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Antibody response to routine measles vaccination among a population of Nigerian children and evaluation of vaccine potency

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Abstract *Background:* Despite a global decline in mortality and morbidity from measles in the last decade, outbreaks continue to occur in some parts of the world including Nigeria.
Objective: To determine antibody response to routine measles vaccination in Nigerian children and evaluate vaccine potency.
Methods: A prospective study of 234 children selected from 3 health centres in an urban area of Lagos, Nigeria. Blood was obtained before and 8-12 weeks after routine vaccination with Edmonston-Zagreb strain of measles vaccine. Antibodies were detected using the measles antibody neutralization test. Reconstituted vaccines samples were analysed for potency on monolayers of Vero slam cells in 96-well tissue culture plates.
Results: Twenty seven(11.5%) had pre-vaccination antibodies.
Seroconversion rate among the 195 who returned for post-vaccination sampling was 69.2%: It was however 74.2% in children with no pre-vaccination antibodies compared to 17.6% in those with antibodies. ($p<0.05$). Only six (50%) of the measles vaccine vials were potent. Seroconversion rate among subjects vaccinated from potent vials was 74.3% compared with 42.9% in those vaccinated from non-potent vials ($p = 0.006$).
Conclusion: Seroconversion to measles vaccination in our environment is sub-optimal, partly attributable to low vaccine potency. Improvement of vaccine handling processes and booster doses of the vaccine are recommended.

Introduction

The burden of measles, a highly communicable, vaccine preventable, disease is highest in developing countries.[1-2] In 2008, there were over 270,000 reported cases with about 197,000 deaths globally, 95% of them in the developing world.[2] Although global measles deaths fell by 74% between 2000 and 2007 largely due to intensified efforts at vaccination,[2] there have been continued reports of outbreaks in some developing countries, including Nigeria.[2] Recently, however, there appears to be a resurgence in measles outbreaks in developed countries like the United States of America where measles was previously reported to be practically eradicated.[3] This has largely been attributed to imported cases of measles as well as waning vaccine immunity in previously vaccinated children[3].

In Nigeria, measles vaccine is routinely administered at the age of nine months. Clinically apparent measles before the 9th month vaccination and in vaccinated children,[4-5] however suggest low passive antibody positivity levels and failure of seroconversion respectively. The World Health Organization (WHO) therefore recommends a second dose in childhood either routinely or through supplementary vaccination activities.[1] Between 2006 and 2007, over 56 million Nigerian children aged 9 months to 15 years were vaccinated through massive nationwide measles supplementary immunization activities.[6] This contributed to the >70% decline in global measles mortality during that period.[7] In spite of this however, outbreaks continue to occur in the country.[2] The Paediatric Association of Nigeria (PAN) as part of it's commitment to reducing the burden of vaccine preventable diseases in Nigerian children, therefore recently recommended a second dose of measles vaccine in children.[8] Sero-epidemiological studies are essential for the monitoring and evaluation of the effectiveness of vaccination programmes. This study is designed to assess seroconversion rates after the nine month vaccination among a group of infants and evaluate vaccine potency in Lagos, Nigeria.

Materials and methods

It was a prospective study conducted between January

Table 1: Comparative distribution of pre-vaccination measles neutralizing antibodies in all 234 subjects at the three primary health centres.

Centre	Pre-vaccination Titres <1:40	1:40	1:80	1:160	1:320	1:640	1:1280	1:2560	Total N	%
A	94	5	-	-	2	1	-	2	104	44.4
B	49	3	1	3	-	1	-	-	57	24.4
C	64	5	2	1	-	1	-	-	73	31.2
Total N	207	13	3	4	2	3	-	2	234	100
%	88.5	5.6	1.3	1.7	0.9	1.3	-	0.9	100	

Table 2: Comparative distribution of post-vaccination measles neutralizing antibodies at the three primary health centres.

Centre	Post-vaccination Titres <1:40	1:40	1:80	1:160	1:320	1:640	1:1280	1:2560	Total N	%
A	12	5	9	6	9	11	7	27	86	44.1
B	12	9	1	5	2	8	5	3	45	23.1
C	20	7	8	2	4	5	8	10	64	32.8
Total N	44	21	18	13	15	24	20	40	195	100
%	22.6	10.8	9.2	6.7	7.7	12.3	10.3	20.5	100	

The percentages shown are of 195 children who returned for the post-vaccination sampling.

Table 3: Distribution of seroconversion following measles vaccination by centre.

Centre	Seroconversion No N (%)	Yes N (%)	Total N (%)
A			
All	19 (22.1)	67 (77.9)	86 (100)
No PVA	13 (16.3)	67 (83.8)	80 (100)
B			
All	17 (37.8)	28 (62.2)	45 (100)
No PVA	11 (36.8)	26 (70.2)	37 (100)
C			
All	24 (37.5)	40 (62.5)	64 (100)
No PVA	22 (36.1)	39 (63.9)	61 (100)
Total			
All	60 (30.8)	135 (69.2)	195 (100)
No PVA	46 (25.8)	132 (74.2)	178 (100)

PVA= Pre-vaccination antibodies. All: x^o x = 4.70, df2, p= 0.095.

Effect of pre-vaccination antibodies on seroconversion

When analysis was restricted to subjects with no pre-vaccination antibodies, there was a rise in seroconversion rates in all the centres while overall rate rose from 69.2% to 74.2%. (Table 3).Of the 27 subjects who had pre-vaccination antibodies, 17 returned for the post-vaccination sampling out of which three (17.6%) seroconverted. Five had a drop in titre, four no longer had detectable antibodies while the titre remained the same in the remaining five. The difference in seroconversion between children with and those without pre-vaccination antibodies was found to be statistically significant. p= <0.05 (Fisher's exact test)

Effect of vaccine potency on seroconversion

Twelve vials were analysed for potency. Six (50%) had potency levels above the minimum WHO required standard of $\log 10^{-3}TCID_{50}$. (Table 4). The vaccine potency rates were 60%, 25% and 66.7% from centers A, B and C respectively. Ninety-four subjects were vaccinated from these vials. Forty-three received potent vaccines of which 35 returned for the post-vaccination with seroconversion in 26. Non-potent vaccines were administered to 51 of which 42 returned for the post-vaccination sampling with seroconversion in 18. The seroconversion rates in the two groups were thus 74.3% and 42.9% respectively. x^o=7.70, df1= 1, p=0.006. The duration of administration of the vials ranged from 45minutes to 118minutes but this was found not to be related to the vaccine titre. (r=0.23, p=0.48).

Table 4: Potency and duration of administration of measles vaccines at the three centres

Centre	Vaccine (CCID50)per dose	Duration minutes
A	4.5	118
A	4.0	75
A	4.0	62
A	2.0	90
A	1.0	65
B	3.5	45
B	2.0	72
B	2.0	55
B	1.5	87
C	3.5	85
C	3.0	105
C	1.0	75

Discussion

The WHO recommendation to administer measles at the age of nine months is predicated upon the need to maintain the delicate balance between the need for early protection of children and the limitation of possible neutralization of the live vaccine by transplacentally acquired antibodies. Expected seroconversion rate at this age under optimal conditions, which is not always achievable is about 85% hence the WHO recommendation of a second dose of the vaccine to provide immunity in non-responders.[1]

The overall seroconversion rate of 69.2% obtained in this study is higher than most previous reports from this country which range from 24% - 69%.[12-14] Higher rates have been reported from similar developing countries such as Turkey(77.6%)[15] and India (>90%),[16] while a rate of 68.8% similar to ours was reported from thailand[17] We also observed variable degrees of seroconversion among study subjects implying variable levels of protection. Bearing in mind that antibody titres wane

and April 2007 at three health centres in an urban area of Lagos State, Nigeria. One centre(A) was chosen because it also serves as the Local Government Area(LGA) vaccine store from where vaccines are dispensed to the other centres. The quality of vaccines there would therefore represent the best offered by the LGA. The others(B and C) were chosen by simple random sampling from a list of all the six health centres managed by the LGA. Ethical approval was obtained from the research and ethics committee of the Lagos University Teaching Hospital (LUTH) and informed consent from parents/ caregivers of study subjects. Calculated sample size was 195. An additional 39(20%) subjects were recruited because of anticipated attrition bringing total sample size to 234.

Data Collection

Children aged 9-12 months who were apparently healthy were consecutively enrolled. Exclusion criteria were parent/caretaker refusal, acute illness and a past history consistent with measles as defined by the centers for disease control.[1] Their names, ages, sex, weights, lengths as well as telephone numbers and contact addresses of caregivers were obtained. About 3mls of blood was obtained via a peripheral vein from each child under aseptic conditions followed by a subcutaneous injection of 0.5mls of the Edmonston-Zagreb strain measles vaccine. The vaccines were both reconstituted and administered by National Programme on Immunization trained nurses. All were from the same batch and within the expiry date of the manufacturer. Subjects were then given an 8-week appointment for repeat blood sampling as above. Defaulters were contacted through telephone calls or home visits and their post- vaccination visits rescheduled within a two-week frame. The last doses of vaccine in alternate reconstituted vaccine vials were transported in an insulated cold box at -4^0C to the laboratory and stored in a freezer at -80^0C till time of analysis. The time interval between reconstitution and delivery of the last dose in each vial was noted.

Laboratory analysis

Measles neutralizing antibodies were detected using the measles antibody neutralization test [9-10] which is based on the ability of the measles antibodies to measure virus infectivity. Serial two-fold dilutions of each serum were prepared starting from 1/40 to 1/2560 on 96-well tissue culture plates. 0.1ml of measles virus stock in Vero slam cells containing 100 TCID50 was then added to each serum diluent and incubated at 37^{oC} for an hour. This was further incubated with 0.1ml of Vero slam cells in a $C0_2$ rich environment for 7 days. Infectivity of the measles virus on tissue cells was visualised after staining with crystal violet. The last column on each plate contained the measles virus stock incubated with Vero slam cells alone and served as controls. The end-point of the virus titration was taken as the highest dilution with visibly stained cells. Plates were read by two of the authors and there was concordance in the judgments of both observers in 421 (98.1%) of 429 readings.

Seroconversion was defined as either a change from negative to positive or a four-fold rise in antibody titre following vaccination. Vaccine potency was determined according to WHO guidelines on monolayers of Vero slam cells in 96-well tissue culture plates. Serial dilutions of the vaccine were incubated with the cells at 36^0C for 7-9 days. Virus end-point titres were calculated in $CCID_{50}$ per vial using the method of Reed and Muench.[10-11] Analysis was conducted in the virology laboratory of the LUTH under the supervision of a virologist trained in WHO assays.

Statistical Analysis

Data was analysed using version 16 of the Statistical Programme for Social Sciences (SPSS) and Microsoft Excel. Measures of central tendency such as mean and standard deviation were generated for continuous variables like weight and height. Chi-square tests was used to test statistical significance between discrete data. Probability(p) value <0.05 considered statistically significant.

Results

Pre-vaccination blood samples were obtained from 234 subjects. Their ages ranged from 8.8 to 12.1 months with a mean of 9.5 ± 0.56 months. Mean weight and length were 8.5 (±1.0) kg and 73.0(±2.7) cm respectively with 231(99%) having weight for age Z scores within two standard deviations of the mean. One hundred and ninety-five returned for the post-vaccination sampling resulting in 16.6% attrition rate. Of the rest, one died following a diarrhoeal illness, six declined the second visit, and five had relocated out of reach while 27 could not be contacted.

Laboratory results

The distribution of pre-vaccination antibody titres by centre is shown in Table 1. Thirteen children (5%) had neutralizing antibodies at the lowest dilution of 1:40, fourteen (5.9%) between 1:80 and 1:2560 while 207 (88.5%) had no detectable antibodies. Following vaccination, 151(77.4%) now had titres at dilutions ranging from 1:40 to 1:2560 while 44 (22.6%) had no detectable antibodies at a dilution of 1:40. (Table 2) Seroconversion rates are as shown in Table 3. The overall seroconversion rate was 69.2% but differed according to centre though this did not reach statistical significance. ($p=0.095$)

with time,[18] children with lower titres may therefore be at risk of losing their protective immunity. Seroconversion increases with age at vaccination, corresponding to reduced levels of maternal antibodies, the highest rates being in children vaccinated after the age of 12months.[15] The role of pre-vaccination antibodies in reducing seroconversion as we observed has been previously documented.[13] This underscores the need for a booster dose of the vaccine especially in a measles endemic environment like ours where early exposure to natural infection may have accounted for the presence of pre-vaccination antibodies in some subjects.

Though still sub-optimal, the 50% vaccine potency rate in this study represents a significant improvement of that from previous reports of 11%- 18.2%[13-14] in the country. This may be a reflection of better vaccine-handling practices, especially maintenance of the cold-chain advocated for by previous workers.[19] This study was however conducted in an urban area with relatively more stable electricity supply and findings may be differ in rural settings where this is not the case. Reconstituted measles vaccines should maintain potency if kept on a foam pad placed in an iced pack at 4°C for 24 hours.[20] It was however obvious during the field study that this temperature was not always maintained. Thus, some children vaccinated towards the end of activities may have received vaccines whose potency had deteriorated during the process.

Incidentally, the duration of exposure was not directly related to vaccine potency implying the role of other factors such as potency of the vaccine prior to reconstitution which is a reflection of the quality of storage and transportation. Investigation of these factors was beyond the scope of this study but supporting evidence can be obtained from the study of Omilabu et al[13] who reported that measles vaccines in the National Central Cold Store maintained potency while only 16.7% of them were potent when traced to the points of service. It is therefore pertinent for the various agencies involved in vaccine handling to address the issues relating to poor transportation and storage. While immune response was mostly directly related to virus titres, an important observation was that seroconversion occurred in some children who supposedly received low potency vaccines. It is possible that they were vaccinated at the start of activities or the differences may be based on individual immunogenicity but this may constitute room for further research.

Conclusion

The low seroconversion to measles vaccination obtained in this study precludes the achievement of a herd immunity of 90% required for measles control. There is thus a build-up of susceptibles in the population with continued risks of outbreaks.[1] As global elimination of measles will be based on successful elimination in all countries, this may continue to compromise present efforts directed at measles control. Improvement of the maintenance of the cold-chain system of vaccine delivery and a second opportunity for measles vaccine are recommended. Seroepidemiological and seroconversion studies which help to highlight the features of the changing epidemiology of the disease are a pre-requisite for effective control and serve as a guide for review of immunization policies.

Author's Contributions
TAL, EMTC, FON, SAO: Conceptualization of the study /critical editing of final draft.
TAL: Data collection.
TAL, EMTC, FON: Data analysis, study write-up
TAL and SAO: Laboratory analysis.

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