Lack of efficacy of the standard potency Edmonston–Zagreb live, attenuated measles vaccine in African infants

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The efficacy of standard potency Edmonston–Zagreb (E–Z) measles vaccine was tested in a randomized trial of Black infants in a rural area of South Africa where a measles epidemic was occurring. The following immunization schedules were used: 48 infants aged 4–8.5 months who received 3.9 log50 infectious units of E–Z vaccine (group A); 48 infants aged 4–8.5 months who received 3.28 log50 infectious units of Schwarz vaccine (group B); and 28 infants aged >9 months who received 3.28 log50 infectious units of Schwarz vaccine and served as controls (group C).

For infants aged less than 23 weeks who were given either the E–Z or Schwarz vaccine, the number of seropositives was low (28%), irrespective of the pre-vaccination level of measles antibody. There was a higher number of seropositives (68%) among those in the age range >23 weeks to <36 weeks who received the E–Z vaccine rather than the Schwarz vaccine (36%). When administered to children aged >36 weeks, the Schwarz vaccine produced a satisfactory, though suboptimal response rate (61%). There was no correlation between seropositivity and pre-vaccination measles antibody status.

Use of the standard dose of E–Z vaccine may have been one of the factors for this poor response, and this supports the WHO recommendation that titres higher than the standard potency vaccine are needed if 6-month-old infants are to be successfully immunized against measles.

Introduction

Measles remains one of the most severe and important infectious diseases of children who live under poor socioeconomic conditions (1).

Until recently, the WHO Expanded Programme on Immunization (EPI) recommended that children in developing countries be immunized against measles as soon as possible after 9 months of age. For children of this age seroconversion rates of 75–98% are achieved with the Schwarz vaccine.8 These rates are significantly lower if the conventional Schwarz vaccine is used to immunize children aged <9 months, because of the presence of circulating maternal antibodies (2). In the southeastern border region of South Africa, 20–45% of cases of measles occur in infants aged less than 8 months (3); effective methods of immunizing the very young against measles in high-density regions of developing countries need to be found.

Attempts have been made to solve this problem by immunizing infants aged 4–8 months. Several studies that administered Schwarz vaccine to young infants consistently reported poor clinical results (4,5). However, vaccination at such an early age with a measles vaccine grown in human diploid cells (Edmonston–Zagreb (E–Z) vaccine) is highly effective (6–12). High titre E–Z vaccine has been reported to provide superior seroconversion than the Schwarz vaccine in 4–7-month-olds, notwithstanding the presence of maternal antibodies. The differences between South Africa and the developing countries where these trials were conducted (Mexico (4, 7, 8), the Gambia (10, 12), and

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Guinea–Bissau (13, 14)) are, however, considerable. We therefore carried out a study with the E–Z vaccine in a southern African setting to determine the serological responses and side-effects and to measure the clinical protection afforded to children given the E–Z vaccine (dose, 3.9 log 50 infectious units) or the conventional Schwarz vaccine (dose, 3.28 log 50 infectious units) at 16–34 weeks of age. A group of children who received the Schwarz vaccine (3.28 log 50 infectious units) at >9 months of age served as the controls. The study was undertaken during a measles epidemic in order to enhance the possibility of measuring the protection given, despite a small sample size.

Subjects and methods

The study was approved by the Ethics Committee of the Faculty of Medicine, University of Natal. Informed consent was obtained from the parent(s) or guardian of each child after explanation of the object of the trial and the number of blood specimens required.

Location

The study was carried out in Qwa-Qwa, an impoverished rural area of South Africa, with little industry and no agriculture (area, 480 km²; estimated population, 385 145 in 1988, 2.9% of whom were aged <1 year; average number of persons per household, 7).

Study design

The study began on 1 February 1988 with the random immunization (using a table of random numbers) of 96 children aged 4–8.5 months: 48 received the E–Z vaccine (3.9 log 50 infectious units, group A) and 48 the Schwarz vaccine (3.28 log 50 infectious units, group B); 28 children aged >9 months who received the Schwarz vaccine (3.28 log 50 infectious units, group C) (Table 1) served as controls.

Blood samples were obtained from the children by venepuncture before vaccination, and at 6–8 weeks, 5–9 months, and 10–14 months post-vaccination. The mother or guardian was instructed to bring the child to hospital if any adverse effects such as fever, rash, cough, conjunctivitis, coryza, diarrhoea, vomiting, ear or skin infections, or neurological complications occurred within 2 weeks of immunization. The children were visited monthly by a healthcare worker, who also provided parents with health education information, especially advice on nutrition, and, if necessary, were subsequently seen by one of the authors, who prescribed treatment.

Vaccines used

The vaccines outlined below were used in the study.

- E–Z live, attenuated vaccine (lot No. 84/2; date of manufacture, December 1986; expiry date, December 1988; titre prior to vaccination (manufacturer’s data), >3.0 log 50 infectious units, and from field samples at the end of the study, 3.9 log 50) grown in human diploid cells. The vaccine was reconstituted in distilled water and a 0.5-ml dose was given subcutaneously.
- Schwarz live, attenuated measles vaccine (lot No. M115N41A; date of manufacture, November 1986; expiry date, November 1988; titre prior to study (manufacturer’s data) >3.0 log 50 infectious units, and from field samples after vaccination 3.28 log 50 grown in chick embryo fibroblasts. This vaccine was also given subcutaneously as a 0.5-ml dose.

Serology

Measles IgG antibody was detected using an enzyme-linked immunosorbent assay (ELISA) kit.

Table 1: Age range of the study children at vaccination

<table>
<thead>
<tr>
<th>Vaccination group</th>
<th>&gt;16 to 23</th>
<th>&gt;23 to 28</th>
<th>&gt;28 to 36</th>
<th>&gt;36 to 40</th>
<th>&gt;40 to 44</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>A*</td>
<td>26</td>
<td>14</td>
<td>8</td>
<td>—</td>
<td>—</td>
<td>48</td>
</tr>
<tr>
<td>B*</td>
<td>20</td>
<td>17</td>
<td>11</td>
<td>—</td>
<td>—</td>
<td>48</td>
</tr>
<tr>
<td>C*</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>27</td>
<td>1</td>
<td>28</td>
</tr>
</tbody>
</table>


\[3.28 \log_{50} \]
ELISA plates coated with measles antigen were supplied from the same batch (OSOK 02103, lot No. 406446A), as was the anti-human IgG-alkaline phosphatase conjugate (OSDH 04/05, lot No. 410955A) and supplementary agents (lot No. 19604). Results are expressed as mIU/ml, with respect to WHO standard anti-measles serum. An absorbance of <0.2 (equivalent to <34 mIU/ml) was considered negative. The pre- and post-vaccination sera of each child and the respective mother's serum were assayed in duplicate and always included in the same tests. The same number of samples from each vaccine group was assayed simultaneously to avoid any bias. As controls, international and local standards (two of which were kindly donated by Dr R. Glass, Centers for Disease Control) were always included in each batch. Seroreponse was defined according to the persistence of antibody (12), while seropositivity was defined as >200 mIU/ml at any time post-vaccination (Dr N. Halsey, personal communication, 1989).

Antibody levels are reported as follows:

- % of seropositives at 6–8 weeks and at 40–56 weeks post-vaccination;
- % of seropositives at 6–56 weeks post-vaccination, according to age at vaccination; and
- % of seropositives at 6–56 weeks post-vaccination, according to both pre-vaccination (i.e., maternal) antibody status and the age at vaccination.

**Statistical methods**

The relationship between the level of measles IgG in mIU/ml and the absorbances was determined using a polynomial regression. The relationship between \(\sqrt{\text{mIU/ml}}\) and the absorbance readings was described very well by the equation:

\[
\sqrt{\text{mIU/ml}} = 3.0031 + 12.794 \text{ (absorbance)} + 5.791 \text{ (absorbance)}^2.
\]

The adjusted coefficient of determination was \(R^2 = 0.97\), i.e., 97% of the variation in \(\sqrt{\text{mIU/ml}}\) is accounted for by the variation in absorbance. The fit for the above equation was limited to the observed range of absorbance values (0–1.5).

The mIU/ml value for a single specimen was calculated by squaring the result obtained after substitution of the appropriate absorbance value into the above equation (control runs were undertaken with each run). Results are reported to the nearest integer.

**Results**

**Rate of follow-up**

Most children returned for the 6–8 weeks post-vaccination examination (41/48 for group A; 46/48 for group B; and 25/28 for group C). This decreased to 23/28, 30/48, and 23/28 for groups A, B, and C, respectively, for the examination at 5–9 months post-vaccination, and to 21/48, 21/48, and 18/28, respectively, for the third examination at 10–14 months post-vaccination. At least two follow-up samples of blood were obtained from no less than 90% of the sample population.

**Health monitoring**

A total of 93/124 children (33/48 in each of groups A and B, and 27/28 in group C) were monitored monthly by the health care worker.

**Development of measles.** During 26 months post-vaccination follow-up, one of the children in group A who was seronegative developed measles 19 months after vaccination. Monitoring of the study children is continuing. Children who were persistently seronegative were revaccinated with the Schwarz vaccine after samples of their blood had been analysed, i.e., 16 months after the initial vaccination.

**Deaths.** There were two deaths in group A: a 6-month-old male who died of unknown causes (pre-immunization antibody level, 224 mIU/ml); and a male who died of pneumonia aged 15 months (pre-immunization antibody level, 25 mIU/ml). The latter child had persistent measles antibody. A 6-month-old male who had diarrhoea died in group B (pre-immunization antibody level 55 mIU/ml). There were no deaths in group C. None of the deaths was associated with measles.

**Seropositivity**

**Seroresponsiveness 6 weeks to 14 months post-vaccination.** The proportion of seropositives 6–8 weeks and 10–14 months post-vaccination are shown in Fig. 1 for groups A, B, and C. In group A two of the five children who underwent late seroresponsiveness had blood taken for antibody determination at 6–8 weeks post-vaccination. Similarly, two of three late seroresponders in both groups B and C had blood taken at the 6–8-week follow-up visit. All but three
Seropositivity rate was poor (28%), irrespective of their pre-vaccination antibody titre.

- For low pre-immunization antibody levels (<40 mIU/ml), children aged >23 weeks to <36 weeks who received the E–Z exhibited better seropositivity than those who received the Schwarz vaccine (60% versus 29%). This advantage of the E–Z vaccine was maintained even if the pre-vaccination antibody levels were 40–90 mIU/ml.
- Although measles antibody levels were high (112–2235 mIU/ml) only two were <200 mIU/ml in all the samples of maternal blood (n = 104) taken when the infants were vaccinated, seropositivity did not correlate with pre-vaccination antibody status.

Maintenance of seropositivity 5–14 months post-vaccination

The length of time that an antibody level ≥200 mIU/ml was maintained was determined for those children from whom at least three samples of blood were taken.

A reduction in measles antibody levels to below the protective threshold was detected 5–11 months after vaccination in six of 12 children from group A, in five of 11 children from group B, and in four of 16 children from group C.

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Table 2: Seroconversion among the study children 6–56 weeks after vaccination, according to pre-vaccination antibody status and age at vaccination

<table>
<thead>
<tr>
<th>Pre-vaccination measles antibody status (mIU/ml)</th>
<th>Proportion who seroconverted, by age at vaccination (weeks)</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>&gt;16 to 23</td>
<td>&gt;23 to 28</td>
</tr>
<tr>
<td>&lt;40</td>
<td></td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>1/8 (13)^a</td>
<td>3/4 (75)</td>
</tr>
<tr>
<td>B</td>
<td>0/2 (0)</td>
<td>2/7 (29)</td>
</tr>
<tr>
<td>C</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&gt;40 to 90</td>
<td></td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>2/3 (67)</td>
<td>4/5 (80)</td>
</tr>
<tr>
<td>B</td>
<td>1/2 (50)</td>
<td>1/4 (25)</td>
</tr>
<tr>
<td>C</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&gt;90 to 199</td>
<td></td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>1/5 (20)</td>
<td>2/4 (50)</td>
</tr>
<tr>
<td>B</td>
<td>3/8 (38)</td>
<td>1/3 (33)</td>
</tr>
<tr>
<td>C</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&gt;200</td>
<td></td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>4/10 (40)</td>
<td>1/1 (100)</td>
</tr>
<tr>
<td>B</td>
<td>1/8 (13)</td>
<td>3/3 (100)</td>
</tr>
<tr>
<td>C</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*a A = Edmonston–Zagreb vaccine; B = Schwarz vaccine given to infants ≤36 weeks of age; and C = Schwarz vaccine given to infants >36 weeks of age.

*b Shown are the number of seropositives/total number in each category.

*c Figures in parentheses are percentages.

*d A versus B ≤36 weeks, P ≤ 0.05, \( \chi^2 = 4000 \).

Discussion

Our results indicate that the E–Z vaccine may be superior to the Schwarz vaccine for infants under 9 months of age, but that at a titre of 3.9log10 50 infectious units it does not produce a satisfactory seroresponse. We also investigated the effects on seroresponse of pre-vaccination antibody levels and age at vaccination.

Age remained a major barrier to effective immunization: for infants aged <23 weeks who received either vaccine, the seropositivity rate was poor (28%), irrespective of pre-vaccination antibody level. This was disappointing, since other studies of the E–Z vaccine have reported conversion rates of 84–100%, and protection against measles for infants in this age range. A better rate of seropositivity (68%), although unsatisfactory for effective control of measles, was achieved for infants in the age range >23 weeks to <36 weeks. Results from other studies suggest that both these deficiencies with the E–Z vaccine could be overcome by using a higher titre formulation.

The seropositivity rate produced by the Schwarz vaccine in infants aged ≥36 weeks was less than optimum (61%), and for children below this age the rate was too low to offer protection against measles.

Although some children showed an inadequate antibody response, only one developed measles over the 26-months follow-up period. Since the attack rate of measles among susceptibles is almost 100%, the single case possibly arose because of an increased herd immunity effect (15); our findings show that fewer measles cases were reported in Qwa-Qwa during 1988 and 1989, although in the latter year this was due to underreporting. It is also likely that, in addition to antibody, cellular factors are necessary for immunity against measles. Antibodies may have been detectable by the plaque neutralization test. For example, Black et al. have shown that many children who had low levels of antibody one year after having received measles vaccine were able to mount a secondary response to revaccination (15). In view of the regular monitoring and reliable recognition of measles by the local population, it is unlikely that any cases were missed during follow-up.

In some children a delayed measles antibody response after vaccination can arise because of their young age, persistence of maternal antibody, or malnutrition (4, 17). We detected such a delay in 11 children for whom none of these factors were directly implicated.

Several explanations have been proposed for the greater seroconversion rates after vaccination of infants aged 7–11 months in developing countries compared with those in industrialized countries (18). The contribution of prematurity can be discounted since such children were excluded from the study. Furthermore, children in developing countries experience immuno-acceleration caused by frequent and occasionally serious infections at an extremely young age (19).
Black et al. have documented that in most developing countries women have similar antibody levels to those of women in the USA (20). All the mothers in our study were from a low-income group and had high levels of measles antibody. Despite this, the number of seropositives had no bearing on the pre-vaccination antibody status. However, our results cannot strictly be compared with those of Black et al. since we used a different test to measure the antibody level and reported the results in mIU/ml and not as titres.

Nutritional status and subsequent illnesses did not influence the seropositivity rates, since in all three study groups equal numbers of well-nourished and undernourished children responded (data not included). This is in accord with observations made by other workers (4, 18).

Inadequate immunity to measles has been demonstrated in children vaccinated at an early age (16). Our data tend to support this finding, since fewer children who were vaccinated with the Schwarz vaccine when they were less than 36 weeks of age maintained their serological status.

The E–Z vaccine is as safe as the Schwarz vaccine. Furthermore, an equal number of children in groups A and B who were seropositive still had protective levels of antibody 5–14 months post-vaccination. Both the E–Z vaccine at standard potency and the Schwarz vaccine appeared to offer clinical protection against measles during the follow-up period. Our study confirms the results reported by Khanum et al. (5), and this endorses the recent WHO recommendation that titres greater than standard potency vaccine are needed if 6-month-olds are to be successfully immunized against measles (21).

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Résumé

Vaccin antirougeoleux vivant atténué Edmonston–Zagreb: manque d’efficacité de la dose standard chez les nourrissons africains

On a testé l’efficacité de la dose standard du vaccin antirougeoleux Edmonston–Zagreb (E–Z) au cours d’un essai randomisé mené chez des nourrissons noirs d’une région rurale d’Afrique du Sud à l’occasion d’une épidémie de rougeole. Le calendrier vaccinal suivant a été appliqué: 48 nourrissons de 4 à 8,5 mois ont reçu 3,9 log 50 unités infectieuses de vaccin E–Z (groupe A); 48 nourrissons de 4 à 8,5 mois ont reçu 3,28 log 50 unités infectieuses de vaccin Schwarz (groupe B); et 28 nourrissons de plus de 9 mois ont reçu 3,28 log 50 unités infectieuses de vaccin Schwarz (groupe C) et ont servi de témoins.

L’efficacité du vaccin a été mesurée par le degré de protection contre la rougeole et par les réponses en anticorps sériques, déterminées par titrage immunoenzymatique (ELISA). On a évalué les effets de l’âge au moment de la vaccination et de la présence d’anticorps avant celle-ci. Un contrôle a ensuite été effectué dans 90% des cas sur au moins deux échantillons de sang prélevés entre 6 et 56 semaines après la vaccination.

Aucun des nourrissons ayant reçu le vaccin Schwarz n’a eu la rougeole; en revanche, un des nourrissons vacciné avec le vaccin E–Z l’a contractée. Deux nourrissons du groupe A et un nourrisson d’un groupe Schwarz sont décédés. Toutefois, ces décès étaient sans rapport avec la rougeole.

Chez les nourrissons de moins de 23 semaines ayant reçu soit le vaccin E–Z, soit le vaccin Schwarz, le nombre des séroconversions est faible (28%), quel que soit le taux d’anticorps antirougeoleux avant vaccination. On a observé une séroconversion plus importante (68%) avec le vaccin E–Z qu’avec le vaccin Schwarz (36%) chez les nourrissons ayant entre 23 et 36 semaines. Administré aux enfants de plus de 36 semaines, le vaccin Schwarz a donné un taux de réponse satisfaisant bien que sous-optimal (61%). Aucune corrélation n’a pu être établie entre la séroconversion et la présence d’anticorps avant vaccination, dans aucun groupe.

La dose standard de vaccin E–Z est peut-être à l’origine de cette faible réponse, ce qui corroborerait la recommandation de l’OMS préconisant l’emploi de doses plus fortes pour vacciner correctement les enfants de 6 mois.

References

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