



RESEARCH PAPER

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Anti-rubella antibodies seroprevalence among pregnant women, attending antenatal care at the Sino-Gabonese friendship hospital in Franceville

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Abstract

Pregnant women represent a population category at high risk of severe rubella infection, capable of adversely affecting their fetus. This study was conducted to determine the anti-rubella antibodies seroprevalence, among pregnant women attending antenatal care at the Sino-Gabonese Friendship Hospital in Franceville. A prospective, cross-sectional study was conducted at the Sino-Gabonese Friendship Hospital in Franceville, from April to June 2023. Using a structured, pre-established and pre-tested questionnaire, sociodemographic, and obstetrical characteristics, were obtained. Three milliliters of serum obtained after centrifugation of the 10 ml of blood sample collected from the participants, were used to measure rubella-specific IgG antibody levels using the OnSite Rapid Test Rubella IgG/IgM COMBO lateral flow immunochromatographic test. Results were considered significant at $p \leq 0.05$. The study included 107 pregnant women attending antenatal care. With a mean age of 27.9 ± 7.1 years, anti-Rubella IgG antibodies were found in 87 pregnant women, indicating an overall seroprevalence of 81.30% (95% CI: [0.72 - 0.87]). Of these, 6.9% ($n = 6$) of the women were serum anti-Rubivirus IgM carriers. In univariate analysis, it was indicated that pregnant women in the study aged between 21-30 years (OR = 0.33; 95% CI: [0.12-0.9] $p = 0.0027$), student (OR = 6.35; 95% CI: [1.39-29.09] $p = 0.001$), or without professional status (OR = 0.05; 95% CI: [0.01- 0.27] $p < 0.001$), high school education (OR = 8.24; 95% CI: [2.83-23.96] $p < 0.001$), single (OR = 0.3; 95% CI: [0.11-0.85] $p = 0.0021$) or cohabiting OR = 3.91; 95% CI: [1.21-12.64] $p = 0.0017$) Residing in an urban area (OR = 49; 95% CI: [1.43-16.75] $p = 0.006$), in the first trimester of their pregnancy (OR = 0.29; 95% CI: [0.09-0.94] $p = 0.033$), with a history of spontaneous abortion (OR = 0.09; 95% CI: [1.04; 11.30] $p = 0.037$), and unvaccinated (OR = 0.13; 95% CI: [0.05; 0.13] $p < 0.000$), had an elevated risk of Rubella, After adjusting of risk factors by logistic regression, residence in a rural area (aOR = 139.87 95% CI : [1.74 - 11236.216] $p = 0.027$), and no vaccination (aOR = 43.3 95% CI: [1.13 -1662.7] $p = 0.043$), were identified as the only significant risk factors for Rubella infection in the present study. The high rate of anti-rubella antibodies seroprevalence among pregnant women in the present study indicates that any short-term vaccination strategy aimed at reducing the number of susceptible women of childbearing age is imperative.

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Introduction

Nowadays, viral infections are increasingly common worldwide (Baker *et al.*, 2022). One of these is rubella. Although this disease is benign, it affects both children and adults. It is responsible for numerous intrauterine complications in pregnancy, which can cause a series of malformations of the embryo, death of the fetus, and premature delivery in the primary infection case among pregnant women (Camejo and Méndez, 2023). In the world, the major epidemics of the disease have occurred in developed countries. This is the case of Great Britain in 1940, Sweden in 1951 and the United Kingdom in 2006, Sweden in 1951 and the United States of America in 1964. In developing countries, an epidemic occurred in Panama in the mid-1980s. Alone in the United States, more than 20,000 congenital rubella syndrome (CRS) cases have been reported (Berg, 2022). In 2010, the incidence burden was estimated at between 90 and 121 cases per 100,000 live births, with the African and South-East Asian regions expected to hold the highest figures (Vynnycky *et al.*, 2016). An integral part of public health, and linked to the risk of infection among pregnant women, the United States of America and Canada, have developed vaccines to combat rubella and congenital rubella syndrome (World Health Organization, 2018). As a result, many countries that have introduced these rubella vaccines into their national programs continue to steadily increase. Indeed, it was reported that as of December 2018, 168 out of 194 countries had introduced rubella vaccine, and global coverage was estimated at 69%. Reported cases of rubella have decreased by 97%, from 670,894 cases in 102 countries in 2000 to 14,621 cases in 151 countries in 2018 (World Health Organization, 2018). In contrast to these countries, rubella remains a real public health problem in developing countries (countries in Africa, Southeast Asia and the Middle East (Lambert *et al.*, 2015). Moreover, it is estimated that the magnitude of congenital rubella syndrome for countries without rubella vaccine policy can be very large (Su *et al.*, 2021).

For example in 1996, there were nearly 22,000 congenital rubella cases in Africa and nearly 46,000 in Southeast Asia (Masresha *et al.*, 2018). Gabon, a central African country is not immune to the antics of this rubella burden. Despite its inclusion in the Expanded Program on Immunization (EPI), implementation of the rubella vaccine in primary health care centers leaves much to be desired, especially in rural areas (Ategbo *et al.*, 2010), there are no rubella screening programs among pregnant women but rather a detection of immunity in them, towards the disease. Thus, the magnitude of the problem is unknown. The lack of data on the seroprevalence of the virus responsible for the disease among pregnant women in this country therefore prompted the setting up of this study, with the general objective of assessing the seroprevalence of rubella antibodies among pregnant women attending antenatal care at the Sino-Gabonese Friendship Hospital in Franceville.

Materials and methods

Study setting

The study was conducted in the medical analysis laboratory of the Sino-Gabonese Friendship Hospital in Franceville, the capital of the second province (Haut-Ogooué) of Gabon in terms of population. This city is divided into four districts. Located in the 2nd district, the Sino-Gabonese Friendship Hospital of Franceville is a support hospital for the Amissa Bongo University Hospital of Franceville, another public health structure, and receives patients from all walks of life.

Type study

This is a prospective and cross-sectional study with descriptive and analytical purposes.

Period study

This was conducted from April to June 2023.

Population study

This study focused on pregnant women attending antenatal care, during the period study, at the Sino-Gabonese Friendship Hospital in Franceville.

Participant and exclusion criteria

Only pregnant women attending antenatal care at the Sino-Gabonese Friendship Hospital, and who had consented to participate, were included in the study. Non-pregnant women and pregnant women who refused to participate in the study were excluded.

Sampling method and sample size

To select pregnant women examined at the Sino-Gabonese Friendship Hospital from April to July 2022, a systematic, random and simple sampling technique was used, and the size of our study population was 107 participants, leading to an inclusion rate of 96.8%.

Questionnaire

A structured, pre-established and pre-tested questionnaire, was submitted to the participants, to collect socio-demographic (age, marital status, professional status, level of education, residence), and obstetrical characteristics such as pregnancies number, age of pregnancy, history of spontaneous abortion, stillbirth, and immunization status.

Definitions

Stillbirth: delivery of a fetus showing no signs of life after 20 completed weeks of gestation.

Fetal death: fetal death in utero after 28 completed weeks of gestation.

Spontaneous abortion: a clinically recognized spontaneous miscarriage prior to 20 weeks gestation.

Previous exposure to rubella virus infection: pregnant women whose blood tests positive for IgG antibodies, thus protective immunity against infection.

Recent rubella virus infection: pregnant women testing positive for IgM antibodies.

Sample collection and storage

After the participants signed the informed consent form, 10 ml venous blood was collected aseptically at the HASG laboratory in Franceville from each pregnant woman in one EDTA-coated tube and one dry tube. Each tube was centrifuged at 3000 rpm for 10 min to remove the plasma, which was frozen at -20°C until use.

Serological diagnosis of rubella

Using the rapid test: OnSite Rapid Test Rubella IgG/IgM COMBO, Rubella was diagnosed with 3 ml of serum obtained after centrifugation of 10 ml of venous blood sample collected from each study participant, as recommended by American manufacturer, CTK Biotech, Inc, California.

Interpretation of the result

Negative result: If only the C line develops, this indicates that anti-Rubella antibodies are not detected in the sample. The result is negative or non-reactive.

Positive result: In addition to the presence of the C line, if only the M line develops, the test indicates the presence of anti-Rubella IgM. The result is anti-Rubella IgM positive or reactive and anti-Rubella IgG negative or non-reactive.

In addition to the presence of the C line, if only the G line develops, the test indicates the presence of anti-Rubella IgG. The result is positive or reactive anti-Rubella IgG and negative or non-reactive anti-Rubella IgM.

In addition to the presence of the C line, if the M and G lines develop, the test indicates the presence of both anti-Rubella IgM and IgG. The result is anti-Rubella IgM and IgG positive.

Invalid Test: If no C line develops, the test is invalid regardless of color development on the test lines (G and M) as indicated below. Repeat the test with a new cassette.

Data quality assurance

To assess its validity and completeness, a questionnaire was pretested with 5% of the study subjects, prior to the actual data collection. This process was methodically and rigorously followed. To verify the accuracy and completeness of the questionnaire data obtained from each study participant, the data were reviewed immediately. The samples were processed and tested by an experienced laboratory professional and according to the recommendations of the supplier and manufacturer. Sensitivity and specificity were 98%

and 97.3%, respectively, for the OnSite Rubella IgG/IgM rapid test.

Ethical considerations

The study was authorized by the Gabon South-East Regional Director of Health, in letter No. 0345/PHO/SG/DRSSE/SGP/D, and endorsed by the hospital administration management. The sampling was designed on the basis of the voluntary participation of the pregnant women in the study, after explaining the rationale, benefits, and ensuring the confidentiality of the study. Participation in the study was finalized by written and informed consent of each participant. To maintain anonymity, code numbers were used instead of nominal identifiers.

Statistical analysis of data

Data were entered into a Microsoft Excel 2013 spreadsheet and analyzed using R version 4.2.1 for Windows. Categorical data were presented as frequencies and percentages. To assess the association between rubella seroprevalence and various sociodemographic and obstetric characteristics of the study's pregnant women, univariate and multivariate analyses using binary logistic regression were performed. In the univariate analysis, only significant variables with a p-value ≤ 0.05 were selected and included in the multivariate logistic regression model. Results were expressed as odds ratios (OR) and 95% confidence intervals (CI). P-values were determined and considered significant when less than or equal to 0.05.

Results

Rubella virus infection seroprevalence among pregnant women in the study (n = 107)

A total of 107 pregnant women seen for prenatal consultation at the Sino-Gabonese Friendship Hospital met the inclusion criteria and responded to the survey. This indicated a 100% response rate for the present study. With a mean age of 27.9 ± 7.1 years for all participants, an overall seroprevalence of 81.30% (95% CI: 0.72 - 0.87) (n=87), of specific anti-Rubivirus IgG was revealed in these women. Among the latter, 6.9% (n=6) of the women were serum anti-Rubivirus IgM carriers.

Rubella virus infection Seroprevalence according to pregnant women sociodemographic characteristics (N = 107)

Univariate analysis of rubella virus infection seroprevalence, according to pregnant women sociodemographic characteristics indicated that, those who were aged between 21-30 years (OR = 0.33; 95% CI: [0.12-0.9] p=0.0027), student (OR = 6.35; 95% CI: [1.39-29.09] p=0.001), or with no occupational status (OR = 0.05; 95% CI: [0.01-0.27] p<0.001), secondary school education (OR = 8.24; 95% CI: [2.83-23.96] p<0.001), single (OR = 0.3; 95% CI: [0.11-0.85] p=0.0021), or cohabiting (OR = 3.91; 95% CI: [1.21-12.64] p=0.0017) and residing in a rural area (OR = 49; 95% CI: [1.43-16.75] p=0.006) were at high risk for rubella virus infection (Table 1).

Rubella virus infection seroprevalence, according to pregnant women obstetric characteristics (N = 107)

Univariate analysis of rubella virus infection seroprevalence, according to pregnant women obstetric characteristics indicated that, unlike risk factors that may be associated with rubella such as stillbirth or gestational age, only pregnant women who were in the first trimester of pregnancy (OR = 0.29; 95% CI : [0.09-0.94] p=0.033), with a history of spontaneous abortion (OR = 0.09; 95% CI: [1.04; 11.30] p= 0.037), and unvaccinated (OR = 0.13; 95% CI: [0.05; 0.13] p<0.000), had a very high likelihood of being infected with rubella virus. These risk factors were significantly associated with rubella antibodies seroprevalence (Table 2).

Multivariate logistic regression analysis of risk factors associated with rubella seroprevalence among pregnant women (n = 107) in the study

Finally, the result of the multivariate logistic regression analysis indicated that pregnant women in the study residing in rural areas (Adjusted OR = 139.87 95% CI: [1.74 -11236.216] p=0.027), and unvaccinated (Adjusted OR = 43.3 95% CI: [1.13 - 1662.7] p= 0.043), were at higher risk of Rubella than other participants (Table 3).

Table 1. Univariate analysis of rubella virus infection seroprevalence, according to pregnant women sociodemographic characteristics (N = 107)

Variables	Rubella virus infection seroprevalence % (n/N)	Crude OR, 95% CI	p-value
Age groups of pregnant women (years)			
≤ 20	80 (12/15)	0.91 [0.23 – 3.58]	0.89
21 – 30	70.73 (29/41)	0.33 [0.12–0.9]	0.027*
31– 40	88.89 (40/45)	2.55 [0.85 – 7.63]	0.09
41 – 43	100 (6/6)	Reference	-
Professional status of the pregnant women			
Pupil/student	94.74 (36/38)	6.35 [1.39 – 29.09]	0.001*
Small job	76.92 (10/13)	0.74 [0.18 – 2.98]	0.67
Public official	81.25 (39/48)	Reference	-
Without	25 (2/8)	0.05 [0.01 – 0.27]	< 0.001*
Education level of the pregnant women			
Primary	92 (23/25)	3.23 [0.69 – 15.02]	0.12
Secondary	91.02 (71/78)	8.24 [2.83 – 23.95]	< 0.001*
University	75 (3/4)	Reference	-
Marital status of pregnant women			
Single	72 (36/50)	0.3 [0.11– 0.85]	0.021*
Cohabiting	81.49 (43/47)	3.91 [1.21–12.64]	0.017*
Engaged	8.33 (5/6)	1.16 [0.13 –10.51]	0.90
Married	75 (3/4)	Reference	-
Residence Area of the pregnant women			
Franceville (Semi-urban)	85.10 (80/94)	4.9 [1.43 –16.75]	0.006*
Other (Rural)	53.85 (7/13)	Reference	-

*= Significant result, OR = Odds ratio, CI = Confidence interval

Table 2. Univariate analysis of rubella virus infection seroprevalence, according to pregnant women obstetric characteristics (N = 107)

Variables	Rubella virus infection seroprevalence % (n/N)	Crude OR, 95 % CI	p-value
Age of pregnancy (Trimester)			
1st Trimester	77.05 (47/61)	0.29 [0.09 – 0.94]	0.033*
2 nd Trimester	87.5 (28/32)	1.9 [0.58 – 6.21]	0.28
3 rd Trimester	85.71 (12/14)	Reference	-
Pregnancies number			
Predigested	82.9 (63/76)	1.41 [0.5 –3.96]	0.51
Multigested	77.42 (24/31)	Reference	-
Number of deliveries after 20 weeks of pregnancy			
≤ 2	95.12 (37/41)	Reference	-
≥3	72.73 (50/66)	0.29 [0.09 – 0.94]	0.061
History of spontaneous abortion			
Yes	33.33 (2/6)	0.09 [0.02 – 0.53]	0.002*
No	82.33 (85/101)	Reference	-
Stillbirth			
Yes	80 (4/5)	0.92 [0.1– 0.71]	0.62
No	81.37 (83/102)	Reference	-
Vaccination status against rubella			
Vaccinated	90.91 (70/77)	Reference	-
Not Vaccinated	56.67 (17/30)	0.13 [0.05 – 0.13]	< 0.001*

*= Significant result, OR = Odds ratio, CI = Confidence interval

Table 3. Multivariate logistic regression analysis of Rubella virus infection risk factors, among pregnant women (n = 107) in the study

Variables	Rubella virus infection seroprevalence % (n/N)	Adjusted OR, 95 %CI	p-value
Age groups of pregnant women (years)			
≤20	80 (12/15)	-	-
21–30	70.73 (29/41)	-	-
31–40	88.89 (40/45)	-	-
41–43	100 (6/6)	-	-
Professional status of the pregnant women			
Pupil/student	94.74 (36/38)	-	-
Small job	76.92 (10/13)	-	-
Public official	81.25 (39/48)	-	-
Without	25 (2/8)	-	-
Education level of the pregnant women			
Primary	92 (23/25)	-	-
Secondary	91.02 (71/78)	-	-
University	75 (3/4)	-	-
Marital status of pregnant women			
Single	72 (36/50)	0.48 [0.02 –11.93]	0.65
Cohabiting	81.49 (43/47)	-	-
Engaged	8.33 (5/6)	-	-
Married	75 (3/4)	1	-
Residence Area of the pregnant women			
Franceville (Semi-urban)	85.10 (80/94)	1	-
Other (Rural)	53.85 (7/13)	139.87 [1.74 -11236.22]	0.027*
Age of pregnancy			
1st Trimester	77.05 (47/61)	0.064 [0.02 –2.41]	0.064
2 nd Trimester	87.5 (28/32)	0.046 [0.00 – 5.00]	0.2
3 rd Trimester	85.71 (12/14)	1	-
History of spontaneous abortion			
Yes	33.33 (2/6)	-	-
No	82.33 (85/101)	-	-
Vaccination status against rubella			
Vaccinated	90.91 (70/77)	1	-
Not Vaccinated	56.67 (17/30)	43.3 [1.13 –1662.7]	0.043*

Discussion

As IgM detection is a better indicator of rubella seroprevalence among pregnant women (Sampedro *et al.*, 2013), the present study revealed that n = 6, or 6.9% of women, carried anti-rubella IgM in their serum.

This result is contrary to that obtained elsewhere (Haabouni *et al.*, 2012), indicating de facto cases of recent and progressive rubella, which can lead to serious fetal complications such as miscarriage, stillbirth or congenital rubella syndrome (CRS) in the child (Mawson and Croft, 2019). Lower than 93%

obtained in a study conducted elsewhere [13], close to those obtained in China (83.3%) (Meng *et al.*, 2018), India (83.4 and 82.3%) (Muliyl *et al.*, 2018; Shanmugasundaram *et al.*, 2021), Cameroon (88.6%) (Fokunang *et al.*, 2010), and higher than those reported in western Sudan (65.3%) (Hamdan *et al.*, 2011) and Algeria (68.6%) [19], an anti-rubella antibody seroprevalence of 81.30% (n=87) was found among pregnant women in the present study. The diversity of these results corroborates with differences in factors such as the serological diagnostic methods used in each study, study sample sizes, environmental characteristics and daily behavioral characteristics of

pregnant women such as level of education, hygiene practices and dietary habits (And *et al.*, 2022). As mentioned elsewhere, the majority of IgG antibody-positive pregnant women in the present study would have already been immunized against rubella, or would have been immunized in the past. It is therefore unlikely that they would infect their fetus outside a state of high immunosuppression (Kolawole *et al.*, 2014). On the other hand, it was shown that in the overall rubella seroprevalence among pregnant women in the study, the proportion of IgM positivity was 6.9%.

Higher than that reported in Ethiopia (2.1%) (Tamirat *et al.*, 2017), this seroprevalence was consistent with the combined prevalence of recent rubella infection in sub-Saharan Africa (5.1%) (Mirambo *et al.*, 2015), as also recently reported in Cameroon (5.5%) (Michel *et al.*, 2018). In contrast, the proportion of IgM positivity in the present study (6.9%), was lower than those reported in Northern Ethiopia (9.5%) (Wondimeneh *et al.*, 2018). This diversity of results in different studies, could be due to the difference in endemicity of rubella virus, variability in sample size of the studies, laboratory methods used, and differences in thresholds of tests used.

Univariate analysis of rubella antibody seroprevalence according to the sociodemographic characteristics of the participants of this study revealed that those aged between 21 and 30, students or without professional status, with a secondary education, single or cohabiting, and living in rural areas, had a high risk of contracting rubella. In contrast to other studies (Ekuma *et al.*, 2022), it was noted that women with low occupational status and single women, probably because of their lower income, poor hygiene practices and dietary habits, or because they live in unfavorable socio-economic conditions, are more likely to be affected by the disease (Ramos-Morcillo *et al.*, 2019). Moreover, in most Gabonese towns, such as Franceville, which is a semi-urban area, people are tending to eat more and more outside the home. The upsurge in street food outlets (grilled meats, fruit and vegetables, fermented

drinks, cakes, etc.) and even the water served for drinking is said to be of dubious origin, quality and hygiene. As a result, an increase in the number of rubella cases can be expected in the years to come, if measures are not put in place to tackle the problem.

This is undoubtedly why the Gabonese Food Security Agency (GFSA) has decided to launch a campaign under the slogan « Eat out, eat clean » to raise awareness of good hygiene practices among street food operators (Ntoutoum, 2019).

In addition, a univariate analysis of rubella antibodies seroprevalence according to obstetrical characteristics of the pregnant women in this study indicated that those who were in the first trimester of pregnancy, had a history of spontaneous abortion, and were unvaccinated, had a very high probability of being infected with the rubella virus. This result is in agreement with a study conducted in Ethiopia that found a higher proportion of IgM positivity in pregnant women who were in their first trimester (Tulu *et al.*, 2018), in Tanzania (Lulandala *et al.*, 2017). Unlike some studies that found a significant association between rubella prevalence and urban settings (Jahromi *et al.*, 2011), further multivariate logistic regression analysis of variables in the present study, showed that rubella seropositivity was significantly associated with rural residence and non-vaccination. These results, similar to those obtained in a study conducted in Canada (Paediatr Child Health, 1999), can be explained by the fact that rubella vaccine is not always administered to unprotected women after delivery, and not all women are tested during pregnancy. Second, in countries in the sub-Saharan region, populations living in the hinterland do not have sufficient information about the appropriateness of vaccination. And the vaccination status of women in Gabon is not checked regularly. Yet, the elimination of congenital rubella syndrome depends not only on effective vaccination in childhood, but also on identifying and vaccinating unprotected women of childbearing age (Dixon *et al.*, 2022).

Study strengths and limitations

Although this study provides preliminary data that can support future research, a few limitations are acknowledged. Firstly, the period covered by this evaluation did not allow for sufficient sample size. Secondly, the OnSite Rubella IgG/IgM COMBO lateral flow chromatography rapid diagnostic, which was used for the rapid and simultaneous detection and quantification of anti-rubella IgG and IgM antibodies, should be combined with sensitivity, specificity and avidity tests for IgG, such as ELISA. In addition, the molecular biology technique (qPCR), which detects Rubivirus RNA, could enhance diagnosis and reflect the true burden of rubella in the study population. Thirdly, as the assessment of risk factors was carried out on the basis of participants' self-reports, the study may be marred by a risk of replication bias. Finally, because it was carried out exclusively in hospital settings, this study may not represent the general public.

Conclusion

The present study, which has the advantage of being one of the few surveys of its kind on rubella among pregnant women in Gabon, and the Haut-Ogooué province in particular, has enabled us to better assess the seroprevalence and risk factors associated with anti-rubella antibodies among pregnant women in Franceville and the surrounding area. The study showed that 18.7% of participants were still at risk of rubella infection during pregnancy, with potential complications for the fetus in the case of congenital rubella. The risk factors revealed in the present study are residence in a rural area and unvaccinated status. To reduce the risk of congenital rubella, preventive measures could be taken by Gabonese health authorities. On the other hand, they should consider awareness campaigns for pregnant women on rubella and its modes of contamination, training health professionals in the diagnosis of prenatal infection, and reducing the cost, or even making diagnosis free of charge, in order to better prevent and control the spread of congenital rubella in Gabon.

What is known about this topic?

1. Despite the existence of a vaccine, rubella is re-emerging in sub-Saharan Africa.
2. In Gabon, there is little research based on the burden of congenital rubella syndrome (CRS).

What this study adds?

1. This study reports on the risk factors associated with rubella complications in Gabonese women living in and around Franceville;
2. This study shows that not being vaccinated and living in a rural environment are predisposing factors for rubella in Franceville (Gabon);
3. The high rate of rubella seroprevalence in the present study suggests that urgent action is needed to prevent this disease and its associated complications in vulnerable populations such as pregnant women and their future babies.

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