Low seroconversion rates to measles vaccine among children in Nigeria*

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The Nigerian Expanded Programme on Immunization (EPI) was assessed with particular reference to measles immunization. Of 150 children who received measles vaccine at the Institute of Child Health, University of Ibadan, Nigeria, 82 (54.7%) seroconverted. The immune response was directly related to the titre of the vaccines used. Vaccines whose titres were 10^{-1} to $10^{1.7}$ stimulated immune responses in 0–25% of vaccinees, those with titres in the range $10^{-2.1}$ to $10^{-2.5}$ stimulated responses in 12–47.6%, while those with titres of $10^{-2.7}$ to $10^{-3.4}$ stimulated responses in 87.5–100% of vaccinees. Only one of the vaccines used had a titre that met the minimum WHO required standard of log 10^{-3} $TCID_{50}$ at the point of vaccination.

Introduction

The Nigerian Expanded Programme on Immunization (EPI), which began operations in 1980, was relaunched in 1984 with huge success in terms of vaccination coverage. Since then, EPI has concentrated on mass vaccination efforts in an attempt to cover a large majority of the target population. What has, however, been lacking is a laboratory back-up system to ensure that the vaccines are potent on arrival in Nigeria and throughout the storage period up to the time of delivery to the vaccinee, and that the majority of the children vaccinated are actually immunized. Any successful vaccination programme must provide a highly potent vaccine, with a long expiry date, a reliable cold-chain system, and ensure that at the point of vaccination the delivery technique does not render the vaccine impotent before it is used. Recent reports of unusually large numbers of cases of measles among vaccinated children point to the urgent need for a laboratory back-up service for EPI in Nigeria.

As part of a collaborative effort between the Department of Virology and the Institute of Child Health, University College Hospital, Ibadan, a programme to monitor vaccines before, during, and after use, as well as the immune responses of the vaccinated children, was begun in Ibadan in 1989. This article reports the initial findings.

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Materials and methods

Study population

The study was carried out between April 1989 and February 1990 on children aged at least 9 months who were attending the Institute of Child Health, University College Hospital, Ibadan, for measles immunization. The children were drawn from the low, middle, and upper socioeconomic classes of the population. All the children were in a good state of health at the time of vaccination. Samples of blood were collected before vaccination by finger puncture on to filter-paper (ROPACO®), as described by Nakano (*1*). Each sheet of filter-paper consisted of a rectangle of 5 cm x 15.2 cm on which four circles (each 12 mm in diameter) had been imprinted. The blood from one child was used to soak each of the circles on a sheet. The name, sex, and age of each child, together with the vaccine batch and date of vaccination were marked on the filter-paper. The filter-papers were then dried at room temperature and stored at –20 °C until used. After the children had received a subcutaneous injection of 0.5 ml of measles vaccine (supplied by the EPI centre), their mothers were advised to bring them back 8 weeks later for post-vaccination evaluation. The vaccinations were performed by nursing sisters who had received additional training in administering EPI vaccines, thereby ensuring that a proper technique was used and that the vaccine was stored at the correct temperature in the clinic. Serum extraction and treatment were carried out as described previously (*1*). The final dilution of the serum was 1:10, and the accuracy of this was cross-checked using the empirical method, as described by Mattews et al. (*2*).

Vaccine potency testing

Both lyophilized and diluted measles vaccines were used in the study. Small aliquots of the diluted vaccines were collected immediately after rehydration at the vaccination centre and stored at –70 °C until they were titrated for potency. Lyophilized, undiluted vaccines obtained from the Federal Vaccine Central Store, Oshodi, Lagos, and stored at –70 °C, were also titrated. Vaccine potency was determined according to WHO guidelines on triplicate samples of confluent monolayers of Vero cells in 24-well tissue-culture plates. The cells were incubated at 37 °C and examined daily for 10 days. The virus end-point was calculated using the method described by Reed & Muench (*3*).

Haemagglutination inhibition test

The haemagglutination inhibition test (HI) was performed as described by Munube (*4*). Serial twofold dilutions of sera were prepared from the 1:10 dilution obtained from the extraction, and 4 HA (haemagglutinating) units per 0.025 ml of commercially available measles antigen[a] was added to each serum dilution plate and incubated for 1 hour at 37 °C, whereupon 0.05 ml of a 1% suspension of monkey red blood cells was added. On each plate, as controls, were also run samples of red blood cells, and known positive and negative sera and antigens. The wells were incubated at 37 °C and the results were read when the reactions in the control wells were satisfactory.

Vaccine stability test

A few of the vaccines were tested for stability at 37 °C, 45 °C, and –70 °C; for this purpose, three ampoules of each batch of vaccine were selected and one ampoule each was stored at one of these temperatures. On days 1, 2, and 3 one ampoule was rehydrated according to the manufacturer's instructions and titrated in Vero cells.

Results

Of the 284 children, only 16 (5.6%) exhibited measles antibody (HI titre, 10–160) prior to vaccination (Table 1). A total of 150 (52.8%) of the 284 children who received measles vaccine returned for post-vaccination screening, and of these, 82 (54.7%) seroconverted (titres, 10–320) following vaccination; 68 (45.3%) did not seroconvert (Table 1). Only 7 (43.8%) of the 16 children with pre-vaccination measles antibodies returned for post-vaccination screening. Five exhibited a reduced antibody titre, while two had an increased titre after vaccination (titre range, 10–40).

Potency test

Only one of the vaccines used in the study met the minimum WHO required standard of $\log 10^{-3}$ $TCID_{50}$. The titres of the various batches are shown in Table 2.

Stability test

The results indicate that the vaccines were not stable at 45 °C, since those vaccines kept at this temperature for 3 days had titres that were 2 $\log_{10}$ units less than at the start of the test. In contrast, vaccines kept at 37 °C or –70 °C for 3 days still retained their original titres (Table 3).

Vaccine titre and immune response

The relationship between measles vaccine titre and the development of measles antibody is shown in Table 2. The immune response stimulated by the different vaccines was dependent on their titre. Four vaccine batches with titres between log $10^{1.0}$ and log $10^{1.7}$ stimulated an immune response in 0–25% of the vaccinated children, while two batches with titres of log $10^{2.1}$ and log $10^{2.5}$ stimulated an immune response in 12.5% and 47.6% of the children, respectively. Seroconversion occurred among 30 (94%) of the 32 children who received vaccines with titres of $\geq$ log $10^{2.7}$.

Discussion

Our results show that only 82 (54.7%) of the children who received measles vaccines seroconverted. Of these children, 71 (86.5%) had low-level antibody titres (1:10 to 1:40).

A total of 16 children had pre-vaccination measles antibodies. These children must therefore have been exposed to the virus before 9 months of age or still exhibited circulating maternal antibodies to measles. Previous studies have reported the presence of such maternal antibodies in children aged over 12 months (*5, 6*). In our study seven such children returned for post-vaccination screening, of whom five had become seronegative, while two had developed an anamnestic response to the vaccine.

During the study only one of the vaccines used had a titre at least log 10^{-3} at the point of vaccination. However, according to the manufacturers all the vaccines had titres in the range $10^{4.8}$ to $10^{5.3}$. The titres were, however, not checked immediately upon receipt of the vaccines. The results of the stability

[a] Virion Laboratories Ltd, Ruschlikon, Switzerland.

Table 1: **Results of the haemagglutination inhibition test on pre- and post-vaccination sera from children who received measles vaccine**

No. of children	Age (months)	Type of sera	No. positive	No. negative	Titre range: <10	10	20	40	80	160	320
284	9	Pre-vaccination	16 (5.6) [a]	268 (94.3)	268	9	2	2	2	1	–
150	9	Post-vaccination	82 (54.7)	68 (45.3)	68	26	20	25	6	4	1

[a] Figures in parentheses are percentages.

Table 2: **Titres and immune responses produced by the measles vaccines used in the study**

Name of vaccine	Manufacturer	Batch/lot number	Date of use	Titre ($TCID_{50}$)	Immune response stimulated
Conpharma	Zagreb, Croatia	153	7 June 1989	$10^{2.1}$	1/8 (12.5) [a]
Conpharma	Zagreb, Croatia	164/4	5 June 1989	10^{-1}	0/1 (0)
Morbilvax	Sclavo, Italy	36A30	30 August 1989	$10^{-1.7}$	0/7 (0)
Bikem Cam.	Osaka, Unijapan	FL12/E60	26 July 1989	10^{-1}	0/7 (0)
Morbilvax	Sclavo, Italy	36A33	6 September 1989	10^{-1}	1/4 (25)
Morbilvax	Sclavo, Italy	36A28	9 August 1989	$10^{-2.5}$	10/21(47.6)
Morbilvax	Sclavo, Italy	33A33	13 September 1989	$10^{-2.7}$	9/9 (100)
Morbilvax	Sclavo, Italy	33A13	27 September 1989	$10^{-3.4}$	14/15 (93.3)
Morbilvax	Sclavo, Italy	33A30	20 September 1989	$10^{2.7}$	7/8 (87.5)

[a] Figures in parentheses are percentages.

Table 3: **Results of the stability tests on selected batches of measles vaccine**

Vaccine batch	Initial titre	Storage temperature (°C)/day	Final titre
30A30	10^{-3}	–70/3	10^{-3}
		37/3	10^{-3}
		45/3	10^{-1}
36A30	10^{-2}	–70/3	10^{-2}
		37/3	10^{-2}
		45/3	10^{-1}
CXV (115)	$10^{2.5}$	–70/3	$10^{-2.5}$
		37/3	$10^{-2.4}$
		45/3	10^{-1}

test showed that the vaccines were relatively stable at 37 °C and –70 °C but not at 45 °C. The vaccinations were performed in the clinic, where the average room temperature was normally air-conditioned to below 30 °C, and the vials of vaccines were usually stored on ice packs provided by EPI.

The immune response stimulated by each vaccine was directly dependent on its titre (Table 2).

The poor response of the vaccinees in this study is attributable to the low vaccine titres. Several reasons could have been responsible and further studies are being carried out to identify the factors that may have led to these subpotent vaccine titres. Meanwhile, we suggest that the cold chain should be evaluated and that the storage conditions of the vaccines should be examined. Also the use of highly heat-stable vaccines should be encouraged, vaccines should be randomly tested before use, and any batch with a titre of $<10^{-3}$ should be discarded.

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Résumé

Faibles taux de séroconversion après vaccination antirougeoleuse chez l'enfant au Nigéria

Le programme élargi de vaccination (PEV) du Nigéria a été évalué en ce qui concerne la vaccination antirougeoleuse. Sur 150 enfants ayant reçu le vaccin antirougeoleux à l'Institut de Pédiatrie de l'Université d'Ibadan (Nigéria), 82 (54,7%) ont présenté une séroconversion. La réponse immunitaire était directement liée au titre du vaccin utilisé. Les vaccins dont le titre était de 10^{-1}–$10^{1.7}$ ($DICT_{50}$) ont suscité une réponse

immunitaire chez 0 à 25% des vaccinés, ceux dont le titre était compris entre $10^{-2,1}$ et $10^{-2,5}$ chez 47,6% des vaccinés, et ceux dont les titres étaient compris entre $10^{-2,7}$ et $10^{-3,4}$ chez 87,5 à 100% des vaccinés. Seul un des vaccins utilisés avait un titre satisfaisant à la norme minimale requise par l'OMS, soit log 10^{-3} $DICT_{50}$ au point d'injection.

Dans cette étude, la médiocrité de la réponse à la vaccination est imputable aux faibles titres des vaccins. Ces faibles titres peuvent avoir plusieurs origines, que l'on cherche actuellement à identifier. En attendant, nous proposons que la chaîne du froid soit évaluée et que les conditions de conservation des vaccins soient examinées. Il faudrait également encourager l'emploi de vaccins de bonne thermostabilité, tester des échantillons de vaccins pris au hasard avant leur utilisation, et rejeter tout lot ayant un titre inférieur à log 10^{-3} $DICT_{50}$.

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