

# Sero-Prevalence Survey of Rubella IgM Antibodies among Pregnant Women in Kano, Nigeria

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## Abstract

Rubella is a generally mild illness and its serious complications are rare. Although a major section of pregnant women are immune, cases of rubella infection occur in Nigeria among pregnant women. This study was aimed at assessing the prevalence of rubella virus IgM antibody among obstetric population between 15 – 44 age group attending Antenatal Clinic Bamalli Nuhu Maternity Hospital, Kano. The samples were analysed using Enzyme-Linked Immunosorbent Assay technique. A well-structured questionnaire was administered to the subjects to obtain socio-demographic data. Of the total 89 samples screened, 7 (7.87%) were reactive to rubella IgM antigens from the coated wells. Out of 7 positive samples, 4 (4.49%) were within 15–24 years age group, representing the most susceptible group, while the infection rate was lowest among 35–44 age group with prevalence rate of 1 (1.12%). Furthermore, women in the third trimesters of pregnancy were recorded to have the highest prevalence rate to the antibody 3(3.37%) while those at first and second trimesters recorded 1(1.12%) and 2(2.25%) respectively. In addition, no case was recorded among women with higher educational background recorded as compared to those who did not acquire western education with prevalence of 4(4.49%). Finally, it was found that rubella infection still occurs in this population, although the prevalence was slightly low, but burden remain alarming due to poor awareness and the ravage of the disease to the foetuses.

## Keywords

IgM, Rubella, Immunosorbent, Bamalli, Obstetric

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## 1. Introduction

Rubella virus is the sole member of the genus Rubivirus in the family Togaviridae. It is spherical in shape, 40 to 80nm in diameter, positive-sense, single-stranded RNA virus with a spike-like, haemagglutinin containing surface projection and are covered by a lipid membrane (viral envelope), derived from the host cell membrane (Kenneth and George, 2004). Rubella, also known as German measles or three-day measles is a disease caused by the rubella virus (Neighbors and Jones, 2010). Rubella virus is the pathogenic agent of the disease rubella, and is the cause of Congenital Rubella Syndrome (CRS) in the newly born when infection occurs during the first weeks of pregnancy (Atreya *et al.*, 2004). The name is

derived from the Latin word and it means little red. It is so called because the disease was first discovered by German physicians in the Mid Eighteen Century (Lee and Bowdeen, 2000). This disease is often mild and attacks often pass unnoticed. The disease can last one to three days hence terms as ‘3-days measles’. Children recover more quickly than adults (Miller *et al.*, 2000).

Infection by rubella virus can be either congenital or acquired. Infection of the mother by rubella virus within the first 20 weeks of pregnancy can be serious, and this causes Congenital Rubella Syndrome (CRS) in the newly born (Atreya *et al.*, 2004). Many mothers who contract rubella within the first critical trimester either have a miscarriage or a still born baby. If the baby survives the infection, it can be

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born with severe heart disorders (Patent ductus arteriosus being the most common), blindness, deafness, or other life-threatening organ disorders. The skin manifestations are called "blueberry muffin lesions" (Da Santis *et al.*, 2006). For these reasons, rubella is included on the torch complex of perinatal infections. The CRS follows intrauterine infection by rubella virus and comprises of cardiac, cerebral, ophthalmic and auditory defects (Atreya *et al.*, 2004). Rubella is also a common childhood infection usually with minimal systemic upset although transient arthropathy may occur in adults. Acquired (i.e not congenital) rubella is transmitted via air borne droplet emissions from upper respiratory track of active cases (Richardson *et al.*, 2001). The virus may also be present in the urine, faeces and on the skin and there is no carrier state. The reservoir exists entirely in active human cases (Richardson *et al.*, 2001), since human is the only known natural host for the virus (Frank and David, 1999). The virus is found in the blood 5 to 7 days after infection and spreads throughout the body.

In 1979, the Federal Republic of Nigeria initiated the Extended Program on Immunization (EPI) which was sustainably revitalized in 1999 to reduce disease burden from vaccine preventable disease but left out rubella (WHO, 2007). Recently, a sero-prevalence of 6.84% of IgM rubella virus infection was recorded in Southern Nigeria, Abia State (Ogbannaya *et al.*, 2012) and also a prevalence of 17.4% was recorded in northern Nigeria (Koki *et al.*, 2014). In south-western Nigeria, a prevalence of 16.3% was observed in Ilorin, Nigeria (Agbede *et al.*, 2011). With epidemics occurring in varied intervals by geographical locations (Dayan *et al.*, 2006), sentinel studies have placed the incidence of rubella on a seasonal distribution, with an average of 5-9 years epidemic pattern that is highly variable in both developed and developing countries (Reddy *et al.*, 2006).

## 2. Materials and Methods

### 2.1. Study Area and Population

This is a cross-sectional study carried out in Bamalli Nuhu Maternity Hospital, Kano between September, 2014 and March, 2015. Bamalli Nuhu Maternity Hospital is located in Kano Municipal Local Government Area (LGA) of Kano State situated between longitude  $10^{\circ} 25' N$  and  $13^{\circ} 53' N$  and latitude  $70^{\circ} 10' E$  and  $10^{\circ} 35' E$  with an altitude range from 400m to 800m above the sea level. Kano state has an estimated of land area of 43,070 Km<sup>2</sup> and estimated population of 9,383,682 million people according to Nigerian census 2006 and has 44 local government Areas. The study comprised of pregnant women of different age groups, locality, trimester of pregnancy, educational and socio-

economical status attending Bamalli Nuhu Maternity Hospital, Kano.

### 2.2. Sample Collection

About 5 ml blood sample was aseptically collected by venipuncture from each pregnant woman. Each blood sample was dispensed into appropriately labelled vacutainer tube, screw-capped and left at room temperature for about 45 minutes to 1 hour, after which it was spun at 3,000 revolutions per minute for 10 minutes to separate serum from blood clot. The serum was dispensed into correspondingly labelled plane containers and stored at  $-20^{\circ}C$  until needed for assay.

### 2.3. Serological Examination

The samples were screened for rubella IgM antibodies using commercially available rubella IgM enzyme immunoassay (EIA) test kit manufactured by Rapid Labs Ltd, United Kingdom with kit product code E-112196.

### 2.4. Assay of Rubella IgM Antibody

A working wash buffer was prepared by diluting the concentrated Wash Buffer with distilled water in the ratio 1 is to 25. A1 of the micro well plate was left as blank. To the wells B1 and C1, 100  $\mu$ L of Negative Controls were added and this was followed by addition of 100  $\mu$ L of Cut-off Calibrator to the wells D1 and E1. Hundred  $\mu$ L of Positive Control were also added to the wells F1 and G1. Hundred  $\mu$ L of Specimen Diluent was added to the assigned wells starting from H1 followed by addition of 5  $\mu$ L of each of the specimens to the assigned wells starting from H1. The unused strips from the micro well plate were removed and stored between 2-8 $^{\circ}C$ . They were mixed gently by swirling the micro well plate on a flat bench for 30 seconds. The micro well plate was cover with a plate sealer and incubated at 37 $^{\circ}C$  for 30 minutes. The plate sealer was removed and each of the wells was washed 5 times with 350  $\mu$ L of working wash buffer solution. The micro well plate was turn upside down on absorbent tissue for 30 seconds to ensure that all wells were completely washed and dried. Hundred  $\mu$ L of conjugate was added to each of the wells with exception of the blank well and it was then covered with a plate sealer and incubated at 37 $^{\circ}C$  for 30 minutes. The plate sealer was removed and each of the wells was washed 5 times with 350  $\mu$ L of working wash buffer solution. The micro well plate was turn upside down on absorbent tissue for 30 seconds to ensure that all wells were completely washed and dried. Fifty  $\mu$ L of both substrate A and B substrates were added to each well, they were mixed gently and covered with plate sealer and incubated at 37 $^{\circ}C$  for 10 minute. The sealer was removed and 50  $\mu$ L of stop solution was added to each well and it was

read at 450nm with a micro well plate reader (Herrman, 1985).

## 2.5. Qualitative Interpretation of the Result

For the IgM ELISA, serum samples with Sample to Cut-off (S/Co) ratio  $>1.1$  were considered positive for anti-RV IgM antibodies while those with S/Co ratio  $<0.9$  were considered negative. Samples with S/Co ratio between 0.9 and 1.1 were considered equivocal as recommended by the kit manufacturer.

## 2.6. Data Analysis

Statistical analysis was carried out using computer database software from the statistical package from social sciences (SPSS version 16) to generate frequency distribution and

percentage prevalence scores of the various parameters. Descriptive analysis of the percentages of continuous variables was reported.

## 3. Results

Of the 89 pregnant women that were screened, 7(7.87%) were positive while 82(92.13%) were negative for the IgM antibodies (Table 1).

**Table 1.** Prevalence of Rubella virus in pregnant women

STATUS	FREQUENCY	PERCENTAGE (%)
POSITIVE	7.0	7.9
NEGATIVE	82	92.1
TOTAL	89	100

**Table 2.** Prevalence of Rubella virus in relation to demographic characteristics

Variable	No. Positive (%)	No. Negative (%)	Total (%)	P – VALUE
Age Group (Years)				
15-24	55	4(4.49%)	51(57.30%)	0.9155
25-34	25	2(2.25%)	23(25.84%)	
35-44	9	1(1.12%)	8(8.99%)	
Total	89	7(7.87%)	82(92.13%)	
Trimester				
1st trimester	11	1(1.12%)	10(11.24%)	0.8690
2nd trimester	33	2(2.25%)	31(34.83%)	
3rd trimester	45	3(3.37%)	42(47.19%)	
Total	89	7(7.87%)	82(92.13%)	
Marital Status				
Married	80	6(6.74%)	74(83.15%)	0.4438
Single	1	0	1(1.12%)	
Divorced	1	0	1(1.12%)	
Widow	5	1(1.12%)	4(4.49%)	
Separated	2	0	2(2.25%)	
Total	89	7(7.87%)	82(92.13%)	
Educational Level				
None	10	4(4.49%)	6(6.7%)	0.0191
Primary	12	0	12(13.5%)	
Secondary	62	3(3.37%)	59(66.3%)	
Tertiary	5	0	5(5.6%)	
Total	89	7(7.87%)	82(92.1%)	
Occupation				
Housewife	50	4(4.49%)	46(51.68%)	0.7164
Trading	21	0	21(23.60%)	
Civil servant	11	2(2.25%)	7(10.11%)	
Farming	7	1(1.12%)	6(6.74%)	
Total	89	7(7.87%)	82(93.16%)	

The highest prevalence of the virus was recorded among the women that fell within the ages of 15-24 years and this tends to decrease with increase in age (Table 2).

IgM antibodies were identified in all the three trimesters of pregnancy (Table 2). Of the 11 women screened in the first trimester, 1(1.12%) was positive for the IgM antibodies while 10(11.24%) were negative. This however decreased during the second and third trimesters with respect to the number of

persons screened where 2(2.25%) out of the 33 screened were observed to be positive for the antibodies and 3(3.37%) were observed to be positive among the 45 patients that were screened (Table 2).

IgM antibodies were only detected in the pregnant women that were married and one woman that was widow. Of the 89 married women sera that were screened, 6(6.74%) were positive while 1(1.12%) out of 5 widows was positive. No

single positive result was found among the singles, divorced, widows and separated pregnant women as indicated in Table 2.

IgM antibodies were detected in women that didn't attend school and those that attended secondary school only. Of the 10 women that didn't attend school, 4(4.49%) were found to be IgM positive while of the 62 that attended secondary, 3(3.37%) were IgM positive as shown in Table 2.

IgM antibodies were detected in all the categories of women studied with exception of 21 women that were trading. The highest prevalence was found in women recorded as housewife (Table 2).

## 4. Discussion

Rubella viruses is known to cause infection in vitro and are often responsible for abortion, still births, premature delivery and congenital malformation. There is considerable variable in the prevalence of the agent among the women of child bearing age in different geographical areas (Usher *et al.*, 2004).

Results obtained from this study indicated that 7(7.9%) out of the 89 pregnant women that were screened from Bamalli Nuhu Maternity Hospital, Kano, were positive to antihuman IgM coated antibodies for IgM antibody, while 82(92.1%) were negative indicating that they lacked specific antibody to the coated anti-human antibodies. This indicated that 7.9% of the study population could be recently infected with the rubella virus as the IgM antibody is the body's first line of defence against an antigen and it is found in the blood and lymph fluids. This confirmed earlier reports by Cutts *et al.* (2000) who reported that the prevalence of women who were sero-positive for rubella virus IgM were less than 10%. This finding also agreed with the findings of WHO which placed the worldwide prevalence rate of rubella susceptibility between 7.5 – 17.4% (Dykewicz *et al.*, 2001).

The study was however slightly lower than the recent study conducted in Kano by Koki *et al.* (2014) with prevalence of 17.4%. This difference may be due to the differences in the sample sizes used in the two studies (Koki *et al.* (2014) n= 288; this study n= 89), since both study sites deliver the same services to the same set of patients with same social status.

In this study, none of the pregnant women had previous history of MMR vaccination, as contained in the questionnaire issued to the subjects during sample collection, possibly due to the non availability of the vaccine in the Nigerian health facilities.

From this study, sero-prevalence of IgM antibody based on age group showed 15–24 age group had highest prevalence

of 4(4.49%) followed by 25–34 age group with prevalence of 2(2.25%). The least prevalence of 1(1.12%) was observed within the age group 34–44. This finding is contrary to the findings of Ogbannaya *et al.* (2012), who reported that the highest sero-prevalence was found within the age group 25–34. This suggests that with increase in maternal age, the prevalence rate of rubella virus infection decreases or possibly the age group involved the sexually active women.

According to this study, IgM antibodies were found in all the trimesters of pregnancy with highest prevalence of 3(3.37%) observed in the third trimester followed by second trimester and first trimester with prevalence rates of 2(2.25%) and 1(1.12%) respectively, (Table 2). This finding was in accordance with findings of Barbara *et al.* (1987) and Agbede *et al.* (2011) in terms of low prevalence rates who reported only 1(1.1%) as prevalence among 92 pregnant women, although contrary since it occurred in the first trimester. This is also contrary to the work reported by Bamgboye *et al.* (2004) and Fokunanag *et al.* (2010) which indicated the highest prevalence rate among pregnant women in their first trimester which increases chances of transmitting the virus from the infected pregnant women to their foetuses with up to 90% chances according to Forbi *et al.* (2009).

Results obtained also suggest that there is a higher prevalence of the infection among married women compared with divorced or singles. This may not be associated with the prevalence rate because the number of divorced and single women was very low compared to the number of married women although it was statistically significant ( $p < 0.05$ ).

IgM antibodies were detected in women that didn't attend school and those that attended secondary school only. Of the 10 women that didn't attend school, 4(4.49%) were found to be IgM positive while of the 62 that attended secondary, 3(3.37%) were IgM positive.

IgM antibodies were detected in all the categories of women studied with exception of 21 women that were trading. The highest prevalence was 4(4.49%) found in women recorded as housewife. There was no significant association between these variable and antibody prevalence of rubella ( $p > 0.05$ ).

## 5. Conclusion

Of the 89 pregnant women that were screened at Bamalli Nuhu Maternity Hospital, 7(7.9%) were positive while 82(92.1%) were negative for the IgM antibodies. Prevalence rate of 3(3.4%) was observed among pregnant women within the age group 15-24. The susceptibility of pregnant women to rubella virus infection in their first trimesters of pregnancy attending Bamalli Nuhu Maternity Hospital, Kano was very low at prevalence rate of 1(1.12%).

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