

Epidemiological evaluation of rubella virus infection among pregnant women in Ibadan, Nigeria

Rubella is a vaccine-preventable, mild rash-inducing viral disease with complications that include a spectrum of birth defects in the developing foetus, especially if the infection is acquired in the early months of pregnancy. Consequently, the primary objective of global rubella control programmes is prevention of congenital rubella infection and associated birth defects often collectively referred to as CRS. Despite the availability of safe and effective vaccines, and elimination of rubella virus in many developed countries, substantial commitment to rubella control has not been demonstrated in the developing countries. This study appraises immunity to rubella, and consequently makes appropriate recommendations aimed at facilitating effective control. A cross-sectional sero-surveillance study was carried out among 272 consenting ante-natal clinic attendees in southwestern, Nigeria. Prevalence rates of 91.54% and 1.84% were recorded for anti-rubella virus (anti-RV) IgG and IgM respectively. Also, 90.7% and 92.3% of the women aged ≤ 30 years and > 30 years respectively had detectable anti-RV IgG. No significant association ($p=0.94$) was recorded between anti-RV IgG detection and age of the women. Previous exposure and susceptibility of significant fraction of the population to rubella infection were confirmed. Considerable political commitment and promotion of free rubella immunization specifically for women of childbearing potential were recommended.

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16 **Introduction**

17 Rubella virus is a member of the *Rubivirus* genus in the family *Togaviridae* [1]. It is a cubical,
18 medium-sized (60 to 70 nm), lipid-enveloped virus with a positive-sense, single-stranded RNA
19 genome. It is the only non-arthropod borne virus in the family and the aetiologic agent of rubella.
20 Rubella is a vaccine-preventable, mild rash-inducing viral disease with complications [2, 3] that
21 include a spectrum of birth defects in the developing foetus, especially if the viral infection is
22 acquired in the early months (first trimester) of pregnancy [3-6]. Birth defects associated with
23 rubella virus infection range from blindness, deafness, and congenital heart disease, to mental
24 retardation and central nervous system (CNS) complications which are often collectively referred
25 to as congenital rubella syndrome (CRS) [4, 7, 8]. Furthermore, in extreme cases, *in-utero*
26 infection of a foetus with rubella virus can cause the death of the foetus [9]. Consequently, the
27 primary objective of rubella-control programmes is prevention of congenital rubella virus
28 infection, and by association CRS [10].

29 Despite the development and administration of effective vaccines for prevention and control of
30 rubella virus infection since the late 1960s, and prevention as well as feasibility of or elimination
31 of the causative agent in many developed countries [11, 12], the infection is still endemic in
32 Nigeria. In fact, it has been shown that a significant number of non-immunized women of
33 childbearing age remain susceptible to rubella virus infection in the country [13]. Also,
34 subclinical or clinical infections as well as continuous circulation of rubella virus have previously
35 been reported in Nigeria [13-18].

36 Efforts to realize significant political commitment and investment in rubella control and possible
37 virus elimination in Nigeria has not yielded significant result. For example, to date rubella
38 vaccine is only accessible at a cost to the informed few in the population. Also, most vaccinees

39 receive monovalent measles rather than rubella-containing vaccines (RCVs) like trivalent
40 measles-mumps-rubella (MMR) vaccine advertised on the platform of National Immunization
41 Programme (NIP).

42 Cutts and Vynnycky [19] in a review of the literature on the prevalence of anti-rubella antibodies
43 from developing countries concluded that CRS is an under-recognized public health problem and
44 that appropriate data need to be collected to estimate the cost-effectiveness of a potential global
45 rubella control program. Furthermore, it had been shown that determination of incidence of
46 rubella and CRS remain important steps to achieve effective prevention and control programme
47 [11]. Accordingly, to appraise immunity to rubella in the population and consequently strengthen
48 the drive for effective prevention and elimination of rubella in Nigeria, this study was designed
49 and conducted to evaluate anti-RV IgM and IgG among pregnant women in the selected facilities.

50 **Materials and Methods**

51 **Study location**

52 This study was carried out among pregnant women attending University College Hospital and
53 Ade-Oyo Maternity Hospital in Ibadan, southwestern, Nigeria. University College Hospital is a
54 tertiary health care facility of the University of Ibadan. The hospital is equipped with facilities for
55 teaching of medical students, research and provision of clinical services to the community.

56 Attendees in the hospital are majorly residents with average economic and educational status.

57 Ade-Oyo state hospital is a secondary health care facility located in the aboriginal nerve of the
58 city, densely populated by indigenes and serving wide range of people with spectra of social,
59 economic and educational background. The hospital serves pregnant women of varied economic
60 and educational status from different parts of the city. The ante-natal clinic records an average of

61 380 ± 20 new subjects per week. Subjects from the University College Hospital and Ade-Oyo
62 Maternity Hospitals were subsequently referred to as RUC (rubella study subjects in UCH) and
63 RAD (rubella study subjects in Ade-Oyo) study groups respectively. The two hospitals were
64 selected to facilitate true representation of the population in the study.

65 **Enrolment of subjects**

66 To achieve our aim and objectives, consenting ante-natal clinic attendees were enrolled from the
67 two selected hospitals described above. Subjects were enrolled between September 2012 and
68 June, 2013. Consenting antenatal clinic attendees were examined for presence of observable
69 rubella-like rash, fever, lymphadenopathy and arthralgia. Subjects presenting with any of the
70 listed clinical presentations were enrolled for the study. Subjects without any of the clinical
71 presentations were excluded from the study. Demographic and other relevant information were
72 obtained using structured questionnaire.

73 **Research Methodology**

74 Blood sample was collected from a total of 272 {median age = 31 years, age range = 17-43 years
75 (RAD: n = 90; age range = 19-42 years; RUC: n = 182; age range = 17-43 years)} consenting
76 (verbal) pregnant women enrolled strictly based on inclusion criteria at the point of registration
77 and routine examination for ante-natal clinic. Ethical approvals for the study were granted by the
78 UI/UCH Ethics Committee (UI/EC/11/0058) and Oyo State Ministry of Health (AD3/479/349).

79 **Sample collection**

80 About 5ml of blood sample was collected via venepuncture of each pregnant woman into an
81 appropriately labeled sterile container free of anticoagulants or preservatives. Thereafter, samples

82 were transported to the laboratory immediately in a cold box with frozen ice packs to maintain a
83 condition of about 4-8°C. Serum samples were separated by low-speed centrifugation at 500g for
84 5 minutes, or direct removal of the serum using a sterile disposable pipette after retraction of the
85 clot. Then, two aliquots of serum were prepared and transferred into labeled sterile cryovials and
86 stored at -20°C until ready for analysis, while the coagulated cells were stored at -20°C in the
87 sterile container.

88 **Laboratory analysis**

89 Laboratory analysis was carried out in the Department of Virology, College of Medicine,
90 University College Hospital, Ibadan. The samples were analyzed for qualitative and quantitative
91 detection of anti-rubella IgM and stable memory IgG using DIA.PRO[®] Diagnostic Bioprobes srl
92 (Sede legale: Via Lucio Giunio Columella, 31-20128-Milano, Italy) Enzyme Immunoassay in
93 accordance with the manufacturer's description. Results of the anti-IgG assay was interpreted
94 with antibody titer ≥ 15 IU/ml as the cut-off point. Both test kits used have diagnostic sensitivity
95 and specificity performance of $>98\%$.

96 **Statistical analysis**

97 Results of the study were analyzed with t-test and χ^2 statistical tests using Statistical Package for
98 the Social Sciences (SPSS) version 15.0 for Windows. P-value ≤ 0.05 was used as indicator of
99 statistical significance. Also, demographic features and other relevant information about the study
100 populations were compared (Table 1).

101 **Results**

102 Overall, prevalence rate of 91.54% (249/272) and 1.84% (5/272) were recorded for anti-rubella
103 virus (anti-RV) IgG and IgM respectively. Further analysis of the results showed that 83 (92.2%)

104 and 1 (1.1%) of the women in RAD had anti-RV IgG and IgM respectively (Table 1). Also, 166
105 (91.2%) and 4 (2.2%) of the women in RUC had anti-RV IgG and IgM respectively (Table 1).
106 Overall, 90.7% (117/129) of women aged ≤ 30 years and 92.3% (132/143) of those aged >30 years
107 respectively had detectable anti-RV IgG. Further analysis of the results for RAD showed that 46
108 (90.2%) of the women aged ≤ 30 years and 37 (94.4%) of those aged >30 years respectively had
109 detectable anti-RV IgG. Also, results for RUC showed that 71 (91.0%) of the women aged ≤ 30
110 years and 95 (91.3%) of those aged >30 years had detectable anti-RV IgG (Table 1). No
111 significant association ($p=0.94$) was recorded between the presence of anti-RV IgG and age of
112 subjects (Table 1). Significant difference ($p=0.0005$) was recorded in educational status of the
113 women by location (woman with tertiary education were more likely to be in RUC); however,
114 similar anti-RV IgG prevalence rates were observed in both locations. Pregnant women enrolled
115 for the study had comparable exposure and presentations of fever, lymphadenopathy and rash
116 (Table 1). Also, Chi square analysis showed no association between location and previous
117 exposure to RV (anti-RV IgG) ($p=0.78$).

118 **Discussion**

119 A high anti-RV IgG prevalence rate was observed in the study. This suggests previous exposure
120 of participants to rubella virus. It also implies previous subclinical or clinical infections with
121 rubella virus. Similarly, detection of anti-RV IgM in a fraction of the study population confirms
122 continuous circulation of the virus. However, the presence of serologically naive pregnant women
123 (8.46%) in the population demonstrates susceptibility of a significant fraction of the population to
124 rubella virus infection. In previous studies [14-18], varied anti-RV IgG prevalence rates have
125 been reported among defined populations of pregnant women in different regions of Nigeria. It is
126 however, pertinent to note that findings from this study corroborate previous reports of
127 subclinical or clinical infection as well as continuous circulation of rubella virus in Nigeria [13-
128 18]. It also supports preliminary report [13] of high prevalence rate of anti-RV IgG among
129 vaccine naïve pregnant women attending ante-natal clinic in one (Ade-Oyo State Hospital) of the
130 study locations.

131 Comparable prevalence rates of anti-RV IgG were recorded despite varied age, age at first
132 marriage, and mean parity (Table 1) of the studied pregnant women. This observation might
133 imply that women in the community possibly become exposed and infected with rubella virus
134 early in life; before reaching childbearing age. However, there is the need for more extensive
135 study on specific variables to facilitate appropriate conclusion. Similar anti-RV IgG prevalence
136 rates were recorded among the women irrespective of their educational status or location of
137 residence (Table 1). Also, comparable rates of presentations of common symptoms of rubella
138 infection including fever, lymphadenopathy, arthralgia and rash were observed among the studied
139 population. These observations might also suggest comparable risks of exposure to and infection
140 with rubella virus irrespective of persons' educational and economic status in region. However, it

141 confirms continuous and consistent circulation of rubella virus in the population. Rubella
142 vaccine is not included in the childhood immunization programme neither is there provision for
143 selective immunization of women of childbearing age in Nigeria. However, it is only available to
144 informed few at a cost, thus high prevalence rates of anti-RV IgG detection in the population
145 suggest previous exposure to the virus.

146 It is pertinent to note that the World Health Organization (WHO) recommended the use of
147 rubella-containing vaccine (RCV) in all countries with national childhood immunization
148 schedules to prevent congenital rubella infection, including CRS in 2000 [20]. The number of
149 WHO member states using RCV increased from 83 (43%) in 1996 to 130 (67%) in 2009.
150 Consequently, the number of rubella cases reported dramatically decreased from 670,894 in 2000
151 to 121,344 in 2009 [21]. However, despite the WHO recommendation and subsequent
152 accomplishments in different parts of the world, rubella vaccine is still available to Nigerians at a
153 cost.

154 It has been recognized [22] with confirmations [23-25] that high childhood immunization rates is
155 essential to achieving effective prevention of CRS [22]. Accordingly, WHO advises a minimum
156 target rate of 80 percent for childhood immunization programs [20]. However, considering the
157 practicability of achieving 80 percent success rates in childhood immunization in Nigeria, *vis-a-*
158 *vis* documented success in prevention of CRS with selective immunization of all women of
159 childbearing age [2] we recommend selective vaccination of women with childbearing potential
160 in the country.

161 **Conclusions**

162 Specifically, the study shows serologic evidence of exposure to rubella virus with a high level of
163 immunity to rubella among the studied women. It also shows that some of the women were
164 currently infected as the time of sampling with a certain proportion revealing susceptibility to the
165 virus. Findings from the study corroborate reports of previous studies in the country and further
166 approve that elimination of rubella virus in Nigeria is feasible since the burden rates is low and
167 the definite susceptible population is defined. Therefore, to facilitate effective rubella control in
168 Nigeria we recommend substantial political commitment and institution of health policy that
169 promotes awareness and free rubella virus immunization programme especially for women of
170 childbearing age. We also recommend that available vaccines should be evaluated to ascertain
171 their potency prior to recommendation for vaccination, and review of antibody response in
172 randomly selected individuals post vaccination to achieve prompt elimination of rubella.

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178 **Author's contributions**

179 AMO conceptualized and designed the study. AMO, OOA, OBA, FTOC and AO were involved
180 in sample and data collection, and laboratory analysis. SWF and AMO were involved in data
181 analysis and interpretation of results. AMO prepared the first draft of the manuscript and all
182 authors read, revised and approved the final manuscript.

183 **Conflicts of interests:**

184 No conflict of interest was reported by the authors.

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Table 1 (on next page)

Profile of the RAD and RUC ante-natal clinics attendees enrolled for the rubella epidemiology study in Ibadan, Nigeria

Table 1: Profile of the RAD and RUC ante-natal clinics attendees enrolled for the rubella epidemiology study in Ibadan, Nigeria

Parameters	RAD (%)	RUC (%)	Remarks
Mean Age (Year)	30.07	31.57	Significantly different (p=0.02)
Age at first marriage (Year)	25.1	27.67	Significantly different (p=0.0005)
Mean parity	1.36	0.97	Significantly different (p=0.03)
Vaccination record	23 (25.6)	56 (30.8)	No association between location and vaccination record (p=0.37)
Education (Primary; Secondary; Tertiary)	8(8.9);39(43.3);43 (47.82)	1(0.6);19(10.5);162(89.0)	Significant association between location and educational status (p=0.0005), that is a woman with tertiary education was likely to be in RUC.
Fever	44.4%	51.1%	Fever not associated with location (p=0.3)
Lymphadenopathy	9 (10)	27 (14.8)	Lymphadenopathy not associated with location (p=0.27)
Ever had rash	18 (20)	46 (25.3)	Ever had rash not associated with location (p=0.34)
Rash (2 weeks before enrolment)	15 (16.7)	40 (22.0)	Rash not associated with location (p=0.31)
Anti-RV IgG prevalence by age (≤ 30 ; > 30 years)	46 (90.2); 37 (94.4)	71 (91.0); 95 (91.3)	Anti-RV IgG seropositivity not associated with age (p=0.94)
Overall anti-RV IgG prevalence	83(92.2)	166(91.2)	No association between location and anti-RV IgG prevalence (p=0.78)
Overall anti-RV IgM prevalence	1(1.1)	4(2.2)	

Key: - RAD: Rubella study subjects in Ade-Oyo Maternity Hospital, RUC: Rubella study subjects in University College Hospital.