPV in a subdistrict, where since 1982 the immunization schedule for infants has consisted of three doses of enhanced potency IPV. A major factor in the outbreak appeared to be the spread of wild poliovirus to susceptible persons via the unprotected intestinal tracts of IPV-immunized children. In contrast, OPV or a combination of OPV and IPV did seem to provide a barrier to the spread of wild virus in this outbreak.

As the momentum to eradicate poliomyelitis grows, attention is being focused on the successes in eradicating the disease in the Americas, a task which is being accomplished using OPV exclusively (7,8). The eradication of poliomyelitis in the Americas will be an essential first step towards achieving global
eradication of the disease by the year 2000—the goal adopted by the Forty-first World Health Assembly in 1988.

Currently, WHO recommends OPV as the vaccine of choice for poliomyelitis eradication: when the interruption of the transmission of the wild virus is being attempted, trivalent OPV must be intensively applied (40). Further studies are, however, needed on the use of the combination of IPV and OPV to take optimum advantage of the properties of both vaccines.

Acknowledgements

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Résumé

Evaluation sérologique et virologique des vaccins antipoliomyélitiques buccaux et inactivés dans une population rurale du Kenya

On a effectué entre 1982 et 1983 une étude visant à comparer l'immunité humorale et intestinale conférée par trois doses de vaccin antipoliomyélitique buccal (VPO; activité: 10^6 (type 1), 10^5 (type 2) et 10^{5.5} (type 3) DITC_{50} par dose), ou par deux ou trois doses de vaccin antipoliomyélitique inactivé à activité renforcée (VPI; activité: 40, 8, et 32 unités antigéniques D des types 1, 2 et 3, respectivement, par dose) dans deux régions rurales du district de Machakos, au Kenya. La vaccination a été commencée à l'âge de 8 à 12 semaines et l'intervalle entre deux doses était de 2 mois.

Chez les enfants présentant de faibles concentrations d'anticorps maternels (ceux à haut risque), la première dose de VPO ou de VPI a suscité une réponse en anticorps, et cette réponse a été en moyenne plus importante avec le VPO. Les enfants ayant de fortes concentrations d'anticorps maternels ont répondu à la première dose de VPO, mais pas à celle de VPI. Les doses suivantes des deux vaccins ont entraîné des augmentations des titres moyens d'anticorps. Après trois doses de VPO, la proportion d'enfants présentant des titres d'anticorps ≥ à 1:8 était de 94% pour le type 1, 88% pour le type 2 et 97% pour le type 3; après trois doses de VPI, 100% des enfants présentaient des titres d'anticorps ≥ 1:8 contre les types 1 et 3, et 98% contre le type 2. La troisième dose de VPI a permis d'obtenir un titre moyen géométrique d'anticorps significativement plus élevé pour les trois types, que celui engendré par les trois doses de VPO.

Les résultats de cette étude indiquent que l'immunité sérologique individuelle a été bonne avec les deux vaccins et n'a pas été modifiée par l'allaitement au sein, la diarrhée ou l'excrétion d'entérovirus non poliomyélitiques.

On a testé l'immunité intestinale en faisant ingérer aux enfants une dose d'épreuve de VPO monovalent de type 1. Au 7ème jour, il y avait plus d'enfants vaccinés par le VPI qui excrètaient le virus d'épreuve que d'enfants vaccinés par le VPO, et seuls les enfants vaccinés par le VPI ont continué à excrêter ce virus plus de 7 jours après son ingestion. Toutefois, la dose d'épreuve (3000 à 7000 DITC_{50}) était trop faible pour qu'on puisse déceler une différence significative entre les deux vaccins. L'évaluation de cette immunité intestinale a été encore compliquée par les taux élevés d'infections par des entérovirus non poliomyélitiques, qui ont été isolés dans 46% des 1111 échantillons de selles examinés. Ces infections par des entérovirus non poliomyélitiques étaient saisonnières, le pic d'excrétion (74%) survenant au cours de la saison sèche (janvier à mars). Il n'a donc pas été possible d'évaluer l'immunité intestinale conférée par ces deux vaccins.

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