Original Articles

A CONTROLLED TRIAL OF MEDIUM TITRE MEASLES VACCINATION AT SIX MONTHS OF AGE: PROSPECTIVE STUDY IN RURAL SUDAN.

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Abstract:

In a rural community comprising 35000 population, 510 infants born within a ten-month period were enrolled from a birth cohort. They were allocated into three equal groups of 170 Two types of measles vaccines, namely medium titre (4.7 log pfu/ml) Edmonston-Zagreb(EZ) or an equivalent dose of Connaught(CON) vaccine were used in a double blind randomized trial to compare their immunological response in infants aged five months. The third group of infants acted as a control and was vaccinated with standard titre (3.9 log pfu/ml) Schwarz vaccine at nine months of age. Measles antibody titres were measured before and 18 weeks after vaccination using plaque neutralization assay. All vaccine groups were monitored for the first three weeks for any adverse reaction and were then prospectively followed up for 60 months.

The mean age of the infants was 20.2 weeks and the three groups were comparable as regards to nutrition. health, family and socioeconomic characteristics. No severe   side effect of any of the vaccines used was reported and there were no significant differences in the rates of adverse reactions between the vaccine groups and the control group. Sera of 153(90%)of the EZ recipients and 149(88%) of the CON group showed seroconversion rates of 71% and 78%, and seropositivity rates of 71% and 69%, respectively. Follow up of the infants showed that early medium titre EZ vaccine conferred adequate clinical protectivity against measles infection for five years.

Thirty months after vaccination, a total of 35 deaths were reported: 14 in the EZ group, of whom 10 were females; 13 in the control group including six females; and eight infants from the Co naught group. The mortality rate among females within the Edmonston-Zagreb vaccinee was just significantly higher than that of males (p<0.05).

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Introduction

For nearly 30 years the means to prevent measles infection has been available in the form of effective vaccine. However, despite this potent intervention, measles still accounts for approximately 1.5 million deaths per year.[1,2] Many of these deaths occur in infants below nine months of age where measles is associated both with an increased mortality and with a higher rate of complications; blindness, pneumonia, prolonged diarrhoea and malnutrition prevail in many children who survive the acute illness.

The Sudan, with its 26.4 million population 80% of whom are rural, 16% of the children are under five and the estimated infant mortality rate is 97 per 1000.[2,3] Measles is prevalent especially during summer and winter.[4,5] The proportion of infants vaccinated against measles was estimated to be 47% and measles epidemics continued to be a problem.[6] The case fatality rate ranged from 4% in hospital cases to 13-33% in epidemics.[4,5,7] It was estimated that measles infection accounted for 20% of the infant mortality rate in the Sudan.[8] Not surprisingly, Sudanese infants aged 6-9 months were found to have no measurable measles antibodies and the rate of seroconversion amounted to 80% when low-titre measles vaccine was administered to this group.[8] In other developing countries such as Bangladesh, Gambia and Mexico, a relatively high degree of seroconversion occurred when measles vaccine was administered in a potent dose to infants as young as five or six months of age.[9-11] Elsewhere, high titre vaccines has been demonstrated to be highly immunogenic at 4-6 months of age, and the vaccine induced antibodies to measles persisted for up to two years.[12-14] However, there was no previous measles vaccine study conducted in rural areas in a Subsaharan region of Africa. Moreover, the effectiveness of administrating some vaccines, namely Connaught, to infants less than nine months of age has not been investigated.

The aim of this study was to compare the immunogenicity and efficacy of medium titre vaccines namely Edmonston-Zagreb (EZ) and Connaught (CON) measles vaccines in infants younger than six months of age, with standard low titre Schwarz (SH) vaccine given at nine months. It is also aimed to compare the clinical protectivity of these vaccines against measles infection. This was coupled with short and long term prospective

follow up to monitor the acute adverse reactions as well as compare the morbidity pattern and mortality rates between the vaccine groups.

Subjects Material and Methods:

The study was conducted in East Nile province of Khartoum State, about 50 kilometres east of the capital Khartoum. This is a rural community with a total population of 35000. Fourteen villages were selected, because they are easily accessible to transport and have stable non-migratory population. An integrated antenatal and perinatal care programme has been initiated in the area since 1985 using the local village midwives(VMs) for data collection and this provided accurate registration of births.[15] The cohort of this study was derived from this register. Twenty educated village girls (interviewers) were recruited, and the study objectives, protocol, and methodology was explained to them. They were trained to do anthropometric measurements and record axillary temperatures using digital thermometers. With the help of the VMs, the interviewers(IVs) explained the study to the parents and encouraged mothers to bring their infants for enrollment in the study. Other main tasks of the IVs was to conduct home visits to the infants vaccinated, alleviate any maternal worries and complete follow up records each time they visit.

Out of a total of 543 infants born within the study area between January 1st and October 1st 1989, 510 were enrolled. Infants for whom consent could not be obtained, those with significant malnutrition (less than 60% weight for age), or past history of measles infection or measles vaccination, as well as infants who were acutely ill at the time of recruitment were excluded from the study. The infants were aged between 17 and 23 weeks at the time of recruitment.

Two measles virus vaccines namely, medium titre Edmonston-Zagreb (Institute of Immunology, Zagreb. lot 158/2 4.7 log pfu/ml) and medium titre Connaught (Connaught laboratories Toronto lot 4488-1 4.7 log pfu/ml) were used in a double blind fashion together with Group A/C polysaccharide meningococcal(M) vaccine as a placebo. The latter was used routinely in the local Expanded Programme of Immunization (EPI). Likewise, the standard titre , Schwarz measles virus vaccine (Smith Kline Laboratories. Brussels, lot M-132 3.8 log pfu/ml TCID), provided by the EPI was also used. Cold Chain monitoring was maintained all along the line and vaccine potency testing for the EZ and CON, as determined by the manufacturer was made initially, midway and at the end of the field work.

The clinic sessions were held in the rural hospital, the health centre or the small health units in the villages. After obtaining informed consent from

the parents, medical history was taken; clinical examination and anthropometric measurements (weight, length and head circumference) were recorded. The infants were randomly allocated into three equal groups of 170 using the method of restricted randomization1.[16] An individual
would therefore have an equal probability of receiving either EZ, CON or a placebo (M) vaccine. Each vaccine was administered as 0.5 millilitre intramuscular injection on the right thigh. Heal prick blood samples collected in microcontainer tubes were taken initially before the vaccines were given and later on the second visit when the infants were nine months old. The blood was allowed to clot and the sera were transferred into small tubes stored in cold boxes to be transported to the laboratory and eventually deep-frozen (-20 0C). Serological analysis using plaque neutralization assay was performed in the Dept of Microbiology, London School of Hygiene and Tropical Medicine. By the time of the second visit decoding of the vaccines administered was completed and accordingly, infants who initially received EZ vaccine were given placebo i.e. meningococcal vaccine while those who had had the CON or meningococcal vaccine were revaccinated with standard (low titre) Schwarz vaccine. All mothers and IVs were instructed to report immediately any adverse event or reaction following vaccination.

During the first three weeks (from day 3 to day 21) post vaccination, the infants were visited at their homes every three days. In each visit, the IVs would inquire about the infants' health during the previous three days, observe the infants, take the axillary temperature, note any adverse event and complete vaccine side effects forms. Subsequent follow up was made biweekly for 18 months and then monthly afterwards for a period of 56-64 months; this was to complete the morbidity/mortality records. Specific inquiry of measles infection or measles contact in the household or neighbourhood was made each time the IVs visit. Study infants suspected of having contracted measles were timely visited and examined by the field supervisor (physician) and measles infection record would then be completed. Details of the circumstances of death of reported mortalities were also noted by interviewing the parents of the deceased.

The data was tabulated using Epiinfo software. Chi square test was used with 95% confidence limit. Statistical methods used to compare the serological data included logistic transformation to calculate the geometric mean, student t-test, correlation coefficient and linear logistic regression analysis.

Results

The infants were vaccinated during the period from June 1989 to July 1990. Out of the 510 infants enrolled in the study 477(94.5%) were aged between 18 and 22 weeks, 8(1.6%) and 25(4.9%) completed 17 and 23 weeks, respectively. The mean (SD) age was 20.2(1.5) weeks; 279(54.7%) of them were males and 231(45.3%) were females with a male:female ratio of 1.2:1.0. The mean (SD) weight was 6.6(0.9) kilograms and 492(96.4%) of

the infants weighed between 5.0 and 9.0 kilograms: 502(98.4%) measured between 56.0 and

70.0 centimetres and the mean (SD) length was 63.1(2.8) centimetres. There was no significant difference between the three groups as regards to infants' age, sex, weight, length, or nutritional status. During the two weeks prior to the time of recruitment, 159 (31.2%) od the infants suffered minor ailments, 97(19%) of these needed medical attention but only 5(1.0%) required hospitalization and all groups were similar in this respect (Table 1). The groups were also comparable with respect to the socioeconomic indicators including mothers' age, parity, education, social class, family income as well as distance of home from the health units (Table 2).

The commonest side effects of the vaccines encountered were diarrhea, fever and rhinitis. There was no significant difference in the incidence of acute adverse effects of vaccination between the three groups (Table 3).

Sera collected before and approximately 18 weeks after vaccination, from 153(90.0%) of EZ vaccine recipients and 149(88.1%) of the CON group were analyzed (Consent for the second blood sample could not be obtained for the remaining infants). The seroconversion rates i.e. four-fold rise in antibody level, for EZ and CON groups were 71.0% and 78.1%, respectively. The seropositivity rates 18 weeks after vaccination with EZ and CON vaccines, were 71.7% and 69.1%, respectively. There was no significant difference in either the rate of seroconversion or the rate of seropositivity between the two groups.

Prospective follow up of all the study infants (except two, who moved outside the area) for 56-64 months showed that 12 out of 14 infants who contracted measles infection were males; only one case developed the disease before the age of nine months and this particular infant received the placebo vaccine at five-month of age. Six and five of the infants affected belong to the EZ and Connaught group, respectively (Table 4). They all had a modified and a mild form of the disease.

A total of 35 deaths were reported - all occurred below the age of three years. For the period of time between six and nine months of age, there

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was no death among infants receiving the medium titre EZ vaccine, one death among the Connaught group, and six deaths among Schwarz vaccinee. Subsequent to nine months of age, there were 14 deaths in the EZ vaccine group, compared to seven in each of the Connaught and Schwarz groups (Table 5). The relative risks of mortality for the infant in the EZ group compared to one in the Schwarz group at 5-35 months and at 9-35 months were 1.06 (CI: 0.05 to 2.25, p=0.89) and 2.00 (CI: 0.75 to 5.54, p=0.19), respectively. The mortality ratio among female Edmonston-Zagreb vaccinee was higher (1.77: CI 0.05 to 6.22. p=0.37) compared to the
Schwarz group. Within the EZ vaccinated group of infants, females had higher mortality rate than did males with a mortality ratio of 3.95 (CI 1.03 to 15.13, p=0.04). No sex difference was detected in the other groups. No sex difference was observed in the other groups.
No deaths were reported after the age of thirty months. The distribution of deaths according to cause of deaths is shown in Tables 6.

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Table 1. **Comparison of vaccine groups according to infant characteristics at time of recruitment**

	Mean (SD)		
Group	Edmonston-Zagreb	Connaught	Schwarz
Criteria			
Age(weeks)	20.1(1.5)	20.3(1.6)	20.1(1.5)
Males/females%	52.9/47.1	60.0/40.0	51.2/48.8
Weight-for-age (z-score)	-0.293(1.065)	-0.206(0.936)	-0.135(1.039)
Length-for-age (z-score)	-0.503(1.195)	-0.417(0.912)	-0.426(0.948)
Breastfed/ Weaned%	95.3/4.7	97.1/2.9	95.9/4.1
%Solids introduced/ not introduced	81.8/18.2	74.7/25.3	75.9/24.1
% Illness previous 2-weeks	30.0	30.0	33.0

Table 2. Comparison of vaccine groups according to family and social characteristics

Group	Mean (SD)		
	Edmonston-Zaqrib	Connaught	Schwarz
Criteria			
Infant birth order	4.5(2.6)	4.3(2.8)	4.4(2.6)
No. of siblings	3.0(2.4)	2.8(2.4)	2.9(2.3)
Mothers' age(years)	26.4(6.9)	25.3(5.8)	26.2(6.8)
% of educated mothers/ illiterates	34.1/65.9	32.4/67.6	34.5/63.5
% Income Satisfactory/ Poor	29.4/70.6	25.3/74.7	23.5/76.5
Distance*	1.9(1.4)	1.6(1.2)	1.8(1.4)

* Distance from home to the nearest health unit in kilometres.

Table 3   **RATES OF ACUTE CLINICAL REACTIONS**
(REPORTED DURING THE FIRST 21 DAYS FOLLOWING VACCINATION)

VACCINE GROUP	NUMBER OF INFANTS STUDIED	PER CENT OF INFANTS EXPERIENCING ADVERSE EVENTS DURING THE FIRST 21 DAYS FOLLOWING VACCINATION								
		ANY ILLNESS	FEVER	DIARRHEA	VOMITING	RASH	COUGH	RHINITIS	CONJUNCTIVITIS	OTHER*
EDMONSTON - ZAGREB (MEDIUM)	73	57 (78.1%)	28 (38.4%)	42 (57.5%)	6 (8.2%)	5 (6.8%)	10 (13.7%)	29 (39.7%)	2 (2.7%)	0 (0.0%)
CONNAUGHT (MEDIUM)	77	57 (74.0%)	28 (36.4%)	44 (57.1%)	12 (15.6%)	5 (6.5%)	11 (14.3%)	28 (36.4%)	9 (12.3%)	0 (0.0%)
SCHWARZ (STANDARD)	70	54 (77.1%)	27 (38.6%)	41 (58.6%)	9 (12.9%)	5 (7.1%)	15 (21.4%)	25 (35.7%)	8 (11.4%)	0 (0.0%)

* OTHER includes deaths, convulsions, or any other life threatening event occurring within 21 days after vaccination.

* Differences between the vaccine groups are not statistically significant.

Table 4.	**Measles Cases**			
Infant ID number	Age (weeks)	Sex	Vaccine given	Outcome
34	32	Male	Placebo	Recovered
140	42	Male	EZ	Recovered
350	93	Male	Schwarz	Recovered
382	98	Male	EZ	Recovered
254	102	Male	EZ	Recovered
120	110	Male	EZ	Recovered
280	131	Male	Schwarz	Recovered
216	145	Male	CON	Recovered
188	151	Female	CON	Recovered
50	156	Male	EZ	Recovered
252	160	Male	Schwarz	Recovered
310	160	Female	Schwarz	Recovered
162	161	Male	EZ	Recovered
249	181	Male	CON	Recovered

CON   = Connaught.
EZ   = Edmonston-Zagreb.
Placebo   = Meningococcal vaccine.

Table 5. Distribution of deaths among vaccine groups according to gender and age of death

Age of death(mon)	Number of deaths			Total male/female
	5 - 9	9 – 24	24 - 35	
Vaccine group				
Edmonston-Zagreb	0	7	7	4/10
Connaught	1	5	2	6/2
Schwarz (Control)	6	5	2	7/6
Total	7	17	11	17/18

Table 6. **Distribution of deaths among vaccine groups according to reported cause of death**

| | Number of deaths | | | |
| | | | | Total |
Group	Edmonston-	Zabreb	Connaught	Schwarz
Cause of death				
Diarrhea/viomiting	7	5	8	20
Chest infection	4	1	1	6
Febrile illness	1		1	2
Malnutrition	1			1
Meningitis			1	1
Accidental			1	1
Unknown	1	2	1	4
Total	14	8	13	35

Discussion

The community-based antenatal perinatal programme running in the study area is unique in the Sudan and it provided an accurate birth register. The sample selected represented about one third of the infants born in the area during the period of the study.[17] The groups were comparable in health, nutrition, familial and socioeconomic parameters. There was an excellent compliance during the first visit. However, many mothers needed persuasion to bring their infants back for revaccination. This was primarily due to the apprehension concerning blood taking which has a deeply rooted concern in our society. Yet, the drop out rate for the second visit was very small.

As shown elsewhere, the vaccines were potent and safe with minimal side effects.[18] There was no severe adverse reaction reported in any of the vaccinated infants. Although nonspecific and transient skin rash was reported, yet none of the infants developed measles infection within one month of vaccination. No significant differences in the minor adverse reaction rates were observed between the infants in the three groups.

The immunological response of the infants to both the EZ and ÇON has been remarkable and is comparable to those reported from other studies.[9-11,18,19] The pre and post serological measurements in infants given EZ and Con vaccines indicated that there was no significant difference between the two vaccines' ability to induce an immune response. Connaught vaccine proved to be immunologically as potent as the equivalent dose of EZ vaccine. There was no published study that used medium or high dose Connaught vaccine administered to infants at any age. However, standard titre Connaught vaccine given to 3-9 months infants in Tanzania and 5-8 months infants in Ghana produced a seroconversion rate of 55% and 100%, respectively[20,21].

The gold standard of vaccine protection is the prevention of measles infection rather than the production of antibodies. Clusters (ranging between 2-13) of measles cases had frequently been occurring in these villages where the coverage of measles vaccination was less than 50%[6] Yet the study infants were the least affected. The number of measles cases among the three study groups is too small to draw conclusions as regard to the protectivity of EZ compared to Schwarz vaccine. Yet the study suggests that EZ measles vaccines given to 4-5 month old infants, was as clinically protective against measles infection for five years as low titre Schwarz vaccine administered at nine months.

Survival

The majority of the children were regularly followed up to their fifth birthday. The drop rate was not statistically significant. Because infants given the CON vaccine at four months had also received the standard titre Schwarz vaccine at nine months of age, the interpretation of the CON data will be difficult. It would be appropriate, therefore, to compare the mortality risks in association with medium titre EZ vaccine and standard titre Schwarz vaccine.

At three years of age, the mortality ratio of the EZ group of infants was comparable to the control subjects. Even when excluding deaths prior to nine months of age, the mortality ratio does not become significant between the two groups. This latter exclusion, however, will give a total childhood mortality of 27.5 and 13.7 per 1000 for the EZ and the control group, respectively. The background mortality in the study area for the period between 1985 and 1990 had recently been studied. The childhood (one to four years) mortality per 1000 were 22.8, 27.4 and 23.0 for the years 1988, 1989 and 1990, respectively, giving a mean mortality of 24.4 per 1000 live births[22] Clearly, the mortality rate among the EZ vaccinee is comparable to the background mortality but it is not significantly higher than that of the Schwarz group ($X^2=0.03$,$p<0.85$).

Although female infants the EZ group had higher mortality ratio than males compared to that in the control group but the differences were not statistically significant ($p=0.37$). However, within the EZ vaccinee, the mortality ratio among females compared to males just reached the significant level ($p<0.05$). No association was detected between mortality and any of the nutritional or socioeconomic indicators.

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