Measles Virus Antibody Responses in Children Randomly Assigned to Receive Standard-Titer Edmonston-Zagreb Measles Vaccine at 4.5 and 9 Months of Age, 9 Months of Age, or 9 and 18 Months of Age

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The World Health Organization recommends administration of measles vaccine (MV) at age 9 months in low-income countries. We tested the measles virus antibody response at 4.5, 9, 18, and 24 months of age for children randomly assigned to receive standard-titer Edmonston-Zagreb MV at 4.5 and 9 months, at 9 months, or at 9 and 18 months of age. At 4.5 months of age, 75% had nonprotective measles virus antibody levels. Following receipt of MV at 4.5 months of age, 77% (316/408) had protective antibody levels at 9 months of age; after a second dose at 9 months of age, 97% (326/337) had protective levels at 24 months of age. In addition, the response at both 9 and 24 months of age was inversely correlated with the antibody level at receipt of the first dose of MV, and the second dose of MV, received at 9 months of age, provided a significant boost in antibody level to children who had low antibody levels. In the group of 318 children who received MV at 9 months of age, with or without a second dose at 18 months of age, 99% (314) had protective levels at 24 months of age. The geometric mean titer at 24 months of age was significantly lower in the group that received MV at 4.5 and 9 months of age than in the group that received MV at 9 months of age (P = .0001). In conclusion, an early 2-dose MV schedule was associated with protective measles virus antibody levels at 24 months of age in nearly all children.

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Keywords. antibody response; early measles vaccination; Edmonston-Zagreb measles vaccine; non-specific effects of vaccines; two-dose measles vaccination.

Following withdrawal of the high-titer measles vaccine (MV) [1], it was suggested to study early 2-dose schedules to lower the age of MV [2, 3]. From 1995 to 2002, we conducted an early 2-dose MV trial in Guinea-Bissau [4, 5]; the children were randomly assigned to receive MV at 6 and 9 months of age or inactivated polio vaccine (IPV) at 6 months of age and MV at 9 months of age. The early 2-dose schedule increased coverage considerably and provided better protection against measles virus infection [5]. Edmonston-Zagreb MV (EZ) and Schwarz MV (SW) were used in 2 different periods, and EZ seemed to boost a secondary immune response better than SW [5]. Previous trials have shown that standard-titer EZ provided good seroconversion at 4–6 months of age [6–8]. We therefore focused on EZ for early measles vaccination.

Following elimination of measles from the Americas, the age of vaccination was raised to 12 months because the antibody response is better at 12 months [9, 10]. A similar policy is recommended in other low-income countries once measles is under control. To implement that policy in Africa could potentially be disastrous.
First, maternal antibody levels have declined following the introduction of MV 25 years ago. Many children are now susceptible already at 2–4 months of age; in a recent epidemic in an urban area with high coverage, the incidence was 19% before 9 months of age in the cohort born just before the epidemic [11]. Increasing the age to 12 months would leave more infants exposed to measles virus infection in situations in which measles is not fully controlled. Second, later vaccination would reduce the period during which the children benefitted from the nonspecific effects of MV [12–15]. During a war period with high mortality, children randomly assigned to receive early MV had significantly lower mortality [13]. Hence, it is justified to examine earlier measles vaccination.

We have recently examined an early 2-dose strategy with standard EZ at 4.5 and 9 months of age; it prevented measles before 9 months and reduced all-cause mortality between 4.5 and 36 months of age [11, 15]. Control children randomly assigned to receive EZ or SW at 9 months of age had 99% protective antibody levels at 24 months of age [16]. In the present article, we examine measles antibodies levels following standard EZ at 4.5 and 9 months of age and compare them to those of children who received 1 dose of EZ at 9 months of age or 2 doses of EZ at 9 and 18 months of age.

METHODS

Subjects and Methods
The study was conducted in the Bandim Health Project (BHP) study area with approximately 102 000 inhabitants. BHP has implemented a routine data collection system; all residents have an identification number, and information on socioeconomic and demographic information is available. All houses are visited every month to register new pregnancies and births. Children are visited every 3 months until 3 years of age; at these visits we collect information on breast-feeding, infections, hospitalizations, vaccination status, and other factors associated with child survival or mortality. There are 3 health centers in the area. The people of Bissau travel frequently to visit relatives living in other parts of the country.

Study Design
The present trial compared different MV strategies, using 2 strains of measles virus and different ages of vaccination. All children were randomly assigned at 4.5 months of age to 1 of 3 groups: group I received EZ at 4.5 and 9 months of age, group II received no vaccine at 4.5 months of age and SW at 9 months of age, and group III received no vaccine at 4.5 months of age and EZ at 9 months of age. At 18 months of age, children in groups II and III were invited back to the health center and after additional maternal consent randomized to a second dose of MV or no booster dose (Figure 1). The primary outcomes were survival at 3 years of age, measles virus antibody levels at 24 months of age, and clinical protection against measles. To detect a 25% difference in mortality between any of the groups we needed to enroll 5755 children and follow them to 3 years of age [15]. The present study compares the antibody response after early EZ at 4.5 and 9 months of age with the standard EZ at 9 months of age (ie, group I and group III; Figure 1). Assuming 85% seroconversion we needed 400 children to detect a 10% difference in the proportion of nonseroconverters in the long-term follow-up at 24 months of age; because of expected loss to follow-up, we recruited around 450 in each group.

On the basis of a community survey in the same community [17], the expected human immunodeficiency virus type 1 (HIV-1) prevalence among mothers was 4%–5%. Children in the trial were not tested for HIV infection. Normally, all children are breast-fed at 4 months of age in Guinea-Bissau, but during the trial period the nongovernmental organization responsible for prevention of maternal HIV transmission recommended that HIV-infected mothers not breast-feed their children. At enrollment, 4% were not breast-fed [15].

Enrollment Procedures

Eligible Children
Newborn infants were identified in the BHP registration system. To ensure that children had received 3 doses diphtheria-tetanus-pertussis vaccine (DTP) 4 weeks before inclusion, we contacted mothers of children aged 6, 10, and 14 weeks and reminded them to go to a health center to receive DTP and oral poliovirus vaccine. Enrollment took place at 4.5 months of age. During morning hours, a field-worker contacted mothers/guardians of eligible children. The field-worker explained the study, filled in a questionnaire on background factors, and obtained verbal consent. The mother/guardian was asked to bring the child to the health center in the afternoon.

Enrollment, Informed Consent, and Randomization
In the afternoon, the mothers/guardians received an oral and a written explanation from a physician. The physician performed a medical examination. The children received treatment according to local standards. Children who were sick could only participate when they had recovered. If mothers consented, they selected an envelope, which specified the randomization group. Clinical examination and treatment was independent of randomization group. At 9 months of age, all children were invited back to the health center; those in group I received a second dose of EZ, and those in group III received standard EZ.

Measles Vaccine
Standard-titer EZ from the Serum Institute of India (Pune, India) was used (batch 2360). According to the manufacturer, the dose was at least 1000 median tissue-culture infective doses, and, when assayed at the MRC Laboratories, batches ranged between 3000 and 6000 plaque-forming units per dose.

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Blood Sampling and Antibody Analysis

We collected blood samples to assess measles virus antibody levels at 4.5, 9, and 24 months of age in group I and at 9, 18, and 24 months of age in group III (Figure 1). The mothers were sampled when their child was sampled the first time to ensure that samples were collected in the same season. The samples at 4.5 months of age in group I and at 9 months of age in group III were obtained before vaccination, to reflect maternal antibody levels. The samples obtained at 9 months of age, in group I, and at 18 months, in group III, were collected before a possible second dose of MV and were therefore used to assess antibody levels after one dose of MV. The sample at 24 months

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Figure 1. Trial profile for collection of blood samples and reasons for loss to follow-up. Abbreviation: MV, measles vaccine.
of age was used to assess long-term maintenance of antibody levels and to examine the possible effect of a booster dose of standard MV. The collection of the first samples was implemented between March and December 2004. If consent was received, we collected blood samples from all trial participants enrolled in this period.

The samples were analyzed by the measles hemagglutination inhibition test (HAI) at MRC Laboratories in Gambia [18, 19]. The HAI test had a sensitivity of 15.6 mIU/mL, and, with a starting dilution of 1:2, the minimum detectable titer was 31.2 mIU/mL. We have previously shown that the protective level is 125 mIU/mL (ie, a positive reading in a 1:8 dilution) [20]. Children with 31–63 mIU/mL of measles virus antibody were considered to have low (but detectable), nonprotective levels.

Statistical Analysis
The statistical analysis was performed with Stata, version 10.0, statistical software. All antibody data were log-transformed. Undetectable antibody levels were counted as 0 on the log-scale. Measles titers are presented as geometric mean titers (GMTs) and 95% confidence intervals (CIs). The subjects reporting measles virus infection before receipt of the first dose of MV were excluded from the analysis (Table 1). The GMT was compared for different vaccination groups, using the nonparametric Kruskal–Wallis test. Additionally, comparisons were conducted by Tobit regression, which is a censored normal regression method [21]. Undetectable levels are not assigned a given value but are instead regarded as censored with the true level being below the detection limit. This did not change the results (data not shown).

RESULTS
The number of samples collected at different ages and reasons for loss to follow-up are indicated in Figure 1. Background variables were compared between group I and group III for children whose antibody samples were analyzed (Table 1); length and arm circumference were significantly shorter in group I; otherwise, there were no differences between the 2 groups.

Measles Virus Antibody Levels in Mothers and Maternal Measles Antibody Levels in Children
Among mothers, 4% (33/846) had a nonprotective titer (<125 mIU/mL); the GMT was 1042 mIU/mL (95% CI, 946–1157).

Among the children tested at 4.5 months of age, 75% had nonprotective levels (72% of boys [165/228] and 78% of girls [162/207]; risk ratio [RR; boys/girls], 0.92 [95% CI, 0.83–1.03]; P = .155). The prevaccination GMT was 43 mIU/mL in the early vaccination group; for 292 children aged 4 months, the GMT was 45 mIU/mL; for 127 children aged 5 months, the GMT was 41 mIU/mL; and for 16 children aged 6 months, the GMT was 23 mIU/mL. In a logistic regression analysis that controlled for maternal antibody level, the antibody level declined significantly with age in days [22]. Antibody levels were significantly lower in the rainy season (GMT, 38 mIU/mL) than in the dry season (GMT, 49 mIU/mL; P = .024; data not shown). The age at enrollment was the same in the dry and the rainy seasons.

At 9 months of age, 92% (382/415) of the unvaccinated children in group III had nonprotective antibody levels. A similar proportion was unprotected among boys (94% [198/211]) and girls (90% [184/204]), with a RR (boys/girls) of 1.04 (95% CI, 0.98–1.10). The GMT before the vaccination was 23 mIU/mL. The GMT did not differ by season (data not shown).

Measles Virus Antibody Levels at 9 Months of Age After the First Dose of EZ at 4.5 Months of Age Versus Prevaccination Levels at 9 Months of Age (Control Group)
Of the 435 children in group I (Figure 1), 409 had follow-up samples collected at 9 months of age; 1 sample had insufficient amount of blood for analysis (Figure 1). In group III, 415 samples were included in the analysis. In group I, 23% (92/408) had

Table 1. Background Factors of Children With Early Receipt of Edmonston-Zagreb Measles Vaccine (EZ) and EZ Receipt at 9 Months of Age

<table>
<thead>
<tr>
<th>Factor</th>
<th>EZ Receipt at 4.5 Mo (Group I)</th>
<th>EZ Receipt at 9 Mo (Group III)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>52 (228/435)</td>
<td>51 (211/415)</td>
</tr>
<tr>
<td>Age at inclusion, d</td>
<td>151 ± 12</td>
<td>150 ± 12</td>
</tr>
<tr>
<td>From Bandim District</td>
<td>40 (175/435)</td>
<td>42 (175/415)</td>
</tr>
<tr>
<td>Risk factors at enrollment at age 4.5 mo</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Not being breast-fed</td>
<td>3 (14/433)</td>
<td>3 (11/412)</td>
</tr>
<tr>
<td>Pigs in household</td>
<td>15 (63/426)</td>
<td>15 (62/411)</td>
</tr>
<tr>
<td>Persons/bed, no.</td>
<td>2.9 ± 0.7</td>
<td>3.0 ± 0.6</td>
</tr>
<tr>
<td>Toilet inside</td>
<td>17 (72/435)</td>
<td>15 (63/414)</td>
</tr>
<tr>
<td>Electricity</td>
<td>40 (174/433)</td>
<td>39 (160/413)</td>
</tr>
<tr>
<td>Functioning electricity</td>
<td>28 (110/390)</td>
<td>26 (103/391)</td>
</tr>
<tr>
<td>Morbidity and anthropometry</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hospitalization before age 4.5 mo</td>
<td>1.4 (6/434)</td>
<td>1.5 (6/411)</td>
</tr>
<tr>
<td>Weight, kg</td>
<td>7.12 ± 0.95</td>
<td>7.24 ± 0.98</td>
</tr>
<tr>
<td>Length, cm</td>
<td>63.6 ± 2.4</td>
<td>64.5 ± 2.8</td>
</tr>
<tr>
<td>Arm circumference, mm</td>
<td>140 ± 10.8</td>
<td>142 ± 11.3</td>
</tr>
<tr>
<td>Maternal arm circumference, mm</td>
<td>273 ± 32.8</td>
<td>272 ± 31.8</td>
</tr>
<tr>
<td>BCG vaccine scar</td>
<td>81 (350/431)</td>
<td>80 (331/413)</td>
</tr>
</tbody>
</table>

Data are % (proportion) of children or mean ± SD, unless otherwise indicated, and they are limited to children with an analyzed blood sample (Figure 1).

a Data are for 434 in group I and 414 in group III.
b Data are for 435 in group I and for 414 in group III.
c Data are for 428 in group I and 401 in group III.
nonprotective levels after receipt of the first EZ dose, in contrast to 92% (382/415) in group III (Table 2), with a RR (group I/group III) of 0.24 (95% CI, 0.20–0.29).

Antibody Levels at 24 Months of Age After 2 Doses of EZ at 4.5 and 9 Months of Age (Group I)

In group I, 337 samples were analyzed at age 24 months (Figure 1). Only 3% (11/337) had nonprotective measles virus antibody levels (Table 3). Nine had low but detectable levels; of the 2 children who had an undetectable antibody level at 24 months, one had a protective level and one had a low detectable level at 9 months after the first dose of EZ. Since no children would have maternal antibodies at 24 months of age, all children with the possible exception of 1 had seroconverted to having specific measles virus antibodies following early MV receipt. The GMT was 657 mIU/mL (Table 3). We did not find any significant difference in nonprotective level between boys and girls, and the GMT did not differ by season of the first MV dose (data not shown).

The titer at 24 months depended on the antibody level at receipt of the first MV dose, with the highest levels for those with no detectable antibody at 4.5 months of age (P = .001; Table 3). Most children with nonprotective levels at 24 months had had protective levels at the initial vaccination, probably because of maternal antibodies. The antibody titer at 24 months depended also on the antibody level at 9 months of age, before the booster dose was administered (Table 4); however, those who had the highest titers at 9 months continued to have the highest titers at 24 months of age (P < .0001). Of those who had undetectable or low detectable levels at 9 months of age, 89% (63/71) had acquired protective levels by 24 months of age. We also tested how many children had a 4-fold increase in antibody titer between 9 and 24 months of age (Table 4). The majority of children who had nonprotective level at 9 months of age (60/71) or medium protective levels (105/210) had a significant boost, whereas only 18% (19/56) of those with a high level had a further boost (P < .0001).

Table 2. Proportion of Children With Nonprotective Antibody (Ab) Levels and Geometric Mean Titers (GMTs), by Age at Receipt of Edmonston-Zagreb Measles Vaccine and Number of Doses

<table>
<thead>
<tr>
<th>Age at Sampling</th>
<th>Vaccination at 4.5 and 9 Mo (Group I)</th>
<th>Vaccination at 9 Mo or at 9 and 18 Mo (Group III)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Nonprotective Ab Level, % (Proportion) [No. Nondetectable]</td>
<td>GMT, mIU/mL (95% CI)</td>
</tr>
<tr>
<td>4.5 mo</td>
<td>75.2 (327/435) [199]</td>
<td>42 (38–48)</td>
</tr>
<tr>
<td>9 mo</td>
<td>22.6 (92/408) [35]</td>
<td>215 (188–243)</td>
</tr>
<tr>
<td>18 mo</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>24 mo</td>
<td>3.3 (11/337) [2]</td>
<td>657 (581–744)</td>
</tr>
</tbody>
</table>

Group I had significantly higher antibody levels at 9 months of age than group III (P < .0001). At 24 months of age, the GMT of group III was significantly higher than that for group I (P < .0001).

Abbreviations: CI, confidence interval; NA, not applicable.

Table 3. Measles Virus Antibody (Ab) Level at 9 Months and 24 Months of Age, by Prevaccination Ab Level at 4.5 Months Age (Group I)

<table>
<thead>
<tr>
<th>Ab Level at 4.5 Mo</th>
<th>Nonprotective Antibody Level, % (Proportion) [No. Nondetectable]</th>
<th>GMT, mIU/mL (95% CI)</th>
<th>Nonprotective Antibody Level, % (Proportion) [No. Nondetectable]</th>
<th>GMT, mIU/mL (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 mIU/mL (undetectable level)</td>
<td>12.8 (24/188) [7]</td>
<td>330 (280–390)</td>
<td>1.3 (2/151) [0]</td>
<td>817 (693–964)</td>
</tr>
<tr>
<td>31.25–62.5 mIU/mL (low detectable level)</td>
<td>19.9 (23/122) [6]</td>
<td>195 (160–237)</td>
<td>1.9 (2/104) [0]</td>
<td>666 (543–816)</td>
</tr>
<tr>
<td>≥125 mIU/mL (protective level)</td>
<td>45.9 (45/98) [22]</td>
<td>104 (76–141)</td>
<td>8.5 (7/82) [2]</td>
<td>433 (323–580)</td>
</tr>
<tr>
<td>Total</td>
<td>22.6 (92/408) [35]</td>
<td>214 (188–244)</td>
<td>3.3 (11/337) [2]</td>
<td>657 (581–744)</td>
</tr>
</tbody>
</table>

Children with a protective level of maternal Ab at 4.5 months of age had a significantly lower Ab level at 9 months of age after 1 dose of EZ (P < .0001) but also at 24 months of age after 2 doses of EZ (P = .001).
antibody levels after 1 dose of EZ, at 9 months of age. As described elsewhere, the GMT was 807 mIU/mL [16]. The antibody titer was analyzed in relation to the prevaccination antibody level. Children who had undetectable or low detectable levels at 9 months of age essentially had the same GMT at 18 months (780 and 757 mIU/mL, respectively). The children who had protective levels at 9 months of age continued to have a high antibody level (GMT, 1219 mIU/mL), possibly reflecting that a protective level at 9 months of age represented subclinical measles virus infection, rather than maternal antibody (Table 5) [16].

Comparison of Early and Later Measles Vaccination at 24 Months of Age (Groups I and III)

In group I, 3% (11/337) had nonprotective antibody levels after they had received 2 doses of MV, in contrast to 1% (4/318) in group III, a difference that was not statistically significant. In group III, the antibody level and proportion with nonprotective antibody levels was similar for children who had received 1 dose at 9 months of age or 2 doses at 9 and 18 months of age (Table 2). The antibody level at 24 months in group III depended on the season of the first MV; children vaccinated in the rainy season had a significantly higher titer (GMT, 1376 mIU/mL) than children vaccinated in the dry season (GMT, 1008 mIU/mL; P = .013). The difference by season of vaccination persisted when we controlled for the antibody level at 9 months of age in a Tobit analysis (data not shown).

The GMT was lower in group I than group III (P = .0001; Table 2). The 4 children in group III who had a nonprotective level at 24 months all had protective levels at 18 months, suggesting that essentially 100% of the children who received EZ at 9 months of age had been protected against measles. In group I, 3 of the 11 children having a nonprotective level at 24 months of age had protective levels at 9 months, and 7 had detectable measles virus antibody levels, indicating that they probably had some protection against measles.

Table 5. Geometric Mean Titers (GMTs) at 18 Months and 24 Months of Age After 1 Dose of Edmonston-Zagreb Measles Vaccine (EV) at 9 Months of Age or 2 Doses at 9 and 18 Months of Age, by Prevaccination Antibody (Ab) Level at 9 Months of Age (Group III)

<table>
<thead>
<tr>
<th>Ab Level at 9 Mo</th>
<th>Nonprotective Level, % (Proportion)</th>
<th>GMT, mIU/mL (95% CI)</th>
<th>≥4-fold Increase From Level at 18 Mo, % (Proportion)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 mIU/mL (undetectable level)</td>
<td>0.9 (3/344) [1]</td>
<td>808 (722–904)</td>
<td>1.3 (3/218) [0]</td>
</tr>
<tr>
<td>31.25–62.5 mIU/mL (low detectable level)</td>
<td>0 (0/28) [0]</td>
<td>1219 (717–2075)</td>
<td>0 (0/27) [0]</td>
</tr>
<tr>
<td>≥125 mIU/mL (protective level)</td>
<td>0 (0/28) [0]</td>
<td>1219 (717–2075)</td>
<td>0 (0/27) [0]</td>
</tr>
<tr>
<td>Total</td>
<td>0.9 (3/344) [1]</td>
<td>808 (722–904)</td>
<td>1.3 (3/218) [0]</td>
</tr>
</tbody>
</table>

The group with protective levels at 9 months of age tended to have higher antibody levels than the other 2 groups at both 18 months of age (P = .108) and 24 months of age (P = .110).

Abbreviation: CI, confidence interval.
We have previously reported that neonatal vitamin A supplementation (NVAS) had a major impact on child survival in group I [15]. However, receipt of NVAS was not linked to measles virus antibody levels at 24 months of age in either group I or group III (data not shown).

**DISCUSSION**

Four percent of mothers had nonprotective measles virus antibody levels. Previous studies in Bissau found a higher GMT among mothers than we did in the present study [5]; there are presumably fewer mothers who have had natural measles virus infection [16]. Hence, children become susceptible to measles virus infection earlier [11, 16, 23]; 75% had nonprotective levels before vaccination at 4.5 months of age, and this proportion increased to 92% at 9 months of age. Many children with protective levels at 9 months of age had probably had subclinical infection [16].

Our study had several important findings. First, EZ receipt at 4.5 and 9 months of age provided protective levels for 97% at 24 months of age, and all children, except possibly 1 child, had responded with measles virus antibody. Second, the antibody response at both 9 and 24 months of age depended on the level of maternal antibodies at the time of the initial measles vaccination. Third, 89% of children who had not obtained a protective antibody level after 1 dose did so after the second dose of EZ. Fourth, children who had received EZ at 4.5 and 9 months of age had lower antibody levels than children who received EZ at 9 months of age or 9 and 18 months of age, there was no significant difference in the proportion with nonprotective levels. Fifth, an additional dose of EZ, at 18 months of age, after receipt of the initial MV dose at 9 months of age, did not boost the antibody response, compared with having received just 1 dose at 9 months of age; a similar observation has been made for SW [16].

A strength of this study is that the cohort experienced a measles epidemic just before blood samples were collected [11]. This provided an opportunity for testing the efficacy of EZ against clinical measles, and the vaccine performed very well [11]. On the other hand, it meant that some samples had to be excluded from the analysis because the children from whom they were obtained already had had clinical measles virus infection. Still, results were clear, so it is unlikely that this had any impact on the overall result, apart from modifying the 95% CIs.

The loss to follow-up was 20%–25% between 4 and 24 months of age (Figure 1). In this community with high mortality, people move to find cheaper accommodations, urban families often go to rural areas to maintain contact with family, and many women are away for several months for the annual harvest of cashew nuts and fruits in the late dry season. Loss to follow-up was similar in group I and group III, and there is little reason to think that this loss should have biased the comparison between groups.

This study has several implications. Nearly all children receiving EZ at 4.5 and 9 months of age had acquired measles virus–specific antibodies by 24 months of age. Since there had been no measles epidemic after the provision of vaccine at 9 months of age, this effect is essentially due to the vaccine. A nonprotective level at 24 months of age occurred mainly among children who had high protective antibody levels at 4.5 months of age; since nearly all had specific measles virus antibodies, they presumably had a modified immune response with a strong cellular response [23] preventing a high antibody response to the booster dose of EZ at 9 months of age. It is not known whether such children are susceptible to measles virus infection.

Achievement of protective antibody levels at 2 years of age among 97% of vaccine recipients should make a 2-dose schedule with standard EZ an attractive strategy in areas with low maternal antibody levels. Although the group that received EZ at 9 months of age had somewhat higher antibody levels, this does not necessarily mean that the early 2-dose cohort is more susceptible to measles over the long term. We have previously shown 94% protection against clinical measles and 100% protection against measles hospitalization or measles death before 9 months of age [11], even though only 77% had protective antibody levels after receipt of 1 dose of EZ at 4.5 months of age. Administering MV early may also increase the period with beneficial nonspecific effects of MV [12–15]. We will continue to follow the children to see whether there is any differential decline in antibody levels between children receiving the first dose of EZ at 4.5 or 9 months of age.

We have previously conducted studies of early 2-dose MV strategies with either EZ or SW administered at 6 and 9 months of age [4, 5, 14]. The level obtained with EZ at 4.5 and 9 months of age was nearly as good as we experienced 10 years ago with EZ at 6 and 9 months of age, presumably reflecting that maternal antibody levels have continued to decline. In the previous study, 1.3% (3/240) had nonprotective levels at 18 months of age after 2 doses [5]. Our result in the present study with EZ was distinctly better than the effect of SW at 6 months of age in the previous study [5].

We only included children who had received DTP before enrollment [11]. If many children receive DTP simultaneously with or after MV, results could be different. We have found that receiving DTP with MV or after MV is associated with increased mortality, compared with having MV as the most recent vaccination [14, 24–29]. However, the impact of such changes for the immune response to MV has not been studied.

In conclusion, EZ receipt at 4.5 and 9 months of age provided protective antibody levels to 97% of recipients, and nearly all had specific measles virus antibodies after vaccination. Since we have previously shown that this strategy is protective against clinical measles virus infection before 9 months of age [11] and reduces all-cause mortality between 4.5 and 36 months of age
[15], this should be a feasible measles control strategy in areas with low maternal measles virus antibody levels.

Notes

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