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Investigation of the effectiveness of measles vaccination in children in Kenya

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SUMMARY

Laboratory studies were performed on 128 children clinically diagnosed as measles when seen at the Infectious Diseases Hospital, Kenyatta National Hospital (IDH), Nairobi (86 cases) and the Rural Health Training Centre, Maragua, Central Province (42 cases) between 9 July and 31 August 1984. A concurrent measles infection was confirmed in 95% of the children seen at IDH and in 85% of those seen at Maragua, with similar proportions of confirmations in children who had, and who had not, received measles vaccine. No differences in the number of sero-conversions nor in the absolute levels of acute or convalescent HI antibody titres could be detected between vaccinated and unvaccinated children. Analysis of the cases seen at Maragua indicates that about two thirds of the children who had received vaccine were protected. A pilot study of vaccinating children at 8 months and again at 12–13 months is suggested in an attempt to eradicate measles.

INTRODUCTION

Measles remains one of the major scourges throughout Tropical Africa, affecting most children before they reach 5 years of age, with mortality rates varying from 4 to 14% (Kenya & Tukei, 1980; Mitchell & Balfour, 1985). Vaccination, using an attenuated live vaccine, administered during the second year of life, has greatly reduced the incidence of measles in Europe and America (WHO, 1983). In Kenya, almost 25% of children contract measles before their first birthday and studies on sero-conversion following experimental vaccination indicated 8–9 months as the optimum age for administration of measles vaccine (Leeuwenburg & Muller, 1980). This is currently accepted as the best compromise age for measles vaccination in Tropical Africa (Heyman *et al.* 1983; Remme, Mandara & Leeuwenburg, 1984). Despite an extensive vaccination programme the incidence of measles remains high in Kenya and a similar situation appears to exist throughout the Third World. During an investigation into the aetiology of acute respiratory disease in children hospitalized in Nairobi, Kenya (Wafula *et al.* 1984) it was found that measles infections accounted for 6% of all the admissions under the age of 5 years, while examination of the case notes indicated that approximately one third of these children had been vaccinated. A survey was therefore designed to investigate the efficacy of measles vaccination in Kenya during the 1984 measles epidemic in Central Province which was expected to occur between July and September (Kenya & Tukei, 1980).

MATERIALS AND METHODS

Selection of children

Children with clinical measles from both urban and rural areas were examined between 9 July and 31 August 1984.

(*a*) On Monday, Tuesday and Wednesday of each week the staff of Ward 3, Infectious Diseases Hospital, Nairobi, selected up to three children with a clinical diagnosis of measles who were believed to have been vaccinated and up to three unvaccinated children also with clinical measles. The laboratory staff then interviewed the parent or guardian and examined the child's vaccination/clinic record card, if available. A full record of vaccination history, date of onset of prodromal symptoms, appearance of rash and a general description of symptoms was made for each child included in the survey. Nasopharyngeal secretions (NPS), a throat swab (TS) in transport medium and acute blood were then collected and the mother asked to return with the child in 2 weeks, bringing the vaccination/clinic record card. On return the mother was informed of the laboratory results, the child examined and a convalescent specimen of blood collected.

(*b*) On Thursday of each week the laboratory staff visited the Rural Health Training Centre, Maragua, where the staff admitted to the survey all children presenting at their clinic on that day with suspected measles. In addition, the parents of children with clinical measles seen earlier in the week were asked to bring their children for investigation on Thursday. Each child was then examined and specimens collected as described above.

(*c*) The collection of convalescent serum specimens continued for 2 weeks after the last new cases of measles were examined.

Demonstration of virus

All NPS and TS were transported to the Virus Research Centre respiratory laboratory on melting ice for immediate processing.

(*a*) An aliquot of each NPS was added to the transport medium containing the TS from the same child, mixed and five drops inoculated onto monolayers of Vero cells in large stationary tubes incubated at 36 °C. The cells were maintained in medium 199 supplemented with 2 % foetal bovine serum and changed weekly. Each tube was examined daily for 21 days and then blind passaged for a further 7 days before discarding as negative. Isolated viruses were identified by examining the cells from tubes showing CPE by immunofluorescence using measles, RSV, herpes simplex and adenovirus antisera. The adenoviruses were subsequently typed by neutralization. All original specimens and isolated viruses were stored at −70 °C.

(*b*) The remainder of the NPS were suspended in PBS and the cells centrifuged into a pellet. This was repeated up to three times until a clean suspension of cells was obtained from which at least four smears, on separate four-spot Teflon-coated slides (C. A. Hendley Ltd), were prepared. After fixing in cold acetone, two smears from each child were used to test for the presence of measles and RSV antigens by the indirect immunofluorescence method (IF) (Gardner & McQuillin, 1980), using specific bovine antisera (supplied by Central Public Health Laboratory, Colindale, London, England) and FITC-conjugated anti-bovine globulin (Wellcome Diagnostics, Dartford, Kent). The remaining slides were stored at −70 °C for future use.

Determination of haemagglutination inhibiting antibody (HI) titres

All sera were tested by standard techniques for the presence of HI antibody, using an antigen prepared from the Edmonston strain of measles virus grown in Vero cells, in microtitre plates. Acute and convalescent specimens from the same patient were tested in parallel.

RESULTS

A total of 128 children with a clinical diagnosis of measles was seen during the 8-week period, 86 at the Infectious Diseases Hospital, Nairobi (IDH) and 42 at the Rural Health Training Centre, Maragua. One child was admitted twice to IDH and each admission has been considered separately. An adequate NPS was obtained from all but one child (at Maragua) who had severe epistaxis and slides suitable for IF were prepared from 128 child admissions. All specimens in which measles antigen was not detected initially were retested and four were then found to be positive for measles. The results of the measles IF tests are summarized in Table 1 and show a good correlation between clinical measles and the detection of measles antigen in the NPS. Measles antigen was detected in the specimen from the second admission of the child admitted twice, but not from the first specimen. Respiratory syncytial (RS) virus antigen was demonstrated in the NPS from 7 children, 4 from IDH and 3 from Maragua. Measles antigen was demonstrated in the NPS from all the IDH admissions, but in only one of those at Maragua, with concurrent RSV.

Paired sera were obtained from 66 children and all were successfully tested for

Table 1. *Correlation between clinical diagnosis of measles and laboratory confirmation of measles virus infection*

	Immunofluorescence			Fourfold or greater rise in HA antibody			Combined methods		
	No.	Positive	(%)	No.	Positive	(%)	No.	Positive	(%)
Maragua	41	30	(73)	23	10	(43)	41	35	(85)
IDH	87	84	(95)	43	24	(56)	87	84	(95)
Total	128	114	(89)	66	34	(51)	128	119	(93)

Table 2. *Correlation between detection of measles virus by immunofluorescence in NPS and rise in HAI antibody*

		Immunofluorescence positive		Immunofluorescence negative	
	HAI	Rise	No rise	Rise	No rise
All children	Maragua	5	9	5	3
	IDH	24	18	0	1
	Total	29	27	5	4
Acute specimen taken < 4 days from onset of rash	Maragua	4	2	3	1
	IDH	17	11	0	1
	Total	21	13	3	2

the presence of HI antibody to measles. Table 1 shows that a four-fold or greater rise was demonstrated in half of the children. Convalescent titres ranged from 32 to 512 and age did not appear to affect the ability of the child to mount a satisfactory antibody response. Table 2 shows the correlation between detection of measles virus by IF and a rise in HI antibody. Children who were first seen fewer than 4 days after the appearance of the rash appeared to show a greater proportion of rising titres than those first seen 4 days or more after the rash, but the difference was not significant. The single IDH child in whom measles virus was not demonstrated by IF did not show a rise in HI antibody. This was the child who was admitted twice and the paired sera refer to the first admission, but a third specimen, convalescent for the second admission was not obtained. Five of the eight Maragua children negative by IF showed a rise in HI antibody. The child from whom an NPS was not obtained did not show a rise in measles antibodies and has been excluded from all further analyses. The last columns of Table 1 summarize the relationship between a clinical diagnosis of measles and the laboratory confirmation of a measles virus infection. In only 9 of the 128 children was the clinical diagnosis not confirmed by the laboratory, and there was a significantly better clinical diagnosis of measles at IDH than at Maragua (chi-squared = 5·334, $0{\cdot}01 < P < 0{\cdot}025$).

The vaccination history was obtained from the parents of all but one of the children, although confirmation by date of birth or examination of the 'clinic card' was only possible in approximately two thirds. As measles vaccination in Kenya

Table 3. *Laboratory diagnosis of measles virus infections from children with a clinical diagnosis of measles*

Vaccination status*	Measles positive			Measles negative			Per cent positive
	IDH	Maragua	Total	IDH	Maragua	Total	
Definitely not vaccinated	24	12	36	2	1	3	92
Probably not vaccinated	19	5	24	0	1	1	96
Vaccinated within 21 days of onset	6	2	8	1	0	1	89
Definitely vaccinated	19	12	31	0	4	4	89
Probably vaccinated	7	4	11	0	0	0	100
Possibly vaccinated	8	0	8	0	0	0	100

* For definition of the groups see text.

Table 4. *Age at which measles observed*

Age range	Unvaccinated*		Vaccinated*	
	IDH	Maragua	IDH	Maragua
Up to 6 months	5	2	—	—
7–8 months	7	6	—	—
9–12 months	10	5	3	1
13–15 months	2	3	5	2
16–18 months	6	0	2	2
19–24 months	3	1	6	3
Over 24 months	10	0	10	8
Total	43	17	26	16

* The vaccinated group comprises the definitely and probably vaccinated and the unvaccinated group comprises the definitely and probably not vaccinated.

is now routinely carried out after 8 months of age, all children less than 8 months old were accepted as being unvaccinated. In older children whose parents were unable to produce a 'clinic card' the vaccination status was classified as (i) probably not vaccinated when the parent stated unequivocally that the child had not been vaccinated; (ii) probably vaccinated when the parent could give the date and place of vaccination; and (iii) possibly vaccinated when the parent said the child had been vaccinated but could not remember when or where.

There was a group of nine children who had received measles vaccine fewer than 21 days before the onset of symptoms in whom the protective effect of the vaccine was uncertain and who are considered separately from the other 'vaccinated' categories. Table 3 details the laboratory findings in relation to the six vaccination categories and shows that the proportions of positives is similar in all of them. Table 4 gives the breakdown according to age of the combined 'definite' and

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'probable' categories and shows that the majority of the unvaccinated children who contracted measles were 9 months or older.

Measles virus was successfully isolated from 9 children, including 3 who had a history of prior measles vaccination. In every case there was a low or undetectable level of HI antibody at the time of collecting the specimen and in only two of the children had the rash been present for more than 2 days.

Although no comprehensive effort was made to identify other viruses in these children, RSV was found by IF in 7, herpes simplex was isolated from 6 and adenovirus from 4. In every one of these 17 children a measles virus infection was also demonstrated.

Several parents claimed that their child had suffered from an illness similar to measles and for five the description appeared to be identical with classical measles. Measles virus was demonstrated in three of the children, all of whom had been vaccinated, while the two in whom there was no evidence of a concurrent measles infection had not been vaccinated.

DISCUSSION

The first objective of this survey was to determine whether or not the illness clinically diagnosed as measles in children who had previously been vaccinated against measles was true measles. Previous investigations, using only serological methods, had concluded that there appeared to be a disease in Tropical Africa which resembled classical measles, but was caused by a different agent (Munube, 1979). Our results show unequivocally that classical measles can occur in children who have been vaccinated.

The principal laboratory method for identifying a measles virus infection was IF, but whenever possible paired sera were also collected for testing by HI. As expected (McQuillin *et al.* 1976), IF proved to be the more sensitive method, although five children in whose NPS measles antigen could not be detected showed a significant rise in HI antibody titre. All these children were seen at the Rural Health Training Centre, Maragua, where the specimens were collected from 09.30 to 12.30 and kept on melting ice until processing started following the return to the VRC laboratory in Nairobi, about 3 h after the last specimen was taken. At the Infectious Diseases Hospital the specimens were collected from 11.00 to 12.30 and were taken directly to the laboratory for immediate processing. It therefore appears that the delay of up to 5 h in the commencement of processing an NPS may result in the loss of the measles-antigen-containing cells from a proportion (15%) of the specimens.

The results show that the vast majority of the children diagnosed as having clinical measles were infected with measles virus. At Ward 3, IDH, where all the children are admitted as suspected cases of measles the correlation between laboratory and clinical diagnosis was very high (95%). The laboratory diagnosis of measles in 85% of the children suspected of having measles at Maragua is particularly gratifying when it is realized that the Clinical Officers have to make their diagnosis while seeing a daily average of some 700 out-patients of all ages and with all manner of clinical conditions.

There were no significant differences detectable between children who had been vaccinated and those who had not, with similar proportions of those clinically

diagnosed as measles being confirmed by the combined laboratory methods. However it did appear as if previous vaccination increased the possibility of failure to detect measles antigen in the NPS specimens transported from Maragua, although the numbers were too small for adequate statistical analysis. No differences in the number of sero-conversions nor in the absolute levels of acute or convalescent HI antibody titres could be detected between vaccinated and unvaccinated children. These observations suggest that the children developing measles after vaccination are those who failed to seroconvert after administration of the vaccine (Leeuwenburg & Muller, 1980). However, the parents using Maragua Clinic stated that the measles vaccine was useful because even when it did not prevent measles the resulting illness was usually mild. It has proved very difficult to make a critical assessment of this proposition, but analysis of the age of the children seen at Maragua suggests that vaccination at 8 months may confer partial or transient immunity. The 33 measles positive Maragua children are a representative selection of all children developing measles in the area served by the clinic. Nine of the 17 unvaccinated children who developed measles were 9 months or older and had therefore missed the immunization programme. It is estimated that over 80 % of all children in this area have received measles vaccine and a batch of the vaccine in use at the time of the survey was taken to the VRC and found to be well above minimum potency. If the vaccine was not effective at all, one would therefore expect to see between 40 and 50 cases of measles in vaccinated children 9 months or older. The actual number seen was 16, indicating that about two thirds of the vaccinated children have been protected. All of the 9 unvaccinated children were less than 2 years old whereas half (8 of 16) of the vaccinated children were over 2 years of age. While this does not prove that the immunity induced by vaccination at 8 months wanes after 2 years, it appears to be the simplest explanation of our figures and a recent outbreak among school children in the USA also indicates that this is probable (Centre for Disease Control, 1984).

In conclusion, the results of this survey indicate that the following pilot study should be set up to investigate the effects of the following. First, attempt to give the primary vaccination at 8 months to over 90 % of the children and reduce the number of susceptible children between 9 and 15 months of age. Second, revaccinate all children at 12–13 months of age to protect those who missed the initial vaccination or in whom it failed and to boost the immunity in the remainder. (In Europe and America it has been found that 12–15 months is the earliest age at which it is possible to achieve almost 100 % protection.) Finally, as a significant number of children develop measles before 8 months, it would be useful to select a second area where the initial vaccination is given from 6 months onwards, followed by revaccination at 12–13 months.

It seems probable that an intensive double-vaccination programme aimed at ensuring that over 95 % of all children are protected could reduce the susceptible population below the level at which measles could be maintained in the community (Mitchell & Balfour, 1985), thereby making the total eradication of measles in Tropical Africa a real possibility.

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