

# STUDY ON MEASLES ANTIBODY STATUS IN CHILDREN BELOW NINE MONTHS OF AGE AND ITS IMPLICATION ON MEASLES VACCINATION

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## Abstract

This cross-sectional, prospective study was done at Armed Forces Institute of Pathology (AFIP), Dhaka to investigate passively transferred maternally derived measles immunoglobulin G (IgG) antibodies in sera of infants below vaccination age and their relation to the immunity status of the mothers. A total of 50 infants aged up to 9 months and their mothers were investigated for the presence of measles IgG antibodies in their sera from July 2006 to January 2007. Cases were selected randomly from Paediatric Ward and out patient department of Combined Military Hospital (CMH) Dhaka, and Bangabandhu Sheikh Mujib Medical University (BSMMU). Among the infants 29 (58%) were boys and 21 (42%) were girls. 92% mothers had measles IgG antibody in their sera. Total 31 (62%) mother infant pairs were positive for measles IgG and 3 (6%) pairs were negative. In 15 (30%) cases mothers were seropositive but infants seronegative and in 1 (2%) case mother was seronegative but infant was seropositive for measles antibody.

The distribution of passively transferred maternal antibody levels in children with respect to age showed a progressive reduction with increasing age. Among infants of up to 3 months age 94.12% were seropositive, whereas 70.59% infants of age up to 6 months and 25% of infants up to 9 months were seropositive. The rate of seropositivity started declining from 5th month and the rate of decline gradually increased as the age of infants advanced.

No significant correlation was found between transplacental transfer of antibody and prematurity or low birth weight as well as with the sex of the infants.

## Introduction

Measles remains a leading cause of death among young children, despite the availability of a safe and effective vaccine for the past 40 years<sup>1</sup>. The infection is second

only to malaria in terms of the number of people who die each year as a result of complications from an infectious disease<sup>2</sup>. Prior to the introduction of measles immunization there were nearly 130 million cases worldwide annually with approximately 3 million deaths<sup>3</sup>. Although an effective vaccine has reduced the numbers dramatically, a significant disease burden exists yet. An estimated 4,54,000 people, the majority of them were children, died from measles in 2004<sup>1</sup>. Of the deaths attributable to measles, 98% occur in developing countries and case-fatality rates in these countries are usually in the range 1-5%<sup>4</sup>.

In industrialized countries, the risk of measles disease in young children is much lower, and measles vaccine is administered at 12-15 months of age, when virtually all children have lost maternal antibody and an optimal immune response is achieved<sup>5</sup>. In developing countries with high rates of endemic measles, routine immunization is often recommended at 9 months of age because of the increased risk of severe infection early in life<sup>2</sup>.

The optimal age for first measles vaccination is defined as the age with the highest proportion of infants responding to the vaccine<sup>6</sup>. It is dependent on the presence of maternal antibodies against measles virus and the maturation of the immune system<sup>6</sup>. The immunogenicity of measles vaccine in infancy is dependent on the rate of decay in maternal antibody since this antibody interferes with vaccine-induced seroconversion<sup>7</sup>. Only after antibody levels are low enough then the vaccine virus can induce an immune response<sup>8</sup>.

The achievement of the goal of eliminating measles is facilitated by vaccination at the earliest possible time after the clearance of maternal antibodies, in order to keep the number of susceptible subjects in the population as low as possible<sup>8</sup>.

A notable proportion of cases and deaths occur in infants younger than the age (9 months) at which measles vaccination is currently recommended in developing

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countries<sup>9,10</sup>. It is well documented that protection of most infants by passively acquired maternal measles virus antibodies is waning before immunization is given<sup>11</sup>. In a study in Argentina only 18.7% of 4 month-old infants showed detectable levels of IgG antibodies against measles virus; this value declined to 4.2% in 6 month-old infants and measles virus antibodies were undetectable in 9 month-old infants<sup>12</sup>. A study in Bangladesh showed that by the age of 5 months, 67% infants had practically no protective antibody left. Only 12% infants at 5 months of age, and 5% at 8 months, had protective levels<sup>7</sup>.

This study was aimed at measuring the level of passively transferred maternal measles antibodies in serum of children below nine months of age and measles antibody level in their mothers and to determine its implication on the current vaccination schedule. Our objectives were to assess decay of maternal derived measles antibody in infants below vaccination age (9 months) by studying the IgG antibodies, to correlate the age of starting the measles vaccination with the antibody status in the infant. We also tried to find out the correlation between antibody titre in infants with prematurity and low birth weight as well as the seroprevalence of measles antibody among women of child-bearing age in Bangladesh.

## Materials and Methods

### Case Selection

The study group consisted of 50 children aged up to 9 months and their mothers. The children were grouped as up to 3 months, 3 months 1 day - 6 months, 6 months 1 day - 9 months. As far as possible equal representative of all age group was ensured; 17 infants from 1st group, 17 from 2nd group and 16 infants from 3rd group were selected. Infants were selected randomly from those admitted in pediatric wards or seeking advice in out patient departments in Combined Military Hospital, Dhaka for diseases other than clinical measles or any exanthematous disease, and infants reporting to One Point Collection Center, Bangabandhu Sheikh Mujib Medical University (BSMMU) for investigation for other diseases. Infants with history of clinical measles or any exanthematous rashes and history of transfusion of blood or blood products were excluded from the study. Blood samples from the infant's mothers were also collected.

### Study Place and Period

The study was carried out at the Department of Immunology and virology, Armed Forces Institute of Pathology (AFIP), Dhaka Cantonment from July 2006 to January 2007.

### Data Collection

All the necessary information and clinical data were

systemically recorded by interviewing the mother on a pre-designed data sheet. Written informed consent form in Bengali was duly filled in by the babies' mother after explaining the purposes and objectives of the study.

### Sample Collection, Preparation and Preservation

About 2 ml of blood was collected aseptically by venepuncture in a sterile dry test tube from each case. The blood was kept for 1-2 hours at room temperature to allow clot formation. Then it was centrifuged at 3000 rpm for 5 minutes. After separation of serum it was kept in Eppendorf tubes, then labeled properly and preserved in deep freeze at -20°C until tests were performed.

### Antibody Detection

All the sera were tested for measles specific IgG class of antibodies in serum by Enzyme Linked Immunosorbant Assay (ELISA). The tests were performed with commercially available kits (Capita Measles IgG, Trinity Biotech) and the manufacturer's instructions were followed strictly for the performance and interpretation of the tests. The results were expressed as immune status ratio (ISR)

### Results

This serological study examined the status of maternally derived measles IgG antibodies in infants below vaccination age (0-9 month) along with the measles IgG seroprevalence in mothers of the infants with a view to find out the window of vulnerability an infant is exposed before the age when vaccination is recommended.

Majority of the mothers (88%) were between 20 and 30

**Table-I :** Distribution of seropositivity of measles virus IgG antibody (Ab) in infants according to age group (n=50)

Measles Ab	Age group		
	1st (up to 3 m)	2nd (>3 - 6 m)	3rd (>6 - 9 m)
Seropositive	16 (94.12)	12 (70.59)	04 (25)
Seronegative	01 (5.88)	05 (29.41)	12 (75)
Total	17	17	16

Figures in the parentheses indicate percentage

years of age. The mean age of mothers was 24.20±3.36 years, lowest age was 19 years and highest age 33 years. Out of these fifty, forty-six (92%) mothers were seropositive and four (08%) were negative for measles IgG antibody.

Table I shows the distribution of serologic status of measles virus IgG antibodies in infants of three age groups. Total 50 mother infant pairs were included in the study. Infants were divided into 3 age groups: 1st group (up to 3 months) included 17, 2nd group (>3-6 months) included 17 and 3rd group (>6-9 months) included 16 infants. In 1st age group out of 17 infants, 16 were seropositive (94.12%); in 2nd age group out of 17, 12

were seropositive (70.59%); in 3rd age group out of 16, only 04 were seropositive (25%). One infant in the 3<sup>rd</sup> group aged 7 months 29 days had positive ISR despite the mother was seronegative.

In this study, 46 out of 50 mothers (92.0%) were found to

**Table-II** : Distribution of serostatus of measles virus IgG antibody in mother infant pair (n=50)

Serostatus	No (%)
Both mother and child seropositive	31(62)
Both mother and child seronegative	3(6)
Mother positive child negative	15(30)
Mother negative child positive	1(2)
<b>Total</b>	<b>50(100)</b>

have measles IgG antibody in their sera. Total 31 mother infant pairs (62%) were positive for measles IgG and 3

**Table-III** : Distribution of seropositivity according to age of the infants (n=50)

Age in month	Total No	Seropositive No	Seronegative No	% Seropositivity
0 - 1	06	06	00	100
1 - 2	06	06	00	100
2 - 3	05	04	01	80
3 - 4	06	06	00	100
4 - 5	05	03	02	60
5 - 6	06	03	03	60
6 - 7	04	02	02	50
7 - 8	07	01	06	14.28
8 - 9	05	01	04	20
<b>Total</b>	<b>50</b>	<b>32</b>	<b>18</b>	<b>64</b>

(6%) pairs were negative. In 15 (30%) cases mothers were seropositive but infant seronegative and in 1 case (2%) mother was seronegative but infant was seropositive for measles antibody (Table II).

Table IV shows the percentage of seropositivity among

**Table-IV** : Comparison of seropositivity before and after 5 months of age in infants of seropositive mothers (n=46)

Age in month	Seropositive No	Seronegative No	Total No	p value
Up to 5 m	25	3	28	x <sup>2</sup> = 121.958 df = 1 (p<0.001)
Over 5 m	7	11	18	
<b>Total</b>	<b>32</b>	<b>14</b>	<b>46</b>	

the infants according to the ascending order of their age.

The rate of seropositivity in infants of seropositive mothers started declining from 5th month and the rate of declining increased as the age of infants advanced. Among infants below 5 months of age 25 out of 28 (89.29%) infants were seropositive whereas among those after 5 months 7 out of 22 infants (31.82%) were seropositive. The decrease in seropositivity was statistically highly significant (p<0.001) (Table IV).

Table V shows the mean Immune Status Ratio (ISR) in the infants of different age group. The mean ISR gradually decreased with the increasing age of the infants.

Out of total 50 infants 17 (34%) were premature. Among the premature infants only 4 (23.53%) were seronegative and rest 13(76.47%) were seropositive. Prematurity was not a significant factor (p>0.10) in case of placental

transfer of measles IgG antibodies (Table VI).

Out of total 50 infants 18 (36%) were low birth weight. Among the low birth weight infants only 4 (22.22%) were seronegative and rest 14 (77.78%) were seropositive. In this study low birth weight also was not a significant factor (p>0.05) in case of placental transfer of measles IgG antibodies (Table VII).

Of the infants of seropositive mothers 28 (60.87%) were boys & 18 (39.13%) were girls. Among 28 boys 19 (67.86%) were seropositive and among 18 girls 12 (66.67%) were seropositive. There was no significant difference in seropositivity among different sexes (p>0.50) (Table VIII).

## Discussion

After the introduction of measles vaccination in Bangladesh in 1980 under Expanded Program on Immunization (EPI) a dramatic decline was observed. Downward trend continued till 2003 but the number of measles cases again increased in 2004 though the percentage of immunization coverage increased from 65% in 1990 to 76% in 2004<sup>13</sup>. In Bangladesh, like many third world countries measles immunization starts at the age of 9 months but a notable proportion of cases and deaths occur in infants younger than this age.

In this study out of fifty mothers, forty-six (92%) were found to be seropositive and four (08%) were negative for measles IgG antibody. High level of seropositivity of mothers indicates that a large number of mothers of childbearing age in this country are seropositive & subsequently passes the antibody to their offspring. As it was a small-scale study, to find out the nation-wide seroprevalence of measles IgG antibody in the women of childbearing age, further studies may be conducted.

Amongst infants of 46 seropositive mothers 31 (67.39%) had protective antibody titer. The proportion of infants with detectable antibodies declined progressively with increasing age. The antibody levels showed a progressive reduction from 5 months of age. Forty percent children aged 5-6 months of age, 50% of age 6-7 months & 80% of age 8-9 months were seronegative indicating that a

titer. They measured measles antibody by plaque reduction neutralization assay and ELISA<sup>14</sup>.

Though plaque reduction neutralization (PRN) assay is considered to the gold standard for measuring concentration of measles antibody, results obtained by ELISA correlates well with the PRN assay results and are reproducible between runs. The haemagglutination inhibition (HI) test is relatively insensitive, and it is likely that any detectable antibody by HI is protective<sup>15</sup>.

**Table-V :** Mean ISR of measles IgG antibodies in infants in ascending order of age (n=50)

Age in month	No of Infants	Positive for IgG (%)	Mean ISR (SD)
0 - 1	06	06 (100)	2.666 / 0.340
1 - 2	06	06 (100)	2.474 / 0.306
2 - 3	05	04 (80)	1.791 / 0.612
3 - 4	06	06 (100)	1.649 / 0.440
4 - 5	05	03 (60)	1.255 / 0.815
5 - 6	06	03 (50)	1.086 / 0.536
6 - 7	04	02 (50)	0.589 / 0.447
7 - 8	07	01 (14.28)	0.871 / 0.834
8 - 9	05	01 (20)	0.756 / 0.791

Another study in Bangladesh which measured the maternal measles antibody decay in rural Bangladesh done in 1994 found that by the age of 5 months, 67% (28/42) infants had practically no protective antibody left. Only 12% infants at 5 months of age, and 5% at 8 months, had protective levels<sup>7</sup>. This study also used PRN assay and ELISA to detect the antibodies. This difference with this study may be attributable to the fact that this study was conducted in infants and mothers mainly from urban areas. The main bulk of the study population was from armed forces personals having a relatively better living condition from rural Bangladeshi peoples.

large number of infants are at risk of acquiring measles before the vaccination age. The decline of antibody with

A longitudinal study done on black South African infant to measure the loss of maternal measles antibody in order to investigate the feasibility of measles vaccination before the age of 9 months found unprotected levels in 88% of 6-month-old infants while at 9 months all were susceptible<sup>16</sup>.

**Table-VI :** Relationship of seropositivity with prematurity

Seroprevalence	Premature infants (n=17)	Full term infants (n=33)	Total (n=50)	p value
Seropositive	13 (76.47)	19 (57.58)	32 (64)	x <sup>2</sup> = 2.965 df = 1 (p>0.10)
Seronegative	4(23.53%)	14 (42.42)	18 (38)	
<b>Total</b>	<b>17 (100)</b>	<b>33 (100)</b>	<b>50 (100)</b>	

Figures in the parentheses indicate percentage.

Another study in Turkish infants found seropositivity rates at six and nine months to be 61.8% and 3.4% respectively and suggested that the measles vaccination could be carried out earlier than just before the critical age of antibody level<sup>17</sup>.

In this study one infant aged 7 months 29 days was found seropositive though his mother

**Table-VII :** Relationship of seropositivity with low birth weight

Seroprevalence	Low birth weight (n=18)	Normal weight (n=32)	Total (n=50)	p value
Seropositive	14 (77.78)	18 (56.25)	32 (64)	x <sup>2</sup> = 0.652 df = 1 (p>0.50)
Seronegative	4 (22.22)	14 (43.75)	18 (38)	
<b>Total</b>	<b>18 (100)</b>	<b>32 (100)</b>	<b>50 (100)</b>	

Figures in the parentheses indicate percentage

the advancing age indicates gradual loss of maternally derived measles antibody in infants as their age advances.

was seronegative. This may be due to sub clinical infection.

In a similar study in Mali the window of vulnerability as detected by the absence of measles IgG antibody was found to begin at 2 months of age and extended up to nine months of age. At 2 months of age 30% had protective titers and among 6-month-old infants none had protective

In the study no significant difference was found with transfer of IgG antibody between mother & infant with prematurity (p>0.10) or low birth weight (p>0.50) (Tables VI and VII).

**Table-VIII : Relationship of seropositivity with sex of the infants**

Seroprevalence	Boys	Girls	Total	p value
Seropositive	19 (65.51)	12 (57.14)	31 (62)	x <sup>2</sup> = 0.410 df = 1 (p>0.50)
Seronegative	10 (34.48)	9 (42.85)	19 (38)	
<b>Total</b>	<b>29 (58.00)</b>	<b>21 (42.00)</b>	<b>50 (100)</b>	

Figures in the parentheses indicate percentage

In a study in the West African country, Gambia where the influence of prematurity and LBW on transplacental transfer of different antibodies was determined, it was found that prematurity was significantly associated with reduced placental antibody transfer for measles antibody. The study concluded that materno-foetal transfer of antibodies is impaired in prematurity and LBW babies in the Gambian population<sup>18</sup>.

In another study in Sri Lanka in which the influence of gestational age, the neonate's birth weight, and maternal age, weight, height and parity on transplacental antibody transfer was assessed in 141 mothers and their neonates, it was found that prematurity and low birth weight may influence the level of maternally acquired immunity in Sri Lankan neonates<sup>19</sup>.

In this study the cause of no significant difference in transfer of antibody between mother & infant with prematurity or low birth weight may be attributable to the small number of study population.

No significant difference was observed between the placental transfer of antibodies in infants of different sex (p>0.50).

### Conclusion

Measles vaccination is one of the most cost-effective public health interventions available for preventing deaths. The most important factor affecting the success of measles immunization is the disappearance of maternal anti-measles antibodies. Again, maternal antibodies at adequate titer can protect infants early in life. Multiple factors influence the quantity and quality of antibodies. As a result, many young infants are exposed to a period of several months during which the titer of maternal antibodies falls below protective level against wild-type measles virus, but may interfere with antibody production in response to measles vaccine. During this window of vulnerability, young infants may develop severe or fatal measles. In this study it was found that the maternal antibodies against measles significantly declined after 5 months of age. As this was a small-scale study, to find out the nationwide picture further large-scale studies may be conducted.

There are many studies where early loss of maternal antibodies was observed and early immunization was recommended. It is therefore important to assess the time of waning of maternal antibodies and serological studies

should be performed periodically, and vaccination programs appropriate for studied country should be determined according to data obtained.

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