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By Mieret Tanguy, Ontsira Ngoyi E. N., Nguessan Koffi, Aloumba A., Doumbia M., Ossibi Ibara B. R. & Faye-Kette H.

Marien Ngouabi University

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Assessing Rubella Immunity: Seroprevalence among Pregnant Women in Brazzaville, Congo

Mieret Tanguy [α], Ontsira Ngoyi E.N. [σ], Nguessan Koffi [ρ], Aloumba A. [ω], Doumbia M. [¥], Ossibi Ibara B. R. [§] & Faye-Kette H. [χ]

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Results: The overall IgG seropositivity of pregnant women was 91.8%. This rate was higher among women over 34 years of age, reaching 100%. The 16-24 and 25-34 age groups had 95.7% and 87.3% seropositivities, respectively. The distribution of the population by gestational age showed maximum seropositivity among women in the second trimester of pregnancy (97.5%), followed by those in the first trimester (88.4%) and third trimester (86.7%). Notably, 62.5% of pregnant women in the first trimester had a negative serology. Mean IgG titers were highest in women aged 25 to 34 years (94.3 IU/L) and in those in the third trimester of pregnancy (109.2 IU/L). IgG seroprevalence showed no statistically significant differences between age groups (p = 0.405) or between trimesters of pregnancy (p = 0.376). No pregnant women have been IgM positive.

Conclusion: IgG seropositivity (91.8%) shows strong immunity against rubella. No pregnant women developed IgM, indicating the absence of recent infections. Vulnerability in the first quarter (62.5%) remains a concern. Surveillance, awareness-raising and vaccination are essential to improve vaccination coverage and protect vulnerable populations in Congo, thereby reducing transmission and protecting future generations.

Keywords: rubella, pregnant women, seroprevalence, immunity.

Résumé- Contexte: La rubéole est un problème majeur de santé publique à cause de ses effets tératogènes, surtout en début de grossesse. Il est crucial de comprendre le profil sérologique des femmes enceintes pour prévenir le syndrome de rubéole congénitale. Cette étude visait à déterminer ce profil chez les femmes enceintes touchées par la rubéole.

Méthodologie: Nous avons mené une étude prospective descriptive sur des plasmas sanguins de femmes enceintes sur une période de trois mois, du 1er janvier au 31 mars 2022. Au total, 98 échantillons de plasmas sanguins de femmes enceintes ont été analysés. L'échantillonnage a été effectué de manière systématique sur l'ensemble des prélèvements sanguins des femmes enceintes ayant fréquenté le laboratoire de l'hôpital spécialisé mère-enfant Blanche Gomez à Brazzaville, Congo, pour des investigations biologiques. Le dosage des IgG et IgM a été réalisé à l'aide de l'analyseur Architect i1000SR (ABBOTT), utilisant la technique immunologique microparticulaire par chimiluminescence.

Résultats: La séropositivité globale en IgG des femmes enceintes était de 91,8 %. Ce taux était plus élevé chez les femmes de plus de 34 ans, atteignant 100 %. Les groupes d'âge de 16 à 24 ans et de 25 à 34 ans avait respectivement des séropositivités de 95,7% de 87,3 %. La répartition de la population selon l'âge gestationnel a montré une séropositivité maximale chez les femmes au deuxième trimestre de grossesse (97,5 %), suivie de celles au premier trimestre (88,4 %) et au troisième trimestre (86,7 %). Notamment, 62,5 % des femmes enceintes au premier trimestre avaient une sérologie négative. Les titres moyens d'IgG étaient les plus élevés chez les femmes âgées de 25 à 34 ans (94,3 UI/L) et chez celles au troisième trimestre de grossesse (109,2 UI/L). La séroprévalence des IgG n'a montré aucune différence statistiquement significative entre les groupes d'âge (p = 0,405) ni entre les trimestres de grossesse (p = 0,376). Aucune femme enceinte n'a été séropositive aux IgM.

Conclusion: La séropositivité en IgG (91,8 %) montre une forte immunité contre la rubéole. Aucune femme enceinte n'a développé d'IgM, indiquant l'absence d'infections récentes. La vulnérabilité au premier trimestre (62,5 %) reste préoccupante. Surveillance, sensibilisation et vaccination sont essentielles pour améliorer la couverture vaccinale et protéger les populations vulnérables au Congo, réduisant ainsi la transmission et protégeant les futures générations.

Mots-clés: rubéole, femmes enceintes, séroprévalence, immunité.

Author α σ ω §: Faculty of Health Sciences, Marien Ngouabi University, Brazzaville, Congo. National Public Health Laboratory, Brazzaville, Congo. e-mails: tmieret@gmail.com, esthernina2009@gmail.com, esther_muller2003@yahoo.fr

Author σ: Bacteriology-Virology Laboratory, Brazzaville University Hospital, Congo

Author ω §: Department of Infectious Diseases, Brazzaville University Hospital, Congo. e-mail: bienvenu_07@yahoo.fr

Author ¥ χ: Pasteur Institute, Abidjan, IVORY COAST. e-mail: doumce1@gmail.com

Author χ: UFR of Medical Sciences of Abidjan, Félix HOUPHOUET-BOIGNY University, IVORY COAST. e-mail: hortensekette@googlemail.com

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I. Introduction

Rubella is an acute illness caused by the rubella virus, manifesting as a maculopapular rash and fever. Highly contagious, it is transmitted through the air through direct contact with an infected person, whose nasopharyngeal secretions contain the virus (1, 2). Generally benign and mainly affecting children, rubella is however a major public health problem due to its teratogenic potential, especially in early pregnancy. Infection during the first trimester can lead to miscarriages, fetal deaths, stillbirths, or birth defects (up to 90% of cases), known as congenital rubella syndrome (CRS). This syndrome can affect various organ systems, including the ophthalmic, auditory, cardiac, neurological, hepatic, and hematological systems (3). Understanding the serological profile is essential to protect the health of mothers and babies, by enabling a timely and effective intervention. The WHO estimates that each year, about 100,000 cases of CRS occur worldwide, including 39,000 in Africa in 2010. The risk of CRS is highest in countries with high rates of rubella susceptibility in women of childbearing age. The incidence of CRS has been significantly reduced or eliminated in many regions due to effective vaccination programs (4, 5). However, rubella remains endemic in several resource-limited countries, particularly in sub-Saharan Africa (6, 7).

The rubella vaccine is an effective prophylactic measure to control the spread of the virus and CRS. However, the devastating consequences of infection persist, not least due to the presence of unprotected people, such as those with ethical or religious objections to vaccination or those who have migrated from areas without adequate vaccination coverage (8, 5). In Congo, the Expanded Program on Immunization (EPI) introduced the combined measles and rubella vaccine in 2019. This first national campaign aimed to reduce morbidity and mortality due to measles and rubella among children, reaching a vaccination coverage rate of 96.9%, although disparities exist between departments (9). Children with CRS can suffer from hearing loss, eye and heart defects, and other lifelong conditions (including autism, diabetes mellitus, and thyroid dysfunction), often requiring expensive treatments and surgeries. The risk of CRS is highest in countries where women of childbearing age are not immune. The seroprevalence of rubella in pregnant women has been studied in several African countries (10-15). A meta-analysis reported a seroprevalence of 89.0% among pregnant women in sub-Saharan Africa (16). In Central Africa, high seroprevalence has been reported, including in Gabon (87.56 per cent) (17), the Democratic Republic of the Congo (84 per cent) (18) and Cameroon (94.4 per cent) (19). Also in Cameroon, another study reported 10.2% of probable cases of CRS (20). In Congo, the epidemiology of rubella remains insufficiently documented, but WHO estimates that Central Africa has high incidences, and Congo is no exception. In 1991, Yala et *al*. reported an 85% seroprevalence among pregnant women (21). Understanding the serological profile of pregnant women is crucial to preventing CRS. The objective of this study was to determine the serological profile of women pregnant with rubella in order to improve prevention and public health protection strategies.

II. Materials and Methods

a) *Ethical Considerations*

This study was carried out using anonymized blood samples, in accordance with current ethical regulations. As the samples are completely anonymous and cannot be linked to any personally identifiable data, an authorization from the ethics committee was not required.

b) *Type, Period and Setting of the Study*

This was a prospective descriptive study to determine the serologic profile of pregnant women with rubella. Such a study provides a valuable basis for future research in public and medical health.

This study involved 98 anonymous blood plasma samples from pregnant women. They attended the laboratory of the Blanche Gomez Mother-Child Specialty Hospital in Brazzaville, Congo, for biological investigations between January 1 and March 31, 2022. The laboratory analysis was designed to detect the presence of IgG and IgM antibodies against the rubella virus. The plasma samples, stored at -20°C, were transferred and stored at the National Public Health Laboratory in Brazzaville. They were then transported by air to the Institute Pasteur de Côte d'Ivoire in Abidjan for analysis. The samples were carefully packaged in triple packaging in insulated bags equipped with cold packs, to ensure their integrity. Maintaining the integrity of plasma samples is essential to obtain reliable and accurate results when testing rubella antibodies. This precaution is essential to obtain reliable and accurate results when testing rubella antibodies.

c) *Sampling*

Systematic sampling was based on all venous blood samples from pregnant women during the study period, collected in tubes containing an anticoagulant (EDTA). Variables analyzed included the age of pregnant women and gestational age. Clinical (gestational age) and epidemiological (age) data were collected using survey sheets including: a code assigned to the sample for the study, the age, and the gestational age.

d) *Methodology*

i. *Collection of blood plasma samples*

After the laboratory tests requested by the patients, the plasma was separated from the whole blood. The venous blood samples, collected in tubes

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containing an anticoagulant (EDTA), were centrifuged at 3000 revolutions per minute for 5 minutes. The obtained plasmas were aliquoted into 1.5 ml Eppendorf tubes and stored at -20°C

ii. *Dosing principle*

Rubella antibody titers were determined using the Architect *i1000SR*analyzer, using chemiluminescent microparticle immunoassay technology. This system relies on paramagnetic microparticles as a solid phase for the quantitative and qualitative detection of rubella antibodies in serum samples. The chemiluminescence signal is measured in relative units of light (RLU), which are directly proportional to the concentrations of immunoglobulins in the serum samples.

The higher the antibody concentrations, the greater the number of photons detected.

iii. *Sample Analysis*

For assays, the samples were sent to the Bacterial and Viral Serology Unit (USBV) of the Institute Pasteur de Côte d'Ivoire. Plasma samples were allowed to thaw at room temperature. Prior to the analysis, the parameters of interest (IgG and IgM) were calibrated on our samples. This calibration, which is stable for several months, must be verified with at least two levels of control. Once the plasmas were thawed, 200 µL of plasma was transferred to the cups of the Architect *i1000SR*analyzer for scheduled sample analysis. After analysis, the results were printed and the samples refrozen at -20°C for possible reuse. Quality control was ensured by introducing two levels of control of known concentrations in each series of analyses, ensuring the precision and accuracy of the analytical system and detecting random (pipetting, mixture quality, cup cleanliness, photometric instability) and systematic (loss of calibration) errors.

iv. *Interpretation*

For IgM, a result was considered positive (reactive) when the sample index was≥ 1.60, negative (non-reactive) when the index was < 1.20, and equivocal when the index was between 1.20 and 1.59. For IgG, a positive result was considered when the IgG titer was≥ 10.0 IU/mL, negative between 0 and 4.9 IU/mL, and equivocal between 5.0 and 9.9 IU/mL. Subjects with IgG titers≥ 10.0 IU/mL were considered immune; those with titers< 10.0 IU/mL, as non-immunized. In this study, equivocal results were considered negative.

e) Data Analysis

Data was collected and analyzed using Microsoft Office Excel 2019. The Fisher exact test was used to assess the relationship between seropositivity and epidemiological and clinical characteristics, with a statistical significance level of 5%.

III. Results

a) Epidemiological and Clinical Data

The study population consisted of 98 blood plasma samples from pregnant women. The distribution by age group made it possible to distinguish three age groups. The mean and median age were 29.04 years and 29 years, respectively. The ages of pregnant women ranged from 16 to 43 years. The most represented age group was 25 to 34 years old (56.1%). The majority of pregnant women were in the first trimester of pregnancy (43.9%) (Table I).

Table I: Distribution of Pregnant Women by Age and Trimester of Pregnancy

Variables	Effective (n=98)	%
Age group (year)		
16 - 24	23	23,5
25 – 34	55	56,1
≥ 34	20	20,4
	Gestational age (Quarter)	
1st	43	43,9
2nd	40	40,8
3rd	15	15,3

b) Epidemiological and Clinical Data by IgG Seropositivity

The overall IgG seropositivity rate was 91.8%. This rate was highest among women over 34 years old (100%), followed by the age groups 16 to 24 years (95.7%) and 25 to 34 years (87.3%). The distribution of the population by gestational age showed a maximal seropositivity among women in the second trimester of pregnancy (97.5%), followed by those in the first trimester (88.4%) and in the third trimester (86.7%). Additionally, 8.2% of women had a negative IgG result, with 62.5% of these women being in the first trimester of pregnancy. No pregnant woman tested positive for IgM. Seropositivity showed no statistically significant association with age (p = 0.405) or trimester of pregnancy (p = 0.376) (Table II).

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Table II: Distribution of Epidemiological and Clinical Data by IGG Seropositivity

Variables	No. of samples	IgG+ (n=90)		IgG- (n=8)	Titter IgG (UI/L)
		n (%)	*P value*	n (%)	mean (SD)
Age group (year)					
16–24	23	22 (95,7)		1 (4,3)	83,2 (69,9)
25-34	55	48 (87,3)	0,405	7 (12,7)	94,3 (104,4)
≥ 35	20	20 (100)		0 (0)	82,8 (61)
Gestational age (Quarter)					
1st	43	38 (88,4)		5 (11,6)	89,7 (83)
2nd	40	39 (97,5)	0,376	1 (2,5)	81,46 (68,8)
3rd	15	13 (86,7)		2 (13,3)	109,2 (143,3)

Table III: Seroprevalence of rubella among pregnant women in selected African countries

Authors, year of publication	Country	Study area	Study population	Sample size	Rubella (%) IgG+	IgM+	Dosing technology
Taku et *al.* 2019	Cameroon	Urban	Pregnant women	522	94,4	5,0	ELISA
Pegha Moukandja et *al.* 2017	Gabon	Urban	Pregnant women	973	87,56		ELFA
Alleman et *al.* 2016	Ground floor	Urban/Rural	Pregnant women	1605	84		ELISA
Zahir et *al.* 2020	Morocco	Urban area	Pregnant women	380	84,7	0	CMIA
AlShamlan et *al.* 2021	Saudi Arabia	Urban	Pregnant women	4328	76,41	1,21	CLIA
Tahita et *al*. 2013	Burkina Faso	Urban/Rural	Pregnant women	341	95		ELISA
Adam et *al.* 2013	Sudan	Urban	Pregnant women	500	95,1		ELISA
Adewumi et *al*. 2015	Nigeria	Urban	Pregnant women	272	91,54	1,84	ELISA

CMIA: Chemiluminescence Microparticle Immunoassay
CLIA: Chimiluminescence Immunoassay
ELISA: Enzyme-Linked Immunosorbent Assay

IV. Discussion

The elimination of congenital rubella and the prevention of congenital rubella syndrome (CRS) are major global public health issues. The World Health Organization (WHO) estimates that about 100,000 cases of CRS occur worldwide each year. In 2010, there were an estimated 39,000 cases of CRS in Africa (5). These alarming figures underscore the critical need for routine rubella screening in pregnant women and widespread rubella vaccination in the population (22).

The ages of the pregnant women studied ranged from 16 to 43 years, with an average age of 29 years. The 25 to 34 age group was the most represented (56.1%) (Table I). Zahir et *al*. in Morocco (23) also reported, in agreement with this study, a predominance in the 25-34 age group (50.8%) among pregnant women. Our average age was higher than the averages reported by Taku et *al*. (27 years old) in Cameroon (19) and Pegha Moukandja et *al.* (25 years old) in Gabon (17).

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In this study, the majority of pregnant women were in their first trimester of pregnancy (43.9%). Trends vary between studies: Taku et *al*. reported a higher frequency of women in the second trimester of pregnancy (59.6%) (19), while AlShamlan et *al*. observed a majority of cases in the first trimester (38.89%) in Saudi Arabia (24). Taku et *al*. in Cameroon and Ekuma et *al*. in Nigeria reported a majority frequency (41% and 45.9%, respectively) in the third trimester of pregnancy (19, 25).

The epidemiology of rubella remains poorly known in Congo, as it is not a notifiable disease. Most acute infections are acquired in childhood and continue to manifest as IgM antibodies, even in adulthood. The results of this study revealed a high prevalence of IgG seropositivity among pregnant women who attended the Blanche Gomez Mother-Child Specialty Hospital. With an overall seroprevalence rate of 91.8%, it appears that the majority of pregnant women are protected against rubella. However, the detailed analysis shows disparities according to age and trimester of pregnancy.

The highest seroprevalence was observed in women over 34 years of age, with a rate of 100%. This finding suggests that older women have been exposed to the rubella virus during their lifetime, which has led to the development of antibodies and lifelong immunity. In contrast, some women under the age of 35 were unprotected, which could be attributed to insufficient exposure to the virus or incomplete vaccination.

The results also indicate that seroprevalence is higher during the first trimester of pregnancy, reaching 97.5%. This observation is crucial, as rubella virus infection in the first trimester can have serious consequences on fetal development, including congenital rubella syndrome.

The national measles and rubella vaccination programme for young children, launched in March 2019 (9), is an important step towards rubella elimination. However, the risk of infection in women of childbearing age does not decrease immediately, as they were not vaccinated as children. The lack of a routine immunization program prior to 2019 means that older women likely acquired immunity through natural infection, creating heterogeneity in the population. With the introduction of the new vaccination program, we anticipate an increase in immunity levels in future cohorts of pregnant women. This program is expected to homogenize protection against rubella and reduce the risk of CRS in the long term. By monitoring the effectiveness of the program, strategies can be adjusted to ensure optimal immunization coverage and improve public health.

Before 2019, the rubella vaccine (Aventis-Pasteur measles, mumps and rubella vaccine) was only available in a few private pharmacies and rarely used. In the absence of a mass vaccination campaign prior to 2019, and based on our clinical information indicating a very low vaccination rate, we conclude that the seroprevalence observed in this study is mainly due to the circulation of wild-type rubella virus rather than vaccination. These data suggest significant previous exposure to the virus and likely significant transmission in the city. Previous studies have shown that the rubella virus is common in several countries in sub-Saharan Africa (16, 26). Our seroprevalence rate was lower than that reported among pregnant women in Cameroon (94.4%) (19), Burkina Faso (95%) (27) and Sudan (95.1%) (28), but similar to that observed in Nigeria (91.5%) (29). In contrast, our prevalence was higher than in Gabon (87.56%) (17) and the Democratic Republic of the Congo (84%) (18). These differences could be due to sample sizes, disease endemicity, diagnostic methods, or test cut-offs.

The results of the statistical analysis, using the exact 5% Fisher test, show that seropositivity has no statistically significant association with either age (p = 0.405) or trimester of pregnancy (p = 0.376). These results suggest several important points to consider in interpreting the data and the implications for public health. The lack of a statistically significant association between seropositivity and the variables age and trimester of pregnancy indicates that other factors may play a more significant role in the presence of rubella antibodies in pregnant women. It is possible that factors such as vaccination history, individual medical history, or environmental exposure may be more influential in the observed seroprevalence.

This study found that 8.2% of pregnant women were not protected against rubella. In addition, 62.5% of non-immunized women were in the first trimester of pregnancy. Our results also showed that all women are at increased risk of rubella infection over the course of their lives. Since up to 90% of rubella infections occurring just before conception and up to the first 8–10 weeks of pregnancy can lead to multiple birth defects, miscarriage or stillbirth (30). These data indicate that a significant proportion of pregnant women are at risk of having a child with congenital rubella syndrome (CRS). The main goal of rubella vaccination programs is to prevent CRS by avoiding infections during pregnancy. To achieve this goal, all women of childbearing age must be vaccinated and vaccination coverage must be achieved above 95% among children. In some developed countries, pregnant women are routinely screened to offer postpartum vaccination to susceptible women (31). WHO recommends that all pregnant women who are HIV-negative or whose immune status is unknown should be vaccinated after delivery before leaving the hospital, in order to achieve 100% seroprevalence (32). In Congo, vaccination of postpartum women is not systematic and vaccination of women of childbearing age is not part of the vaccination programme. Reducing the risk of CRS will only be possible if the circulation of the virus is interrupted by

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mass vaccination of women of childbearing age and school-age girls, routine vaccination of nonimmunized women after childbirth, vaccination of children against measles and rubella, as well as the establishment of a national surveillance system for rubella infection during pregnancy.

Specific IgM can be detected not only in cases of recent primary infection, but also in cases of reinfection, non-specific polyclonal stimulations of the immune system, or cross-reactions with rheumatoid factors in systemic disease (33). During this study, no pregnant women were IgM positive and there were no acute infections. Zahir et *al.* (23) also reported a positivity rate of 0%, in line with our study, while low rates were recorded among pregnant women in Cameroon (5%) (19) and Nigeria (1.84%) (29).

The lack of knowledge about the epidemiology of rubella in Congo is crucial. It would be appropriate to launch specific epidemiological studies and to set up continuous surveillance programmes. These initiatives would provide a better understanding of the dynamics of rubella transmission in the region and strengthen efforts to prevent and control the disease.

Limitations of the study: It is essential to consider the limitations of this study. First, the small size of our sample, although relevant to the study of pregnant women in Congo, may influence the generalization of the results. Second, the lack of a confirmed history of rubella vaccination precludes an assessment of the impact of vaccination on HIV status. In addition, unmeasured variables, such as socioeconomic status or antenatal care practices, could affect the findings. Finally, the results concern only women who attended the Blanche Gomez Mother-Child Hospital, thus limiting the scope of the conclusions. Despite these limitations, the study highlights the need for further research and effective prevention strategies to protect pregnant women and their children.

V. Conclusion

No pregnant women developed IgM, indicating the absence of recent or active infections. IgG seropositivity was high (91.8%), indicating strong immunity to rubella in these women. These findings highlight the importance of ongoing surveillance and vaccination for long-term protection. The vulnerability of women in the first trimester of pregnancy (62.5%) is of concern due to risks to fetal development and the high risk of congenital rubella syndrome. Awareness campaigns, partnerships with health care providers and continued immunization efforts are key to improving immunization coverage and protecting vulnerable populations. Achieving high levels of immunity in women of reproductive age is crucial for public health, as it can reduce rubella transmission and improve overall health in Congo, while preventing future outbreaks and protecting future generations. Monitoring the impact of vaccination campaigns and assessing the epidemiology of rubella is essential to adjust public health strategies and ensure continued protection.

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