Original Research Article

The susceptibility to Rubella virus infection among pregnant women attending antenatal clinics in Unguja, Zanzibar: the need to employ multiple strategies to control Congenital Rubella Syndrome in Tanzania

.Abstract

Aims: This study was conducted to determine the proportion of pregnant women susceptible to Rubella virusinfection inZanzibar, the information that might be useful in future control interventions.
Study design: A cross-sectional hospital-based study.
Place and Duration of Study: The study was conducted in Unguja Zanzibar between May 2023 and July 2023
Methodology: Sociodemographic and other relevant information were collected from pregnant women attending antenatal clinics using structured questionnaires. Blood samples were collected from each consented woman. Detection of rubella IgG antibodies was done by an indirect Enzyme Linked Immunosorbent Assay. Data analysis was done by using STATA version 15.
Results: The median age of enrolled participants was 27[Interquartile range (IQR): 23-31] years while the median gestation age was 28 [IQR: 20-32] weeks. Out of 171 participants, 10/171(5.8%) [95% CI:2-9.6%] were negative to Rubella virus IgG antibodies indicating susceptibility to active Rubella virus infection that can lead to Congenital Rubella Syndrome. On multivariate analysis, history of congenital malformations (OR: 27.4, 95% CI: 1.29-580.5, p=0.033) was significantly associated with susceptibility to Rubella virusinfection.
Conclusion: Despite high level of natural immunity, there is a considerable proportion of pregnant women in Zanzibar susceptible to Rubella virusinfection necessitating the need for multiple strategies to reach a goal Congenital Rubella Syndromeelimination.

Keywords: Rubella, susceptible pregnant women, CRS, Zanzibar

1. INTRODUCTION

Rubella virus (RV) infection usually causes a mild fever and rash in children and adults. However, if contracted in the first trimester of pregnancy the virus can cause teratogenic effects that can lead to Congenital Rubella Syndrome (CRS) characterized by multiple birth defects. The most common manifestations include heart diseases, bilateral cataracts and deafness among many others [1-3].CRS occurs in about 90–100% of the infants whose mothers were infected with the virus in the first trimester while fetal manifestations become rare when maternal infection occurs after 16 weeks of gestation[4]. In the year 2010, an estimated 105,000 CRS cases occurred globally[5]. Before the introduction of Rubella vaccine in 1969, RV caused epidemics every 6–9 years. Few data are available on the global burden and epidemiology RV and CRS before 1969, while in the USA, 60% of RV cases were among children younger than 10 years and 23% among individuals older than 15 years[6]. In Africa the RV natural immunity reported to range from 52.9% to 97.9 % while the proportion of susceptible pregnant women ranged from 2.1% to 47.1%[7]. In Tanzania mainland IgG seropositivity was reported to be 50.8%, 90.4% and 92.6% among children, adolescent girls and pregnant women respectively while prevalence of active infection was found to be 13%, 10.9% and 8.9% among older children, under five children and pregnant women respectively [3, 8, 9]. A previous report in Mwanza documented 46 out of 55 pregnant women with RV active infection in the first trimester to have adverse pregnancy outcomes with 10.9%

having newborns with CRS[3]. This implies that RV active infection can cause serious undesirable pregnancy outcomes necessitating the need to ensure all women of reproductive age are immune to RV.
Tanzania government introduced a catchup campaign on Rubella vaccine in October 2014 which was followed by the introduction of Rubella vaccine in January 2015(about 8 years ago) targeting children under five years of age. A recent report of surveillance data showed a notable decrease in RV active cases in different regions of Tanzania[10]. The world Health Organization (WHO) recommends multiple strategies to be employed including vaccinating susceptible women of reproductive age to reach a goal of CRS elimination[11]. Most of high-income countries (HICs)have achieved thisand are nearly close to reach the goal of CRS elimination. However, in most of low and middle income countries (LMICs) cases of CRS are still reported whereby more than 100,000 children are born with CRS each year [12-14]. Seroepidemiological studies and surveillance data are important in identifying specific groups eligible for vaccination[15]. Despite high level of natural immunity observed in women of reproductive age in Tanzania; there is a considerable proportion of pregnant women susceptible to RV infection that can lead to CRS. Therefore, there is a paramount need to investigate proportion of these women so as to produce information that can be useful in devising appropriate control interventions. In Zanzibar there is limited data on the current status of RV among different population including pregnant women. In a view of that, this study was conducted to determine the proportion of susceptible pregnant women. This information might be useful in future control interventions to reach a goal of CRS elimination.

2. MATERIAL AND METHODS

Study Design, duration, study area and study population

From May to July 2023, a cross-sectional study involving pregnant women attending antenatal clinics in Unguja Zanzibar was conducted. Women were enrolled from Mwembeladu and Rahaleo hospitals. Approximately 1500 women attend antenatal clinics per month at Mwembeladu hospital while approximately 30 women attend gynecological clinic per day atRahaleo hospital.All women enrolled in the study had no prior history of Rubella vaccination.

Sample size estimation, sampling procedures and selection criteria

Sample size was calculated by using Kish Leslie formula using the prevalence of 92.6% from a previous study in Mwanza[16]. A minimum sample size was 105, however a total of 171 pregnant women were enrolled. All pregnant women attending antenatal clinics and consented to participate in this study were included.

Data and sample collection and specimen analysis

The structured pre-tested questionnaire was used to obtain sociodemographic, clinical and other relevant information. Under aseptic procedures approximately 3-5mls of whole blood was drawn from medial cubital vein of the forearm and transferred into plain vacutainer tubes (Becton & Dickson Co.LTD, Nairobi) and allowed to clot for serum extraction. The extracted sera were transferred into well labelled cryovials and kept at a -20^{0}C. Sera were analyzed for the presence of RV IgG antibodies by using indirect Enzyme Linked Immunosorbent Assay (ELISA) as per manufacturer's instructions (Vircell, S.L. ParqueTechnologico de la Salud, Avicena 8, Spain).The assay has sensitivity of 95% and specificity of 95% for the detection of IgG antibodies. A water bath was set to 37^{0}C and all reagents were brought to room temperature before use, approximately 1 hour without removing the plate from the bag. The plate was removed from the package and the numbers of wells to be used were determined. Four wells were used for quality control (two for the cut off values and the other two for the negative and positive control). A100µl of serum diluent was added to all wells, then, 5 µl of each sample, 5 µ of positive control, 5µl of cut off control (in duplicate) and 5 µl of negative control were added into the corresponding wells. The plate was shaken in a plate shaker for 2 minutes until the homogenous mixture of the reagents was achieved. The plate was covered with a sealing sheet and incubated at 37^{0}C for 45 minutes, then the seal was removed and the liquid was aspirated from all wells then washed 5 times with 0.3 ml of washing solution per well. The remaining liquid was drained off. Immediately, 100 µl of IgG conjugate solution was added into each well; plates were covered with a sealing sheet and incubated at 37^{0}C for 30 minutes. The seal was removed, and the liquid was aspirated from the wells,then washed with 0.3 ml of washing solution per well, any remained liquid was drained off. Immediately, 100µl of substrate solution was added to each well and incubated at room temperature for 20minutes followed by addition of 50µl of stopping solution into all wells. Plate was read at 450/620nm with a spectrophotometer within 1 hour of stopping.

Data management and analysis
Laboratory results were recorded into laboratory logbook then sorted and transferred into Microsoft excel sheet for cleaning and coding. Descriptive data analysis was done using STATA version 15 (College Station, TX: Stata Corp LLC). Percentage or fraction was used to summarize categorical variables while median with Interquartile range (IQR) was used for continuous variables. Logistic regression was used to determine factors associated with susceptibility to RV infection and a P-value of <0.05 was considered as statistically significant.

3. RESULTS

Sociodemographic and clinical characteristics
A total of 171 participants were enrolled from Mwembeladu and Rahaleo hospitals in Unguja, Zanzibar and analyzed in this study. The median age of the participants was 27 [IQR: 23-31] years and median gestation age was 28 [IQR: 20-32] weeks. Majority of the participants 152/171 (88.9%) were fromurban areas and most of them 158/171 (92.4%) were married. Moreover, more than two thirds 125/171 (73%) attained formal education. Furthermore, more than a half 111/171 (64.9%) of the participants were housewives. The median number of household members was 5 [IQR:3-6] members while the median parity was 2[IQR:1-3] children. Regarding the clinical history of the participants during the current pregnancy, 16/171 (9.4%) of the participants reported to have fever and 19/171 (11.1%) reported to have rashes. Majority of them166 (97.08%) reported no history of stillbirth (Table 1).

Table 1: Sociodemographic and clinical characteristics of the enrolled participants (n=171)

Variables	Category	Frequency	Percentage (%)
Residency	Urban	152	88.9
	Rural	19	11.1
Marital status	Married	158	92.4
	Unmarried	13	7.6
Education level	No formal education	46	26.9
	Primary school	58	33.9
	Secondary school	55	32.2
	Tertiary	12	7
Occupation	Housewife	116	67.84
	Employed	55	32.16
Water source	Tap water	171	100
House type	Brick	168	98.25
	Mud	3	1.75
Fever	Yes	16	9.4
	No	155	90.6
Rash	Yes	19	11.1
	No	152	88.9
Median [IQR] age in years		27[23-31]	
Median gestation age in weeks		28[20-32]	
Median parity [IQR] children		2[1-3]	
Median [IQR]household members		5[3-6]	

Prevalence of women susceptible to RV infection and associated factors among pregnant women attending antennal clinics in Unguja, Zanzibar (n=171)
Out of 171 participants, 10/171(5.8%, 95% CI:2 – 9.6%] were negative to Rubella IgG antibodies indicating that they are susceptible to active RV infection. On univariate analysis, history of congenital malformations (Fishers exact 7.16, p=0.007) was significantly associated with susceptibility to RV infection. On multivariate analysis; history of congenital malformations (OR:27.4, 95%CI:1.29-580.52, p=0.033) remain to be significantly associated with susceptibility to RV infection (Table2).

Table 2: Factors associated with RV susceptibility among pregnant women in Zanzibar (n=171)

Variable	Total	Rubella susceptibility N (%)/ median (IQR)	Univariate analysis		Multivariate analysis	
			Fisher exact	P value	OR [95%]	P Value
Age (years)	10	27(24-37)		0.503	1.09(0.966-1.23)	0.155
Gestation Age (weeks)	10	30(28-32)		0.379		
Household number	10	5.5(3-8)		0.544		
Rash						
No	152	9(5.92)				
Yes	19	1(5.26)	0.014	0.908		
Stillbirth						
No	166	9(5.42)				
Yes	5	1(20.0)	1.975	0.171	4.2(0.35-50.55)	0.254
Occupation						
Formal employed	55	1(1.82)				
Housewife	116	9(7.76)	0.171	0.111	4.7(0.555-39.57)	0.156
Marital status						
Not married	13	0(0.0)				
Married	158	10(6.3)	1.00	0.443		
Congenital malformation						
NO	169	9(5.33)				
Yes	2	1(50)	7.16	**0.007**	27.4(1.29-580.52)	**0.033**

DISCUSSION

Seroepidemiological studies are important in understanding the burden of disease and its associated consequences in different populations. This study documents the proportion of pregnant women susceptible to RV attending antenatal clinics in Unguja-Zanzibar, Tanzania. Despite the high level of natural immunity observed among pregnant women in Zanzibar, there is a considerable proportion of these women susceptible to acute RV infection that can lead to CRS.The data from the current study are in agreement with a previous report in Tanzania mainland which reported the prevalence of RV susceptible pregnant women to be 7.4%[16]. Moreover, in comparison to previous reports from other African countries, the prevalencereported in the current study is comparable to reports from Eldoret-Kenya, Zaria-Nigeria, Osogbo-Nigeria, Maputo-Mozambique andKhartoum state-Sudan which reported prevalence of 7%, 2.4%, 12.5%, 4.7%, and 4.9% respectively[17-21]. Considering the consequences of acute RV in pregnant women, there is a great risk of these women to contract RV infection that can results into adverse pregnancy outcomes as previously documented in Mwanza[3].

The Tanzania government through Immunization and Vaccination Development Programme (IVDP)introduced RV vaccine in January 2015 targeting children under five years of age which is given in combination with Measles vaccine (MR) in two doses, however, the WHO recommends multiple strategies to be employed to reach a goal of elimination of CRS. Most of the countries employing these strategies including the Russian federation and some of the Pacific countriesare in CRS elimination phase[22, 23]. Despite the efforts made by Tanzania government to introduce RV vaccine and the impact of its implementation[10], there is a paramount need to consider other strategies such as screening and vaccinating women of child bearing age and susceptible pregnant women during postpartum period as recommended previously[24].This has been reported to be more cost-effective in High income countries (HICs) than vaccinating all children and adolescents blindlywhich is mostly practiced by low and middle income countries (LIMCs). Furthermore, introducing screening in the antenatal package can be useful strategy to identify susceptible women so that they can be vaccinated after delivery as previously recommended[25].This can be archived by using existingscreening services in placeto reduce implementation costs.In addition, sustained surveillance system to monitor early occurrence of outbreaks and CRS cases across the country is recommended.Combination of these strategies has been found to be effective in other countries that are in the CRS elimination phase[22].

Among the factors studied, history of congenital malformations was significantly associated with susceptibility to RV infection. This particular subpopulation requires further studies to investigate their vulnerability to other pathogens that can cause congenitalmalformations [26-28].

4. CONCLUSION

In conclusion, the Tanzania government should ensure sustained high vaccination coverage across the country to break RV transmission cycle and employ multiple strategies to reach a goal of CRS eliminationsuch as; screening and vaccinating susceptible women of reproductive age, introduce screening of RV in the existing antenatalpackage and vaccinate susceptible pregnant women during postpartum period, maintaining surveillance system to monitor the trends of outbreaks across the country as well as monitoring CRS cases.

CONSENT

The importance of the study was explained well to the participants and those agreed to participate were asked for the consent by signing in the consent form. Confidentiality was maintained throughout of the study.

ETHICAL APPROVAL

Ethical clearance to conduct the study was sought from CUHAS/BMC research ethics and review committee (CREC) with clearance number 2603/2023 and Zanzibar health research institute (ZAHRI) with reference number 2023/100. Permission to conduct the study was sought from relevant authorities.

ACKNOWLEDGEMENT

The authors of this article are grateful to all pregnant women participated in this study and all relevant authorities for their cooperation.

REFERENCES

1. Grant GB, Reef SE, Patel M, Knapp JK, Dabbagh A: **Progress in rubella and congenital rubella syndrome control and elimination—worldwide, 2000–2016**. *Morbidity and Mortality Weekly Report* 2017, **66**(45):1256.
2. Mwambe B, Mirambo MM, Mshana SE, Massinde AN, Kidenya BR, Michael D, Morona D, Majinge C, Groß U: **Sero-positivity rate of rubella and associated factors among pregnant women attending antenatal care in Mwanza, Tanzania**. *BMC pregnancy and childbirth* 2014, **14**(1):1-5.
3. Mirambo MM, Aboud S, Majigo M, Groβ U, Mshana SE: **Adverse pregnancy outcomes among pregnant women with acute Rubella infections in Mwanza city, Tanzania**. *International Journal of Infectious Diseases* 2019, **78**:72-77.
4. Gershon AA: **Rubella virus (German measles)**. *Principles and Practice of Infectious Diseases Philadelphia: Churchill Livingstone* 2010:2127-2132.
5. Murhekar M, Verma S, Singh K, Bavdekar A, Benakappa N, Santhanam S, Sapkal G, Viswanathan R, Singh MP, Nag VL: **Epidemiology of Congenital Rubella Syndrome (CRS) in India, 2016-18, based on data from sentinel surveillance**. *PLoS neglected tropical diseases* 2020, **14**(2):e0007982.
6. Patel MK, Antoni S, Danovaro-Holliday MC, Desai S, Gacic-Dobo M, Nedelec Y, Kretsinger K: **The epidemiology of rubella, 2007–18: an ecological analysis of surveillance data**. *The Lancet Global Health* 2020, **8**(11):e1399-e1407.
7. Mirambo MM, Majigo M, Aboud S, Groß U, Mshana SE: **Serological makers of rubella infection in Africa in the pre vaccination era: a systematic review**. *BMC research notes* 2015, **8**(1):716.
8. Mirambo MM, Aboud S, Mushi MF, Seugendo M, Majigo M, Groß U, Mshana SE: **Serological evidence of acute rubella infection among under-fives in Mwanza: a threat to increasing rates of congenital rubella syndrome in Tanzania**. *Italian journal of pediatrics* 2016, **42**(1):1.
9. Mirambo MM, Aboud S, Groß U, Majigo M, Mushi MF, Mshana SE: **Rubella seromarkers and determinants of infection among tanzanian children and adolescents in prevaccination Era: Are we in the right track?***International journal of preventive medicine* 2017, **8**.
10. Michael F, Mirambo MM, Lyimo D, Kyesi F, Msanga DR, Joachim G, Nyaki H, Magodi R, Mujuni D, Tinuga F: **Reduction in Rubella Virus Active Cases among Children and Adolescents after Rubella Vaccine Implementation in Tanzania: A Call for Sustained High Vaccination Coverage**. *Vaccines* 2022, **10**(8):1188.
11. WHO: **Position paper on rubella vaccines**. *Weekly epidemiological record* 2000, **75**:161-172.
12. WHO: **Rubella vaccines: WHO position paper—Recommendations**. *Vaccine* 2011, **29**(48):8767-8768.
13. Binnicker M, Jespersen D, Harring J: **Multiplex detection of IgM and IgG class antibodies to Toxoplasma gondii, rubella virus, and cytomegalovirus using a novel multiplex flow immunoassay**. *Clinical and Vaccine Immunology* 2010, **17**(11):1734-1738.
14. Katow S: **Rubella virus genome diagnosis during pregnancy and mechanism of congenital rubella**. *Intervirology* 1999, **41**(4-5):163-169.
15. Mirambo Mariam M. HA, Mrutu Neema B, Majigo Mtebe, Aboud Said, Groβ Uwe, Mshana Stephen E. : **Strategic immunization to control rubella and congenital rubella syndrome in Tanzania**. In: *Policy Brief.* Catholic University of Health and Allied Sciences; 2016.
16. Mwambe B, Mirambo MM, Mshana SE, Massinde AN, Kidenya BR, Michael D, Morona D, Majinge C, Groß U: **Sero-positivity rate of rubella and associated factors among pregnant women attending antenatal care in Mwanza, Tanzania**. *BMC pregnancy and childbirth* 2014, **14**(1):95.
17. Amina M-D, Oladapo S, Habib S, Adebola O, Bimbo K, Daniel A: **Prevalence of rubella IgG antibodies among pregnant women in Zaria, Nigeria**. *International health* 2010, **2**(2):156-159.
18. Kolawole OM, Anjorin EO, Adekanle DA, Kolawole CF, Durowade KA: **Seroprevalence of Rubella IgG Antibody in Pregnant Women in Osogbo, Nigeria**. *International journal of preventive medicine* 2014, **5**(3):287.
19. Barreto J, Sacramento I, Robertson SE, Langa J, Gourville E, Wolfson L, Schoub BD: **Antenatal rubella serosurvey in Maputo, Mozambique**. *Tropical Medicine & International Health* 2006, **11**(4):559-564.

20. Adam O, Makkawi T, Kannan A, Osman M: **Seroprevalence of rubella among pregnant women in Khartoum state, Sudan**. *EMHJ* 1995, **19**(9).
21. Kombich J: **Seroprevalence of natural rubella antibodies among antenatal attendees at Moi teaching and Referral Hospital, Eldoret, Kenya**. *Journal of Infectious Diseases & Immunological Techniques* 2012.
22. Chekhlyaeva T, Tsvirkun O, Turaeva N, Erokhov D, Barkinkhoeva L, Tikhonova N: **Assessing the 2019 rubella elimination status in the Russian Federation**. *Russian Journal of Infection and Immunity* 2022, **12**(1):85-94.
23. mondiale de la Santé O, Organization WH: **Roadmap to elimination standard measles and rubella surveillance**. *Weekly Epidemiological Record= Relevé épidémiologique hebdomadaire* 2017, **92**(9/10):97-105.
24. Mirambo MM, Hokororo A, Mrutu NB, Majigo M, Aboud S, Gross U, Mshana SE: **Strategic immunization to control rubella and congenital rubella syndrome in Tanzania**. *CUHAS/PB/001* 2016:1-6.
25. Hattoufi K, Aguenaou H, Kharbach A, Barkat A: **Seroprevalence of Rubella in a Group of Moroccan Parturients and Physicians' Opinions**. *Iranian Journal of Neonatology* 2022, **13**(3).
26. Mirambo MM, Chibwe E, Mushi MF, Majigo M, Mshana SE: **Cytomegalovirus, Parvovirus B19 and Rubella Co-infection among Pregnant Women Attending Antenatal Clinics in Mwanza City: The Need to be considered in Tanzanian Antenatal Care Package**. *Epidemiology: Open Access* 2016, **2016**.
27. Chibwe E: **Seroprevalence and associated factors of human cytomegalovirus among pregnant women attending antenatal clinics in Mwanza city, Tanzania**. *Catholic University of Health and Allied Sciences (CUHAS-Bugando)* 2016.
28. Chibwe E, Mirambo MM, Kihunrwa A, Mshana SE: **Magnitude of the Cytomegalovirus infection among pregnant women attending antenatal clinics in the city of Mwanza, Tanzania**. *BMC Research Notes* 2017, **10**(1):489.

DEFINITIONS, ACRONYMS, ABBREVIATIONS

BMC	Bugando Medical Centre
CRS	Congenital rubella Syndrome
ELISA	Enzyme linked immune sorbent Assay
CUHAS	Catholic University of Health and Allied Sciences
IgG	Immunoglobulin G
OR	Odds ratio
CI	Confidence interval
IQR	Interquartile range
IVDP	Immunization and Vaccination Development Programme