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Long-term survival in trial of medium-titre Edmonston–Zagreb measles vaccine in Guinea-Bissau: five-year follow-up

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SUMMARY

A trial of protective efficacy which compared medium-titre Edmonston–Zagreb (EZ) measles vaccine ($10^{4.6}$ p.f.u.) from the age of 4 months with the standard Schwarz (SW) measles vaccine given from the age of 9 months was started in an urban community in Guinea-Bissau in 1985. Because trials of high-titre measles vaccine have found increased mortality among female recipients, we examined whether EZ medium-titre vaccine was associated with any long-term impact on mortality, suppression of T-cells, or growth. The mortality rate ratio over 5 years of follow-up was 1·12 for EZ children compared with children in the standard group ($P = 0{\cdot}63$). Seventy-five percent of the children still residing in the area at 5 years of age took part in an immunological and anthropometric examination. There was no difference in T-cell subsets between the two groups. There was no difference in mid-upper-arm circumference, but EZ children were significantly shorter than the children in the standard group. In conclusion, medium-titre EZ was not associated with reduced survival or persistent immunosuppression.

INTRODUCTION

Measles immunization administered before 9 months of age could contribute significantly to the reduction of measles-related mortality in many developing countries where there is a high incidence of measles before 9 months of age [1–4]. Thus, following the demonstration in trials of medium and high-dose Edmonston–Zagreb (EZ) that infants in West Africa and Mexico had satisfactory antibody

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responses [5–7] and clinical protection [8], the Global Advisory Group of the Extended Programme on Immunisation (EPI) recommended vaccination at 6 months of age with high-dose EZ measles vaccine in countries with a high incidence before the age of 9 months [9]. However, only few studies had examined side-effects [5, 7], and antibody persistence [10], long-term clinical protection [5, 8] and long-term survival following early measles immunization [8, 10].

In a trial started in Guinea-Bissau in February 1985, we compared the efficacy of medium-titre EZ measles vaccination given from the age of 4 months with Schwarz measles vaccine (SW) given from the age of 9 months, the standard regime for developing countries. EZ vaccine was found to provide significant protection against measles [8]. In a subsequent trial in Guinea-Bissau using high-titre EZ vaccine, mortality among female recipients was significantly higher than among the standard group [11]. This observation was supported in studies from Senegal [12] and Haiti [13] which used high-titre EZ and Schwarz measles vaccines. EPI has therefore rescinded the recommendation of high-titre vaccines ($> 10^{4.7}$ plaque forming units (p.f.u.)) [14].

Following the identification of the problem of differential survival in the high-titre trial [11], in 1990 we undertook an investigation of possible long-term consequences of medium-titre EZ vaccine. We assessed survival for all children who had entered the trial when the children had reached the age of 5–6 years. Because it was suspected that increased mortality for recipients of high-titre vaccine could be related to persistent immunosuppression [13] and impaired growth due to increased susceptibility to infections, we examined T-cell subsets and anthropometry among the children still residing in the community.

SUBJECTS AND METHODS

Background

The trial was carried out in Bandim 1, a district in the capital of Guinea-Bissau, which has had a routine registration of pregnancies, births, infections, immunizations and childhood deaths since 1978 [1, 9–11]. Children under 3 years of age are called for weighing and vaccination every third month. The measles vaccination study, which has been described in detail elsewhere [8], included children born between 1 August 1984 and 30 September 1985 and registered in Bandim 1 before 4 months of age.

Study design

In addition to standard EPI vaccines, children were randomized to receive from the age of 4 months either EZ or inactivated polio vaccine (IPV) (kindly donated by Institut Mérieux) in the standard group; at 9 months of age the standard group received SW standard vaccine and the EZ group IPV. Children were called for vaccination once a month until they received these vaccines. If not vaccinated by the age of 9 months, children of both groups then received only one vaccine, this being either the EZ or the SW measles vaccine. Blood samples were collected before the first vaccination (at the age of 4 months or over), before the second vaccination (at 9 months or over) and again at 18 months of age.

Vaccines

Children in the EZ group received a dose of $10^{4.6}$ p.f.u. of the EZ vaccine, lot 529 [15]. The children in the standard group received a standard dose of the attenuated SW vaccine ($10^{3.8}$ p.f.u.). Measles vaccines used in the study were tested regularly for potency at the MRC Laboratories, The Gambia [15].

Long-term follow-up and survival

The present assessment of mortality is based on a revisit to all the children in May–June 1990. If the child was present and the parents consented, a blood sample was collected in order to assess measles antibody levels and T-cell subsets. These children also had their height and arm-circumference measured.

Survival had been monitored regularly as the children have been included in the rouine registration system in operation in the area. We also inquired about children who had moved out of the area since relatives or neighbours in the original residence are usually aware of the fate of previous residents [11]. There was no major loss to follow-up, as only 7·8% of the surviving children (31/398) had to be censored before the general control in May–June 1990 due to lack of information on their current whereabouts. All comparisons of survival are based on total mortality and not cause-specific mortality [16].

Laboratory methods

After a finger prick, slides were prepared for examination of malaria parasitaemia, and capillary blood was collected in a heparinized microtainer. On the day of collection, the white blood cell count (WBC) was obtained by haemocytometer. Smears were prepared as for conventional haematological examination [17]. Immunocytochemical labelling was carried out with monoclonal antibodies CD4 and CD8 against T-helper and T-suppressor cells using alkaline phosphatase, conjugated with Avidin–Biotin Complex [17]. All smears were coded and read by one author (I.M.L.); 200 lymphocytes were counted for each of the CD4 and CD8 slides. This method has been found to give T-cell subset counts closely correlated with facs scanner results.

Malaria thick drop slides and smears were analysed by laboratory technicians at the National Public Health Laboratory in Guinea-Bissau.

Statistical methods

Since information on dates of birth, vaccination and death or follow-up was available, it was possible to apply survival analysis techniques. Children were included in the analysis from the age of their first vaccination until age at death or follow-up, respectively. Thus, children randomized to one or the other group who did not receive a vaccination have not been included, but children have been included regardless of whether they received a second vaccination or not. To control for effects of sex, age at vaccination, season at birth, season (rainy/dry), and measles infection, multivariate Cox regression analyses [18] were performed. Mortality is often higher during the rainy season from June to November, and the time at risk has therefore been divided into rainy and dry season.

Age is used here as the time scale in the Cox regression model, thus describing

mortality as a function of age. Effects are expressed as the ratio of mortality rates, i.e. mortality ratio (MR) (95% confidence intervals). The study compares total mortality from age at first vaccination, thus evaluating both the effect of earlier immunization against measles in the EZ group than in the SW group and the effect of any difference in efficacy of the two vaccines. A separate analysis of survival from the time of second vaccination, if any, has been performed to assess the relative efficacy against mortality of EZ vaccine compared to SW vaccine. Since we have previously reported survival to the age of 24 months [8], we also made a separate analysis of mortality after 24 months.

All haematological results are presented with median values and Wilcoxon rank sum test has been used to measure statistical significance. Weight-for-age (w/a) and height-for-age (h/a) z-scores were calculated using ANTHRO programme (WHO).

RESULTS

Study children

A total of 470 children were recruited for the study, 234 in the EZ group and 236 in the standard group. There was no difference in age at vaccination between the children in the EZ group and the standard group. The median age at first vaccination, and entry into the study, was 5 months. The median age of measles vaccination in the standard group was 10 months. Two hundred and eleven of the children were weighed at the time of their first vaccination. There were no significant differences in mean weight-for-age (w/a) at the time of immunization for children in the EZ group (median z-score: $-0{\cdot}02$) and the standard group (median z-score: $-0{\cdot}07$), nor was there a significant difference in w/a between the children who survived and those who subsequently died.

During the period of follow-up, 72 children had died (37 EZ, 35 standard) and 165 had moved (79 EZ, 86 standard). Of the 233 children (118 EZ, 115 standard) still living in the area in 1990, 174 (75%) participated in the re-examination. The children who participated in the re-examination were not different in w/a at time of entry into the study (median w/a z-score: 0·08, $N = 80$) compared with those who were not re-examined (median z-score: $-0{\cdot}09$; $N = 131$).

Long-term mortality

Survival curves are shown in Fig. 1 and cumulative mortality in Table 1. Over the 5-year period, there was no difference in mortality between the two groups, the MR being 1·07 (0·67–1·70). If follow-up was censored at time of migration, the MR was 0·82 (0·48–1·41). Since previous analyses have shown higher mortality for girls receiving high-titre EZ, survival was also analysed by sex in this trial. There were no significant mortality differenes by sex between the EZ and the standard group. Though not statistically significant, contrary to what has been observed in the high-titre trials [11–14], girls receiving EZ vaccine tended to have lower mortality than boys receiving EZ.

In a multivariate analysis, the variables sex, age at first vaccination and season of birth had no significant impact on mortality when age, vaccine status and season were controlled. However, season (MR (rainy/dry) = 2·30 (1·39–3·80), $P =$ 0·001) and measles infection (MR (measles/no measles) = 2·04 (1·06–3·93), $P =$

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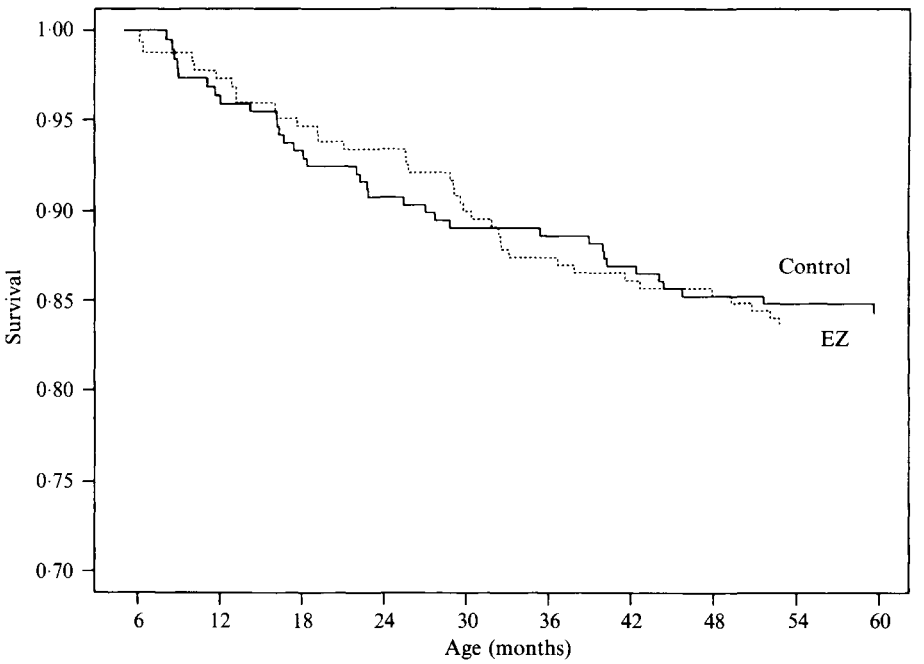

Fig. 1. Survival curves according to vaccination group. Children born 1 August 1984 to 30 September 1985. Bandim cohort 1 (from first vaccination).

Table 1. *Cumulative mortality between 4 and 60 months of age and number of deaths by sex and vaccinated group. Children born 1 August 1984 to 30 September 1985. Follow-up until May–June 1990*

	Cumulative mortality (%)					
	EZ			Standard group		
Until age (months)	Males ($N = 131$)	Females ($N = 103$)	Total ($N = 234$)	Males ($N = 115$)	Females ($N = 121$)	Total ($N = 236$)
12	2·8 (3)	2·6 (2)	2·7 (5)	3·1 (3)	4·1 (4)	3·6 (7)
18	6·8 (5)	3·6 (1)	5·3 (6)	5·8 (3)	7·6 (4)	6·7 (7)
24	8·3 (2)	4·6 (1)	6·6 (3)	7·6 (2)	10·9 (4)	9·3 (6)
36	15·9 (10)	8·4 (4)	12·6 (14)	11·0 (4)	11·8 (1)	11·4 (5)
48	19·8 (5)	— (0)	14·7 (5)	14·5 (4)	15·0 (4)	14·8 (8)
60	20·5 (1)	11·3 (3)	16·4 (4)	15·4 (1)	16·1 (1)	15·7 (2)
Deaths	(26)	(11)	(37)	(17)	(18)	(35)

0·033) were significantly related to mortality when age was controlled. The MR of EZ vaccines compared with SW vaccines was 1·12 (0·70–1·80) ($P = 0·63$) when age, season and measles infection were controlled. After 24 months, the mortality ratio of the two groups was 1·77 (0·90–3·46) ($P = 0·097$).

Of the 470 children in the study, 423 (90%) received the second vaccination, IPV in the EZ group or Schwarz in the standard group. The MR (EZ/SW) from the time of the second vaccination when children in the standard group had also been immunized against measles was 1·22 (0·70–2·13) ($P = 0·482$) controlling for the background factors mentioned above.

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Table 2. *Mortality (deaths/person-years-at-risk) according to number of vaccines, type of measles vaccine and sex. Children born 1 August 1984 to 30 September 1985. Follow-up May–June 1990*

	Mortality (deaths/person-years-at-risk)		
	Females (F)	Males (M)	F/M ratio
EZ group			
Only one vaccine (EZ)	0·046 (3/64·9)	0·078 (7/89·3)	0·59 (0·15–2·28)
Two vaccines (EZ+VIP)	0·021 (8/380·4)	0·044 (19/434·8)	0·48 (0·21–1·10)
Total			0·51 (0·25–1·03)
Standard group			
Only one vaccine (VIP)	0·106 (7/66·2)	0·044 (3/68·9)	2·43 (0·63–9·38)
Two vaccines (VIP+SW-std)	0·025 (11/435·3)	0·033 (14/423·1)	0·76 (0·35–1·68)
Total			1·05 (0·54–2·03)

In the EZ group, there was no difference in the mortality ratio of females compared with males between those who received only the first vaccine and those who received both vaccines (Table 2). The pattern was the opposite in the standard group; mortality tended to be higher for girls than for boys among those children who had not yet received measles vaccine whereas girls had lower mortality than boys among recipients of SW-std vaccine. However, these differences were not statistically significant.

T-cell subsets

There was no difference between the 86 EZ children and the 88 children in the standard groups in malaria parasitaemia (69 *v.* 60%) or any of the haematological indices (WBC 7·5 *v.* 7·5 ($\times 10^9$/l); lymphocyte percentage 59 *v.* 59; CD4% 43 *v.* 44; CD8% 23 *v.* 21; CD4/CD8 ratio 1·840 *v.* 1·965; total CD4 count 1·785 *v.* 1·833 ($\times 10^9$/l)).

Anthropometric measurements

Among the children who took part in the follow-up examination in 1990, mid-upper-arm circumference (muac) was not different between the two groups, median muac being 158 (150–164) in the EZ group and 158 (150–166) in the standard group. However, the EZ children had a significantly lower *z*-score for height-for-age (−1·31) than children in the standard group (−0·95) ($P = 0·01$), a difference which was significant for the girls (−1·30 *v.* −0·94) ($P = 0·019$) but not for boys (−1·34 v. −1·05) ($P = 0·220$).

DISCUSSION

Following the observation of reduced survival among female recipients of high-titre EZ vaccine in several studies [11–13], WHO has recommended [14] that vaccines with a titre of $10^{4·7}$ p.f.u. should no longer be used in routine immunization programmes. In the first study in Guinea-Bissau, we used a medium titre of $10^{4·6}$ p.f.u. and it was therefore of interest to examine whether such titres are safe. Given the measurement variation in any titre assessment, the children could in

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fact have received a titre above $10^{4.7}$ p.f.u. There have been only two studies reporting from medium-titre trials. In The Gambia, where mortality is extremely low for a developing country, there was no death among 120 children immunized with EZ during 3 years of follow-up and only one child died out of 119 who had received SW-std [10]. In a previous report from Bissau, EZ children were found to have an accumulated mortality of 6·3% from 4 months to 2 years of age compared with 9·5% in the SW group [8]. Subsequent follow-up of this cohort as reported in this paper showed EZ vaccinated children to have somewhat higher, but statistically insignificant, mortality after 2 years of age than the SW children. At the age of 5–6 years, there was no difference in survival between the two groups.

Furthermore, there was no sign of different T-cell levels among the children still surviving. Mid-upper-arm-circumference measurements were similar for the two groups. However, the EZ children were significantly shorter than children in the standard group. Changes over time could not be monitored since the children had not previously been examined for T-cell or height. However, weight-for-age had not been different when the children entered the study. Since we found no difference in arm circumference and other studies of medium-titre EZ have found no differences in weight for age [10], the difference in height may be due to chance.

Considering that several studies have found lower survival for female recipients of high-titre measles vaccines compared with girls in the standard group as well as male recipients of high-titre vaccines [11–14], it is noteworthy that female recipients of medium-titre EZ vaccine in the present study had borderline significant lower mortality than the EZ males (Tables 1 and 2). In Senegal, girls receiving Schwarz measles vaccine had a significantly lower mortality rate than boys whereas there was no difference for the unimmunized children [12]. As shown in Table 2 there was also lower mortality for girls who had received medium-titre EZ or standard vaccine though these differences were not stastically significant. Hence, there seems to be important interactions between sex and measles immunization for which we have no explanation. In the present cohort we found that girls had lower levels of antibodies than boys (authors' unpublished observation). This could potentially explain differences in mortality between the sexes if higher pre-immunization antibodies provided some protection against the possible negative effects of the high-titre EZ vaccine. However, this would not explain why female recipients of SW-std have better survival than males in some studies [12]. Future studies of measles vaccines have to investigate possible interactions with sex and maternal antibody levels.

On balance, there was no difference in mortality between children in the medium-titre EZ group and the standard group. Though medium-titre EZ vaccine had provided clinical protection against measles infection before 9 months of age [8], it did not provide a long-term benefit in terms of survival, nutritional or immunological status. In order to improve measles control and reduce child mortality, we may need new vaccines or a two-dose strategy with SW-std to protet children before 9 months of age. While there has been no epidemiological study of the protective efficacy provided by a two-dose strategy with standard vaccine, SW-std administered before 9 months of age has been found to be highly effective in terms of reducing mortality [2, 19].

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