# Passive Immunity to Measles in the Breastmilk and Cord Blood of Some Nigerian Subjects

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**Summary**

**Maternal and cord blood collected from 33 Nigerian mother–child pairs were tested for measles-sepcific IgG. All 33 had protective measles antibodies at the time of delivery with a positive correlation of $r = 0.87$. Determination of the rate of waning of these antibodies revealed that 58 per cent of these children had lost the protective maternal antibody by the age of 4 months and only 3 per cent of the children had enough antibody to protect them between the ages of 6–9 months. Fifty-five colostrum samples from the same mothers and 347 breastmilk samples collected at various periods of breastfeeding also showed that anti-measles IgA had dropped below the protective cut-off within the first 2 weeks of birth. It is evident that the Nigerian child is born with solid anti-measles antibody but the rate of waning has left a large number unprotected before the first dose of the vaccine. There is an urgent need to review the measles vaccination programme in Nigeria to protect these susceptible infants.**

## Introduction

Measles is one of the vaccine-preventable diseases of childhood. Unfortunately the disease has continued to be a source of serious concern in Nigeria as it has continued to be a source of high morbidity and mortality among Nigerian children year after year. Epidemiological reports showed that there were about 35 000 cases of measles last year alone with 420 deaths.[1] Although vaccination coverage has not been so impressive, most of the children involved were below the 9-month age target at which the measles vaccine is usually administered in Nigeria. This has therefore put into serious question the immune status of these children before the age of vaccination.

The source of measles immunity in the first few months of birth is the passive immunity passed to the infants from their mothers, either transplacentally or through colilstrum and breastmilk. Life-long immunity is usually conferred by natural measles infection and this is transferred from mothers to their offspring through active placental transportation and from colilstrum and breastmilk.[2–4] However, the quality and quantity of such passive immunity depends on the level of measles antibody passively passed to the babies through these sources. In a study conducted recently by Hartter, *et al.*,[5] comparing the measles antibody level between Nigerian and German children, a rapid decline of passively acquired measles-specific antibodies in Nigerian children was observed, with about 70 per cent of the children loosing maternally acquired immunity by 4 months. Several questions were then raised as to the quality of the measles immunity acquired at birth and the likely factors responsible for this rapid decline in immunity.

We therefore decided to investigate further the relationship between the measles virus-(MV) specific antibodies of mothers and their babies by measuring the level of MV-specific antibodies, not only in maternal and cord blood but also in colostrum and breastmilk of some Nigerian mothers.

## Subjects and Methods

*Sample collection*

Four major hospitals in Ibadan metropolis were used as study sites. These are the Adeoyo State Hospital (ASH), the University College Hospital (UCH), St Mary's Hospital, Eleta (SMH), and Oni Memorial Hospital (OMH).

Maternal and cord blood samples were collected

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from 33 newborns and their mothers at the Department of Obstetrics and Gynecology of UCH during delivery, while colostrum was collected from the same mothers before they were discharged. An additional 22 samples of colostrum were collected from other mothers bringing the total number of colostrum samples to 55. A total of 347 breastmilk samples collected at various periods of breastfeeding were collected from mothers bringing their children for vaccinations. Two hundred and sixty-two sera samples were also collected by the finger prick puncture method from unvaccinated children aged 0–9 months. Informed consents of mothers were sought after the purpose of the study was explained to them.

## Serology

*Flow cytometry for IgA antibodies in breastmilk*

Measles-specific IgA was measured in colostrum and breastmilk samples by the fluorescence activated cell scanner (FACS) measured immunofluorescence assay, as described by de Swart[6] in 1997 with little modification. The FACS scan assay detects antibodies directed against the haemagglutination (H) and fusion (F) glycoproteins of measles, which had been transfected into human melanoma cells lines (mel-juso). These cells expressing the transfected genes in their native conformation served as the positive target cells, while the untransfected cells served as the negative controls. Breastmilk and colostrum samples were diluted 1:10. The negative and positive control monoclonal antibodies (BH67 and BH216)[7] were diluted 1:1600 and 1:1000, respectively, with FACS buffer. The mel-juso cells, which had been previously diluted to contain $2 \times 10^6$/ml in FACS buffer, were plated in 96-V well plates and centrifuged at 1200 rpm for 10 min at 4ºC. Breastmilk and colostrum samples were then incubated with the cells on ice at room temperature for 30 min. The pre-diluted florescent labelled anti-IgA conjugate was then added to all the wells and incubated on ice at room temperature for 15 min. The fluorescence was measured after the plates were washed, centrifuged, and resuspended in 150 µl FACS buffer. Fluorescence signals were expressed as average fluorescence units (AFU) at a positivity cut-off of 1:8. The FACS measured fluorescence signals correlated with the amount of specific immunoglobulins present in the samples.

*Plaque reduction neutralization test on maternal and cord blood*

Maternal and cord blood samples were tested for MV-specific neutralizing antibodies by the plaque reduction neutralization test as described by Albrecht.[8] Sera were inactivated at 56ºC for 15 min. Starting with a 1:8 dilution, a two-fold serial dilution of sera was done and 120 µl of a low passage wild strain of Edmonston MV, diluted to contain between 25 and 35 plaque forming units (PFU) per 50 µl, was added to each test serum and serum control wells. Plates were then incubated for 1 hr 45 min at 37ºC after which 240 µl of the virus/serum mixture were inoculated on a monoplayer of the Vero cell. Plates were read on day 7pi (post-inoculation) and the PRN titers was calculated as the serum dilution that gave a 90 per cent plaque reduction. Each PRN titer represents the reciprocal geometric mean titer (GMT) of two assays.

*IgG ELISA on mother/child-paired sera*

The level of MV-specific IgG was determined in the mother/child-paired sera using the commercial ELISA Enzygnost kit (Dad Behring, Marburg, Germany) according to the manufacturer's instructions. The OD was measured at 450 nm and the results were expressed in milli-International Units/ml (mIU/ml). A cut-off of OD titer >120 mIU/ml was chosen as the protective MV antibodies for the paired sera.[9]

Using the same ELISA technique, MV-specific antibodies were determined in the sera of the 262 unvaccinated Nigerian children aged between 0 and 9 months to determine the level of antibody waning.

*Neutralization test*

Measles neutralizing antibody levels were measured in the mother/child-paired sera using the adapted neutralization assay in B95a cell lines as described by Ward, *et al*.[10] A 100 $TCID_{50}$ of the Edmondston wild-type virus was used as the challenge virus. Briefly, the test sera, positive and negative controls were diluted in 96-well plates starting from a 1:4 dilution. Seventy-five micolitres of the pre-titrated virus suspension containing 100 $TCID_{50}$ of the challenge virus was added to all wells and incubated at 4ºC for 3 h after which 75 µl of the B95a cell lines diluted to contain 35 000 cells were then added. Plates were incubated at 37ºC in an atmosphere of 5 per cent $CO_2$ for 4 days. The second International Standard Serum used at a concentration of 1000 mIU/ml and two in-house controls were included in each assay to ensure reproducibility. A neutralization test titer greater than 1:8 was designated as the protection level.[10]

## Results

All the 55 colostrum samples were positive for anti-measles IgA with a mean value of 5.37 AFU. However, breastmilk AFU varied with the time of collection. From day 6–15, the mean AFU value had dropped to 2.11 ($n$ = 15) while between days 15 and 30, the value had dropped below the cut-off point of 1.8–1.48 ($n$ = 10). All the remaining samples ($n$ = 322) tested 30 days to 1 year had a mean AFU value of 1.32 (Fig. 1). There was a statistically significant difference in the AFU values of the different

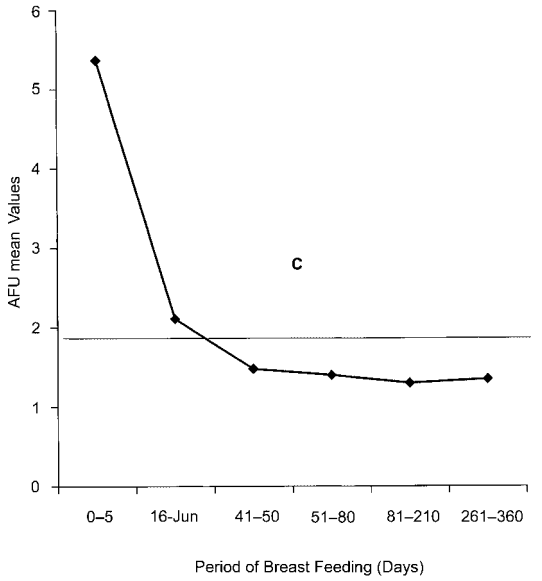


FIG. 1. Measles-specific IgA levels in colostrum and breastmilk of Nigerian mothers. Solid line signifies AFU cut-off.

TABLE 1
*Measles specific antibody levels in mother–child pairs showing percentage protection*

| Assay | Mothers | Newborns |
|---|---|---|
| MV-ELISA (mIU/ml) | 2528 (200–10 706) | 2503 (280–6950) |
| NT ($log_2$) | 80 (8–512) | 69 (8–512) |
| PRNT PRN titer | 2141 (616–4520) | 2120 (632–3939) |
| % | 100% | 100% |

Ranges are given in parentheses.

samples ($p < 0.05$). To investigate further the transfer of MV-specific IgA from colostrum and breastmilk, breastmilk was collected from 12 mothers who had donated colostrum at puperum.

Results of the paired samples showed that all 12 mothers were positive for MV-specific IgA with a mean AFU value ranging from 1.85 to 15.00, while only one of the breastmilk samples was positive (0.26–216).

*Passive immunity from cord blood*

All 33 mother/child serum pairs were positive in the three assays used. Using the previously described protection level for the different assays, all 33 mother/child pairs had a antibody level greater than

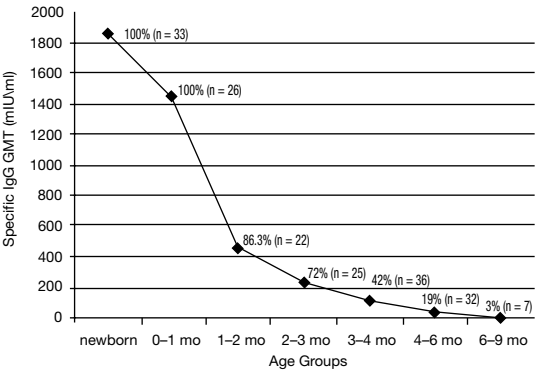


FIG. 2. Specific-measles IgG levels in Nigerian children aged between birth and 9 months showing the weaning of maternally acquired immunity. The percentage of children protected against the number of children tested are shown.

the cut-off titers (Table 1). Neutralization test titers for mothers were between 1.16 and 1≥1024 while ELISA titers for the same mothers ranged from 200 to 10 706 mIU/ml. The PRN titer ranged from 600 to 4250. Among the newborns, neutralization test titer was 1:16 to 1:1024, ELISA titer was between 280 and 6950 mIU/ml and PRN titer ranged between 632 and 3939. There was a positive correlation in titer between the mothers and their newborns ($r = 0.87$).

*Waning of maternal antibodies in Nigerian children*

The waning of measles-specific maternal antibodies in the 262 Nigerian infants was investigated by measuring specific-MV IgG. There was a statistically significant difference in the IgG level of the different age groups ($p < 0.05$). The newborn had the highest anti-measles IgG (GMT 1864 mIU/ml) with a protection level of 100 per cent. This gradually decreased to GMT 1448.39 for children aged between 0 and 1 months ($n = 26$). A sharp drop in titer was recorded for children aged 1–2 months (GMT 458 mIU/ml) ($n = 22$) with a protection level of 86 per cent. Only 42 per cent of children aged between 3 and 4 months had MV antibodies over the protective level (GMT 112.26). Infants aged between 4 and 6 months ($n = 32$) had a GMT of 40.33 with only 19 per cent of them protected. By the age of 6–9 months, which is the age of vaccination in Nigeria, the GMT had decreased to 3.6 leaving only 3 per cent of the children protected (Fig. 2). There were significant differences in the titers of the different age groups.

## Discussion

There is evidence from this study that the Nigerian infant is born with a solid anti-measles passive immunity. In the investigation of passive immunity

from mother to child via cord blood, all the mothers in this study were seropositive for MV antibodies. Also, all the newborns had maternally derived antibodies to measles with anti-MV IgG titer ranging between 280 and 6950 mIU/ml. This is in agreement with the finding of Halter, *et al.*[5] who observed that the maternal measles titer could be used as the intitial antibody value of a Nigerian infant. In this study a correlation of $r = 0.87$ was found between the antibody levels of the mothers and those of their babies. However, the most disturbing issue is the rate of waning of this antibody with time. In the present study we observed a rapid waning of anti-measles specific IgG from 1864.64 mIU/ml at birth, with 100 per cent protection, to a rate as low as 3.65 between the ages of 6 and 9 months, with only 3 per cent of the infants protected against any attack by the virus. In fact less than half of the children had fallen below the protective threshold before the age of 3–4 months. This finding is in total agreement with those of Halter, *et al*.[5] The implication of this is that more than half of the Nigerian children born with solid anti-measles passive immunity become susceptible to the disease before the age of 4 months. This is a serious matter in the face of continuous circulation and endemicity of the measles virus in Nigeria and the perpetual low routine coverage. There is, therefore, a need for increased and sustained routine vaccine coverage and a possible vaccination regime.

There has been a marked interest in the role of breastfeeding in the last two decades. Breastfeeding has been shown to protect neonates and infants from various infections.[9] The role of breastmilk in the transfer of measles antibody from mother to child was therefore investigated in this study. A flow cytometry method was adopted in the determination of measles-specific IgA levels in the colostrum and breastmilk samples of mothers. Our result showed that all colostrum samples had measles-specific IgA with a mean AFU of 5.37, far above the protective level of 1.8. However, a very sharp decline was observed starting from day 6, and by 1 month the IgA level had fallen below the positive AFU cut-off. This is similar to the findings of Rahman, *et al.*[11] and de Swart, *et al*.[6] Paired breastmilk samples collected from 12 mothers at delivery and 6 weeks after delivery showed the same result as that obtained in the general population. All the colostrum samples collected at birth were positive for measles-specific IgA with an AFU value ranging from 1.85 to 15.00, while the AFU value had fallen below the AFU cut-off point for the breastmilk samples. From both observations, the period most critical for transfer of anti-MV IgA from maternal milk to child is considered to be the first 2 weeks of life, as maternally derived IgA is no longer available from the second week onwards.

With the rapid waning of maternal anti-MV antibodies among Nigerian and other children from developing countries, associated with lower specific MV antibodies and poor transplacental transfer efficiency due to elevated total IgG,[2,5,13,14] it appears the role of this short-lived IgA from colostrum and breastmilk is not significant in changing the immune status of the Nigerian child. This fact was also observed in this study. Babies who were breastfed in the first few weeks after birth still experienced a waning of their MV-specific antibody within 3–4 weeks.

In the continuous face of increased total IgG among Nigerian mothers[5] and the wide spread of the wild measles virus in Nigeria, there is an urgent need to review the measles vaccination programme in Nigeria with the aim of protecting these susceptible infants during this period.

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