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Seroprevalence and Factors Associated with Rubella Immunity: An Epidemiological Study of Pregnant Women in Kinshasa

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Abstract

Introduction: Every year, 100,000 infants worldwide are born with CRS. Routine vaccination of pregnant women can prevent this complication. In the Democratic Republic of the Congo, this vaccine was not yet available at the time of the study. This research aims to determine the seroprevalence and analyze the factors associated with rubella immunity in pregnant women in Kinshasa. **Methods:** A cross-sectional study was conducted from January to February 2025 among 376 pregnant women recruited from two centers (urban-rural and urban). Serological analyses were used to detect specific IgG and IgM antibodies. Multivariate logistic regression was performed to identify factors associated with seropositivity. **Results:** The anti-rubella IgG seroprevalence was 79.0% (n = 297), with no cases of IgM detected. Factors associated with immunity included age ≥ 25 years (aOR = 4.3; 95% CI = 1.98 - 9.8), marital status (married) (aOR = 2.35; 95% CI = 1.24 - 4.53), and late first pregnancy. Multiparity (aOR = 0.22) and consultation in the third trimester (aOR = 0.11) were inversely associated with seropositivity. **Conclusion:** Despite high overall immunity, 21% of pregnant women remain exposed to rubella, putting their fetuses at severe risk. These results reinforce the call for routine vaccination of women of childbearing age and targeted prenatal screening in the DRC, in the context of the recent introduction of the vaccine.

Keywords

Rubella, Seroprevalence, Pregnant Women, Kinshasa

1. Introduction

Rubella is a viral rash disease that is generally benign in children and young adults [1] [2]. However, rubella infection occurring early in pregnancy can have dramatic consequences for the fetus, leading to spontaneous abortions, stillbirths, or malformations grouped under the term congenital rubella syndrome (CRS) [3] [4]. The risk of fetal transmission and congenital abnormalities reaches up to 90% when maternal infection occurs in the first trimester [5] [6]. Due to this tropism for the product of conception, rubella is recognized as one of the leading preventable causes of congenital malformations [4] [7].

To prevent these serious outcomes, rubella vaccination is the cornerstone of public health strategies [8]. The World Health Organization (WHO) recommends the introduction of a combined measles-mumps-rubella (MMR) vaccine into expanded immunization programs, targeting not only infants but also young adults and women of childbearing age who have not been vaccinated or exposed, in order to eliminate rubella and CRS [2]. In industrialized countries with high vaccination coverage, the circulation of the virus has been virtually interrupted: in Germany, where rubella vaccination is mandatory for school entry, the seroprevalence of immunity reaches 99%, and cases of congenital rubella have become exceptional [9]. Similarly, the Americas have been declared rubella-free thanks to intensive vaccination and prenatal screening campaigns [4] [10]. Conversely, in many low- and middle-income countries, rubella vaccination has only recently been introduced or remains unavailable, maintaining endemic transmission of the virus [2].

This is the case in the Democratic Republic of the Congo (DRC), where the rubella vaccine had not been implemented until very recently [11]. Serological surveillance data have confirmed that the virus is actively circulating there: for example, a retrospective analysis in Kinshasa from 2010 to 2012 detected rubella in 27% of patients tested as part of measles screening [1]. However, seroprevalence data on rubella immunity among women of childbearing age in the DRC remain limited. A national study conducted in 2013 by Alleman *et al.* estimated that 84% of pregnant women had anti-rubella IgG antibodies [8]. This corresponds to the high average observed in Africa. Indeed, a recent meta-analysis in sub-Saharan Africa reports an average IgG seroprevalence of 89% among pregnant women, with approximately 5% of recent IgM cases [12]. However, there are significant geographical disparities: for example, a local study conducted in Kisantu (DRC) found that only 59% of women were immune, suggesting that some populations remain much more susceptible than others [13]. Similarly, in Chad, Makemgue Louise Stéphanie *et al.* reported a rubella seroprevalence of 85.5% in 2024 among 90 pregnant women in N'Djamena [14], while in Nigeria, studies have found rates ranging from ~68% to 88% depending on the region and urban/rural context [15].

As the DRC had not yet implemented the rubella vaccine during the study period, it was essential to assess the proportion of pregnant women who were still unimmunized and therefore at risk of contracting rubella, as well as the factors that could be linked to this lack of immunity. Such an assessment provides a pre-

vaccination baseline and informs prevention priorities (catch-up vaccination, awareness-raising, etc.) for the general population, and in particular for maternal and neonatal health.

The present study aims to determine the seroprevalence of rubella antibodies (IgG and IgM) and to analyze the factors associated with rubella in pregnant women attending prenatal consultations in Kinshasa and associated with adjusting rubella control strategies through the systematic vaccination of women of childbearing age.

2. Materials and Methods

We conducted a cross-sectional analytical study from January 14 to 28, 2025, at the Kingasani Hospital Center and the Renaissance University Hospital Center (formerly Mama Yemo) from February 5 to 19 in the city of Kinshasa (DRC). The Kingasani Hospital Center (KHC) is located in the Kingasani health zone, west of the Kinshasa health province in DR Congo. It is a medical facility operating within the Diocesan Office of Medical Works in Kinshasa. It has a maternity ward and qualified staff. This hospital center was selected because it provides clinical and diagnostic services to a large number of pregnant women. It receives 100 new pregnant women each week from different municipalities in Kinshasa. The Renaissance University Hospital Center (formerly Mama Yemo) is located in the Gombe Health Zone. It offers three main functions: medical training, clinical research, and tertiary care. It is one of the largest hospitals in the city of Kinshasa and ranks first in terms of the number of laboratory-confirmed cases of rubella since the beginning of this year (20 confirmed cases). Pregnant women attending selected prenatal care clinics during the study period were included consecutively, regardless of gestational age, and provided they consented to participate. Exclusion criteria included lack of consent and refusal to provide a blood sample. A total of 376 pregnant women were recruited, covering all three trimesters of pregnancy (≈34% in the first trimester, 56% in the second, and 10% in the third) and a range of ages (from 15 to 49 years). A standardized questionnaire was used to collect sociodemographic data (age, age group, marital status, level of education, occupation, municipality of residence, religion) and obstetric data (number of deliveries, number of pregnancies including the current pregnancy, age at first pregnancy, term of the current pregnancy, history of spontaneous abortion or miscarriage, episodes of fever during pregnancy, etc.).

This information was supplemented by data from the prenatal record. Each participant had a blood sample taken in a dry vacutainer tube (5 ml of venous blood) by a trained nurse. The samples were transported daily by vehicle in a cooler with ice packs to the virology laboratory of the National Institute for Biomedical Research (INRB) in Kinshasa and stored at −20˚C. Serological tests were performed to detect IgG and IgM specific to the rubella virus. IgG was tested for in all samples collected. Those that tested IgG negative were tested for anti-rubella immunoglobulin M (IgM) using Vidas Rub IgG (RBG) rubella-specific enzyme immunoassay kits, following the manufacturer's recommendations. 100 µl of serum was

placed in each well. The results were read after 40 minutes. Serums with titers of ≥15 IU/ml of blood were considered seropositive for rubella antibodies, while those with an equivocal determination (10 - 14.99 IU/ml) or with titers < 10 IU/ml were considered seronegative (For each sample, a quantitative result (IU/mL) was obtained for IgG, allowing subjects to be classified as seropositive (immunized) or seronegative (susceptible)).

Positive and negative controls were included in each series of tests to ensure the quality of the results.

Data were entered and analyzed using R software (4.3.2). An initial descriptive analysis was performed: quantitative variables (e.g., age) were presented as mean ± standard deviation or median (IQR), and qualitative variables as counts and percentages. The seroprevalence of rubella immunity was calculated as the percentage of IgG-positive women in the overall sample, with its exact 95% confidence interval. To identify factors associated with IgG seropositivity (acquired immunity), we first performed bivariate analyses: comparison of the percentages of IgG+ women according to the modalities of each explanatory variable, using the χ^2 test of independence (or Fisher's exact test when appropriate). Unadjusted odds ratios (OR) and their 95% CI were calculated to estimate the strength of association between each factor and serological status. In a second step, all variables deemed relevant ($p < 0.20$ in bivariate analysis or a priori interest) were included in a multivariate logistic regression model (stepwise downward method). This model identified the independent factors associated with rubella immunity, controlling for confounding effects between variables. The results are presented as adjusted OR (aOR) with 95% CI and p-value (significance threshold: $p < 0.05$). The quality of the model was assessed using Tjur's pseudo-R^2 and the Hosmer-Lemeshow test.

The study protocol was approved by the National Health Ethics Committee (No. 665/CNES/BN/PMMF/2025). Written informed consent was obtained from each participant. Individual serology results were communicated to the women and the referring physician; seronegative women were informed of preventive measures and advised to undergo postnatal vaccination, while the rare suspected cases (none in this study) would have been subject to special follow-up. Anonymity and confidentiality of data were respected throughout the research.

3. Results

3.1. Population Characteristics

A total of 376 pregnant women were included in the study, with a mean age of 27.4 years (±6.5) and a median age of 27 years (IQR: 23 - 31). The majority were in the 25 - 34 age group (61%), followed by 15 - 24 (23%) and 35 and over (16%). Two-thirds of participants were married (66%), and more than one-third were single (34%). The level of education was relatively high: 36% had higher education, 56% had secondary education, and only 8% had primary education or no

schooling. In terms of occupation, 40% were housewives, 25% were unemployed, 14% were civil servants, 6% were shopkeepers, and 6% were still pupils or students. The majority (79%) belonged to revivalist churches, followed by Catholics (15%) and Protestants (5%). In terms of obstetric characteristics, 24% were primigravidas, 27% were paucigravidas, and 49% were multigravidas; parity followed a similar distribution. The age at first pregnancy ranged from 14 to 38 years (mean 20.9 years), with 17% having started their motherhood before the age of 18%, 52% between the ages of 18% and 24%, and 31% after the age of 25. The distribution by trimester of pregnancy showed that 34% of women were in the first trimester, 56% in the second, and 10% in the third. Finally, 22% reported having had a spontaneous abortion, and none had received the rubella vaccine or been informed of their previous serological status.

3.2. Seroprevalence of Rubella and Immune Status

Of the 376 samples tested, 297 were positive for anti-rubella IgG, representing an IgG seroprevalence of 79.0%, as shown in Figure 1. Conversely, 79 women (21%) were seronegative for rubella and therefore considered non-immune to the virus (having probably never contracted rubella). None of the participants tested positive for IgM serology, indicating that no cases of recent rubella infection were detected during the study. This absence of IgM+ cases suggests that there was no ongoing rubella epidemic in the study population during the collection period, as rubella can circulate sporadically or seasonally with epidemic outbreaks several years apart, as is typically observed in tropical areas.

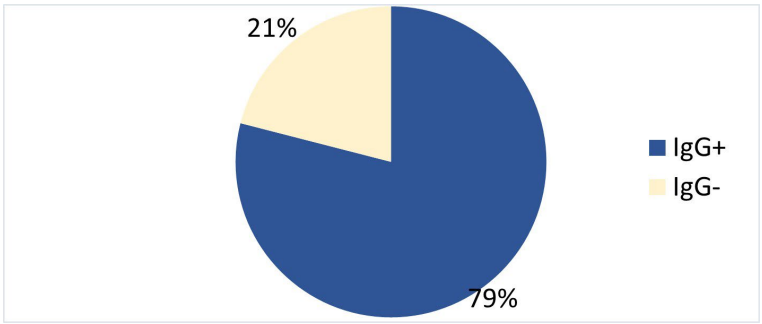

Figure 1. Distribution of anti-rubella IgG antibodies in pregnant women.

The bivariate analysis of sociodemographic characteristics according to IgG anti-rubella serostatus is presented in Table 1. The mean age of participants did not differ significantly between seropositive and seronegative women (26.7 ± 6.5 years vs. 26.9 ± 6.6 years; $p = 0.64$). However, the distribution by age group showed a clear association ($p < 0.001$): the proportion of seronegative women was higher among those aged 15 - 24 (41% vs. 19%), while those aged 25 - 34 constituted the majority of immunized women (65% vs. 47%). Marital status was also associated with immunity ($p < 0.001$). Among seronegative women, more than half were single (57% vs. 28% among seropositive women), while married women

predominated in the immune group (72% vs. 43%).

Table 1. Sociodemographic characteristics of pregnant women according to rubella antibody seropositivity.

Feature	Rub IgG+ N = 297[1]	Rub IgG- N = 79[1]	Total N = 376[1]	p-valeur[2]
Age				
(Min; Max)	(15; 45)	(16; 48)	(15; 48)	
Mean ± ET	26.7 ± 6.5	26.9 ± 6.6	27.4 ± 6.5	
Median (Q1; Q3)	27.0 (23; 32)	26 (23; 30)	27 (23; 31)	
Age group				<0.001
15 - 24years	56 (19%)	32 (40%)	88 (23%)	
25 - 34 years	192 (65%)	37 (47%)	229 (61%)	
35 - 49 years	49 (16%)	10 (13%)	59 (16%)	
Civil status				<0.001
Single	83 (28%)	45 (57%)	128 (34%)	
Married	214 (72%)	34 (43%)	248 (66%)	
Level of education				<0.001
Without level	7 (2.4%)	3 (3.8%)	10 (2.7%)	
Primary	9 (3.0%)	10 (13%)	19 (5.1%)	
Secondary	158 (53.2%)	52 (66%)	210 (56%)	
Superior	123 (41.4%)	14 (18%)	137 (36%)	
Occupation				<0.001
Unemployed	86 (29%)	8 (10.1%)	94 (25%)	
Housekeeper	109 (36.7%)	43 (54.4%)	152 (40%)	
Merchant	16 (5.4%)	8 (10.1%)	24 (6.4%)	
Student	16 (5.4%)	5 (6.3%)	21 (5.6%)	
Civil servant	46 (15.5%)	5 (6.3%)	51 (14%)	
Other jobs	24 (8.1%)	10 (12.7%)	34 (9.0%)	
Religion				0.4
Other religions	3 (1.0%)	2 (2.5%)	5 (1.3%)	
Protestant	13 (4.4%)	5 (6.3%)	18 (4.8%)	
Catholic	48 (16%)	8 (10%)	56 (15%)	
Revival church	233 (78%)	64 (81%)	297 (79%)	

[1]n (%); [2]chi-square test of independence.

Level of education emerged as a determining factor ($p < 0.001$). Women with higher education had a higher proportion of immunity (41% vs. 18%), while primary and secondary education levels predominated among seronegative women (79% vs. 56%). Occupation was significantly associated with serostatus ($p <$

0.001). Housewives accounted for more than half of seronegative women (54% vs. 37%), while civil servants and other formal occupations were more common among immune women (15% vs. 6%). In contrast, religious affiliation was not significantly associated with IgG seroprevalence (p = 0.40), with the majority of participants belonging to revivalist churches in both groups (≈80%).

From an obstetric perspective (Table 2), age at first pregnancy was significantly associated with serological status (p = 0.005). Women who had their first pregnancy at an advanced age (≥25 years) had a higher proportion of immunity (35% vs. 16%), while those whose first pregnancy occurred at an early age (13 - 17 years) were more often seronegative (23% vs. 15%). Parity was not associated with seropositivity (p = 0.70). The proportions of nulliparous, primiparous, and multiparous women were comparable between the two groups. Similarly, gestation showed no significant difference (p = 0.50), with a similar distribution between primigravid, oligigravid, and multigravid women.

Table 2. Obstetric history of pregnant women according to rubella antibody seropositivity.

Feature	IgG+ N = 297[1]	IgG- N = 79[1]	Overall N = 376[1]	p-valeur[2]
Age at first pregnancy				0.005
13 - 17	46 (15.5%)	18 (22.8%)	64 (17%)	
18 - 24	146 (49.2%)	48 (60.8%)	194 (52%)	
25 years old and older	105 (35.3%)	13 (16.4%)	118 (31%)	
Parity				0.7
Nulliparous	103 (34.7%)	25 (31.7%)	128 (34%)	
Primiparous	81 (27.3%)	25 (31.6%)	106 (28%)	
Multipara	113 (38%)	29 (36.7%)	142 (38%)	
Gestational age				<0.001
Quarter 1	117 (39.4%)	11 (13.9%)	128 (34%)	
Quarter 2	161 (54%)	50 (63.3%)	211 (56%)	
Quarter 3	19 (6.4%)	18 (22.8%)	37 (9.8%)	
Gestation				0.5
Primigravida	71 (24%)	20 (25%)	91 (24%)	
Paucigravida	83 (28%)	17 (22%)	100 (27%)	
Multigravida	143 (48%)	42 (53%)	185 (49%)	
Abortion				0.027
Non-abortion	225 (76%)	69 (87%)	294 (78%)	
Spontaneous abortion	72 (24%)	10 (13%)	82 (22%)	

[1]n (%); [2]chi-square test of independence.

However, gestational age was strongly linked to serological status ($p < 0.001$). The proportion of seropositive women was significantly higher in the first trimester (39% vs. 14%), while seronegative women were overrepresented in the third trimester (23% vs. 6%).

Finally, a history of spontaneous abortion appeared to be associated with immunity ($p = 0.027$). Seropositive women reported a history of spontaneous abortion more often (24% vs. 13%), while the majority of seronegative women reported never having had an abortion (87% vs. 76%).

In summary, the sociodemographic factors and obstetric variables associated with rubella immunity in bivariate analysis were age, marital status, educational level and occupation, age at first pregnancy, gestational age, and history of abortion, while religion, parity, and gestation did not show any association. These variables were selected for multivariate analysis.

3.3. Multivariate Analysis—Factors Associated with Rubella Immunity

In the univariate analysis, the study site was significantly associated with rubella seropositivity: women recruited at Mama Yemo Hospital had a lower prevalence of anti-rubella IgG compared with those from the Kinshasa Hospital Center (OR = 0.23; 95% CI 0.12 - 0.42; $p < 0.001$). However, this association disappeared after adjustment for maternal age, marital status, gestational age, and parity (aOR = 0.44; 95% CI 0.14 - 1.23; $p = 0.14$). This suggests that the apparent difference between sites was mainly due to differences in the demographic and obstetric profiles of the women rather than to the center itself (Table 3).

Table 3. Factors associated with anti-rubella IgG seropositivity in pregnant women.

		Univariate regressions			Multivariate regressions		
Characteristic	**IgG+[1]**	**OR[2]**	**95% IC[2]**	**p-value**	**OR[2]**	**95% IC[2]**	**p-value**
Hospital Center							
Kingasani Hospital Center (KHC)	91.3% (137/150)						
Mama Yemo Hospital	70.8% (160/226)	0.23	0.12 - 0.42	0.001	0.44	0.14 - 1.23	0.14
Age group							
15 - 24	63.6% (56/88)	—	—		—	—	
25 - 34	83.8% (192/229)	2.97	1.69 - 5.20	<0.001	4.30	1.98 - 9.79	<0.001
35 - 49	83.1% (49/59)	2.80	1.28 - 6.54	0.012	4.54	1.56 - 14.2	0.007
Marital status							
Single	64.8% (83/128)	—	—		—	—	
Married	86.3% (214/248)	3.41	2.05 - 5.73	<0.001	2.35	1.24 - 4.53	0.010
Level of education							
No education	70.0% (7/10)	—	—		—	—	

Continued

Primary	47.4% (9/19)	0.39	0.07 - 1.86	0.3	0.33	0.05 - 1.93	0.2
Secondary	75.2% (158/210)	1.30	0.27 - 4.87	0.7	1.04	0.19 - 4.61	>0.9
Superior	89.8% (123/137)	3.77	0.75 - 15.3	0.075	2.57	0.43 - 12.6	0.3
Gestational age							
1st trimester	91.4% (117/128)	—	—		—	—	
2nd trimester	76.3% (161/211)	0.30	0.14 - 0.59	<0.001	0.40	0.18 - 0.83	0.018
3rd trimester	51.4% (19/37)	0.10	0.04 - 0.24	<0.001	0.11	0.04 - 0.28	<0.001
Gestation							
primigeste	78.0% (71/91)	—	—		—	—	
Paucigeste	83.0% (83/100)	1.38	0.67 - 2.85	0.4	0.72	0.30 - 1.69	0.4
multigesture	77.3% (143/185)	0.96	0.52 - 1.74	0.9	0.22	0.09 - 0.52	<0.001

Mean dependent var	0.718	SD dependent var	0.486
Pseudo r-squared	0.213	Number of obs	376
Chi-square	53.776	Prob > chi²	0.000
Akaike crit (AIC)	257.215	Bayesian crit, (BIC)	284.25

The Hosmer-Lemeshow goodness-of-fit test

Statistic	5.71591
p-value	0.67902

Reading: OR > 1 means a higher probability of being immune to rubella for the category considered compared to the reference; OR < 1 indicates a lower probability. *Significant at $p < 0.05$; *$p < 0.01$.

After confirming the absence of site-related heterogeneity, we proceeded to examine individual-level predictors of rubella immunity. The multivariate results confirm several associations already suggested in the crude analysis. Maternal age remains a major predictor of rubella immunity: a woman aged ≥ 25 is approximately 4 to 4.5 times more likely to be IgG positive compared to a woman under 25 (Ora = 4.30 for 25 - 34 years; Ora = 4.54 for 35 - 49 years). This dose-response relationship with age is consistent with the idea that in endemic situations, cumulative exposure to the virus over the years is a key determinant of natural immunization. Marital status also retains a significant effect after adjustment: at the same age, married women are more than twice as likely to be immune (ORa = 2.35, $p < 0.01$). Level of education does not appear to be significant in itself in the final model, suggesting that there is no clear difference in immunity between, for example, a woman with no formal education and a woman with a higher education, once other factors are taken into account. An association with illiteracy was reported, as these authors observed a significantly higher proportion of rubella-negative women among those who had never attended school.

An interesting result of the multivariate model is the negative relationship between multiparity and rubella positivity (ORa ≈ 0.22 for multiparous women compared to primiparous women, $p < 0.01$). In other words, at the same age, women

who have had multiple pregnancies appear to be less often immune than those who are pregnant for the first time. This finding may seem counterintuitive, since one might expect that a woman who has had several children (and is therefore older on average) would have had more opportunities to contract rubella. However, this result is linked to a phenomenon of confusion due to age at first pregnancy: the sample in this study showed that women who have had multiple pregnancies are overwhelmingly those who started having children early. However, these early pregnancies and motherhood may have reduced their years of schooling and potentially limited certain social interactions outside the home, compared to women of the same age who did not yet have children. In other words, among women aged 30, for example, those who are in their sixth pregnancy may have spent their youth in a different context (rural environment or out of school) than those who have only had one late pregnancy and a professional career, which may influence their exposure to the virus. It is therefore plausible that this is an effect of the structure of our population: multiparous women in this cohort include many women from peri-urban areas (e.g., N'Sele) where rubella may have circulated less intensely in the past, while older primigravidas are often educated city dwellers who may have contracted rubella during adolescence or in their workplace.

The final model confirms the impact of gestational age at the time of testing, as already mentioned. After adjustment, being in the second trimester rather than the first reduces the probability of being immune by approximately 60% (ORa = 0.40; $p = 0.018$), and being in the third trimester reduces it by nearly 90% (ORa = 0.11; $p < 0.001$). This result obviously does not mean that the progression of pregnancy causes a loss of immunity (!) but reflects, as discussed, a recruitment bias: high-risk profiles (young, primigravida, non-immune) are overrepresented at the end of pregnancy among those who had not been monitored earlier. This temporal marker highlights the importance of identifying these women as soon as they enter the prenatal care system for possible interventions (counseling, postpartum vaccination). It also shows that the longer a woman delays seeking care, the more likely she is to be among those who were not initially immune.

In terms of obstetric factors, age at first birth and multiparity were found to be linked to immune status, highlighting the importance of individual reproductive history in exposure to the virus.

4. Discussion

This study provides a snapshot of the rubella immune status of pregnant women in Kinshasa in 2025, just before the introduction of the combined measles-rubella vaccine in the DRC. The results reveal a contrasting situation: on the one hand, a large majority (approximately 79%) of women have IgG antibodies against rubella, indicating widespread previous exposure to the virus in this population; on the other, nearly one-third (21%) remain susceptible to rubella, which is a significant proportion of women of childbearing age without immune protection.

Although the crude analysis suggested a lower immunity rate among women recruited at Mama Yemo Hospital, this difference was no longer significant after adjustment, indicating comparable immunization profiles across both hospitals. This homogeneity supports pooling data from the two sites in the multivariate model.

The overall seroprevalence of 79% observed in Kinshasa is relatively high compared to some older African studies, but it remains lower than the levels reported in several countries in Africa and elsewhere. For example, Alleman *et al.* estimated that 84% of pregnant women in the DRC were immune in 2013 [8], perhaps reflecting a national trend or differences in methodology. A meta-analysis by Kassa *et al.* (2020) combining 28 studies in sub-Saharan Africa calculated an even higher average seroprevalence of 89% among pregnant women [12]. Countries such as Ghana [16] and Ethiopia reported more than 90% of pregnant women immune at the end of pregnancy. In comparison, our rate of 79% appears rather low. However, it is in line with the rates observed in certain communities: for example, in N'Djamena (Chad) in 2024, Stéphanie *et al.* found 85.5% IgG+ in a small sample [14], and in Nigeria, local surveys showed rates ranging from 54% (rural north) to 87.5% (urban Osogbo) [15]. It should be noted that rubella transmission is heterogeneous: it often occurs in intermittent epidemic waves, which may leave certain age groups or geographical areas relatively unaffected for a time. Thus, the lower immunity measured in Kinshasa could reflect the fact that certain cohorts of young women have not been exposed recently in the absence of an outbreak. The literature typically describes rubella cycles every 4 to 7 years in Central Africa [1]. If the last significant wave in Kinshasa occurred more than 5 years ago, for example, a significant proportion of women now aged 20 - 24 may have gone through adolescence without encountering the virus. This hypothesis would be consistent with the total absence of IgM cases detected in our sample, suggesting that 2024 was not a year of notable rubella activity in Kinshasa. It should also be noted that in the DRC, integrated measles/rubella surveillance reported very few confirmed cases of rubella in 2024 [17], raising fears of a new epidemic wave in the near future given the accumulated pool of susceptible individuals.

The consequence of this high susceptibility (21%) is that nearly one in three pregnant women remains at risk if a rubella epidemic occurs, with potentially cases of CRS as a result. This risk is not theoretical: it is estimated that, without intervention, thousands of children are born each year with congenital rubella syndrome worldwide (4.7). Our data also confirm that a history of miscarriage was more common among immune women (22% vs. 13%, $p < 0.05$), which could reflect the past impact of undiagnosed rubella infections on previous pregnancies. A Sudanese study reported that approximately 20.5% of women with rubella antibodies had a history of spontaneous abortion. Of course, miscarriage has multiple causes, but this finding reinforces the argument in favor of prevention: every case of rubella prevented in a pregnant woman is potentially a fetal life saved or not disabled.

In terms of associated factors, our results are broadly consistent with the existing literature. Advanced age is one of the most consistent determinants of seropositivity, as the longer a woman has lived in a setting where rubella circulates, the greater the likelihood that she has contracted it at some point in her life. Several African and Asian studies have found this association with age. For example, Zanga *et al.* in the DRC (Kisantu) already noted a seroprevalence climbing from 45% in those < 20 years old to 68% in those > 30 years old [13]. Similarly, in India, Banik *et al.* (2021) report that immunity increases from ~55% in women < 25 years old to ~85% in women ≥ 30 years old [18]. We therefore confirm that in the absence of vaccination, immunity to rubella is acquired gradually with age, depending on exposure opportunities. This finding fully justifies the targeted vaccination approach for adolescent girls and young women before pregnancy, in order to bridge this "immunity gap" among younger children.

Marital status is less frequently discussed in the literature, but the data from this study suggest that it is a relevant marker: being married (and therefore generally older and a mother) is associated with more frequent immunity. Njiki Bikoï *et al.* in Chad observed a similar difference (86% immune among married women vs. 67% among single women), although it was not statistically significant in their small sample [14]. In Cameroon, Michel Noubom *et al.* (2018) also found the same results (68.13% immune among married women vs. 31.87% among single women) [19]. This points to the role of children in transmission: a married woman, especially a multiparous woman, often has young children who are likely to catch rubella at school and transmit it to their mother (if she is not yet immune). Married women, often mothers, may have been exposed through their children or spouses, or because they belong to more stable households where childhood diseases circulate (schoolchildren potentially bringing the virus home). This result is consistent with some local studies in Central Africa, although marital status is not always highlighted as an independent risk factor [14]. On the contrary, an unmarried woman without children may go through her twenties without contracting the virus until the day she catches it, sometimes during her first pregnancy—a high-risk situation. It is therefore understandable that married women/mothers are on average more immune, and this encourages us not to neglect women without children in catch-up vaccination strategies, as they may reach motherhood still naive to the virus.

Level of education and socioeconomic status do not appear to play a major direct role in this study, which is consistent with several previous studies. In a country where rubella circulates in all strata of society, the difference between affluent and disadvantaged backgrounds may be blurred: children from affluent backgrounds may contract it at school, while those from more disadvantaged backgrounds may contract it in their neighborhood—ultimately, everyone is exposed before adulthood. A paradoxical trend has sometimes been described: in some countries, educated urban women had lower immunity because they had escaped epidemics in childhood (due to better early childhood conditions, fewer siblings,

etc.) and were therefore susceptible later in life [14]. However, this pattern is more common in contexts of epidemiological transition or low endemicity. In the DRC, rubella remains sufficiently prevalent to affect the majority of children regardless of their status, which explains why we have not seen a clear difference between levels of education or between working women and stay-at-home mothers. Different socio-cultural contexts could explain these disparities: in Sudan, uneducated women may have lived in isolated rural areas with less viral transmission, whereas in the DRC, the urbanization of Kinshasa exposes almost everyone, regardless of educational level, to crowds and contact at some point (markets, churches, etc.). In any case, in the present cohort, education was not a major discriminating factor in rubella immunity.

With regard to obstetric factors, we highlighted the importance of age at first pregnancy as a risk indicator: women who delayed their first pregnancy had better immunity. This factor is rarely mentioned explicitly elsewhere, but it ties in with the issues of marriage and multiparity. A study conducted in Sudan found that multiparity was associated with stronger immunity (contrary to the present study) [20], while in Ethiopia, Tulu *et al.* (2016) did not observe any effect of parity but noted a difference between urban and rural areas (urban women being more often immune than rural women) [21]. A woman who starts her reproductive life late has often pursued higher education or worked, increasing her social interactions for a decade longer than a woman who becomes a mother at 17; as a result, she is more likely to have contracted rubella before her pregnancy. This finding highlights those early pregnancies (<20 years of age) frequently occur in young girls who have not yet encountered the virus and who find themselves pregnant without immune protection. Ultimately, this argues for intensifying vaccination of adolescent girls (middle school/high school students) before they reach childbearing age, in order to protect them in the event of early pregnancy.

In terms of public health, this study provides an important baseline as the DRC introduced the rubella vaccine into its routine immunization program (in combination with measles) in 2023. The data from this study highlight the urgency and relevance of this vaccine introduction. With nearly 30% of pregnant women not immune, the DRC is likely to continue to record a significant number of preventable CRS cases each year. By comparison, in countries that have eliminated rubella, the proportion of non-immune women of childbearing age has generally fallen below 5% [12]. We are therefore still a long way off, but routine vaccination of infants (accompanied, if possible, by a catch-up campaign for young women) should gradually reduce this susceptibility rate. The results of this study strongly support the need for a routine vaccination program for all women of childbearing age, as recommended by the WHO, and confirm that the lack of vaccination left a significant immunity gap in the Congolese female population.

Furthermore, the data from this study argue in favor of including rubella in prenatal screening activities. Currently, in the DRC, screening for syphilis and HIV is recommended during prenatal care, but not for rubella. Given the propor-

tion of seronegative women and the danger posed by infection during pregnancy, it would be appropriate to add rubella serology at the beginning of pregnancy. Of course, finding that a woman is seronegative during pregnancy will not allow her to be vaccinated immediately (live vaccine contraindicated during pregnancy), but it would make her aware of preventive measures (avoiding contact with sick children, consulting a doctor in case of febrile rash) and, above all, allow her postpartum vaccination to be organized before she leaves the maternity ward. This strategy of postpartum vaccination of non-immune women has been used successfully in several context of epidemic cycles. Early detection of rubella cases, and even more so of congenital rubella syndrome (CRS) cases in newborns, therefore remains essential for assessing the impact of interventions. The experience of the Americas, where vaccination has eliminated rubella while maintaining vigilance over pockets of unvaccinated individuals (particularly among certain migrant communities), illustrates the importance of continuous surveillance. Similarly, in the DRC, despite the upcoming introduction of the vaccine, it will be crucial to monitor areas with low coverage and unvaccinated age cohorts. Enhanced reporting of skin rashes in pregnant women, as well as systematic diagnosis of neonatal malformations compatible with CRS (deafness, congenital heart disease, cataracts, etc.), should be encouraged. Such strategies were successfully implemented in several countries prior to the widespread introduction of the rubella vaccine, helping to close the immunity gap without putting current pregnancies at risk.

The results of this study, combined with those of the few previous studies in the DRC, suggest that rubella has circulated at a moderate endemic levelin recent years: a high proportion of pregnant women have IgG, but very little IgM has been detected. However, this situation may change rapidly in the context of epidemic cycles. Early detection of rubella cases, and even more so of congenital rubella syndrome (CRS) cases in newborns, therefore remains essential for assessing the impact of interventions. The experience of the Americas, where vaccination has eliminated rubella while maintaining vigilance over pockets of unvaccinated individuals (particularly among certain migrant communities), illustrates the importance of continuous surveillance. Similarly, in the DRC, despite the upcoming introduction of the vaccine, it will be crucial to monitor areas with low coverage and unvaccinated age cohorts. Enhanced reporting of skin rashes in pregnant women, as well as systematic diagnosis of neonatal malformations compatible with CRS (deafness, congenital heart disease, cataracts, etc.), should be encouraged. These measures will make it possible to measure the progress achieved through vaccination and to quickly detect any residual outbreaks.

5. Conclusions

This study, conducted in urban areas of the Democratic Republic of the Congo before the introduction of vaccination, shows that approximately 79% of pregnant women have immunity to rubella, acquired naturally during their lifetime, while 21% remain unimmunized and therefore at risk of rubella infection during preg-

nancy. The factors associated with immunity are mainly related to age and reproductive history: older, married, multiparous women are significantly more likely to be immune than young, unmarried primigravidas, who constitute the main vulnerable group. These results illustrate the phenomenon of progressive immunity in a context of endemic exposure, while highlighting a worrying reservoir of susceptibility among young women. In a pre-vaccination context, this translates into a continuing risk of congenital rubella cases during future outbreaks.

This study provides quantitative justification for recent efforts in the DRC to introduce the rubella vaccine into the routine immunization program. It demonstrates the importance of this measure in protecting cohorts of girls and preventing them from reaching adulthood without immunity (as is currently the case for nearly one-third of them). The data also suggest additional courses of action: raising awareness among young women (especially those pregnant for the first time) about the dangers of rubella, and considering prenatal strains circulating in the DRC (through rubella strain genotyping) would be useful for monitoring transmission dynamics and documenting the future elimination of this virus from the territory.

In summary, this study provides a solid basis for guiding rubella control policies in the DRC. It confirms the extent of naturally acquired immunity while pointing to gaps in a significant subgroup of women. Systematic vaccination of children, coupled with catch-up strategies for non-immune women, appears to be the essential measure for protecting mothers and their future children, thereby breaking the cycle of infection and avoiding the tragedies of congenital rubella syndrome. The results obtained in Kinshasa call for a rapid extension of these efforts nationwide, so that rubella—a preventable disease—ceases to be a threat to Congolese mothers and newborns. The challenge in the coming years will be to translate these recommendations into effective action, ensuring the widest possible vaccination coverage and maintaining epidemiological vigilance until rubella and CRS are completely eliminated in the DRC.

The challenge for the coming years will be to translate these recommendations into concrete actions, ensuring the widest possible vaccine coverage and maintaining epidemiological vigilance until the complete elimination of rubella and congenital rubella syndrome (CRS) in the DRC, where serological screening could help identify and vaccinate seronegative women in the postpartum period.

Looking ahead, now that rubella vaccination is being implemented in the DRC, it would be useful to conduct post-introduction studies to assess changes in seroprevalence in a few years' time and verify that the proportion of non-immune women is indeed declining. Monitoring CRS rates in obstetric facilities will also make it possible to measure the real impact on the health of newborns. In addition, qualitative studies could explore women's knowledge, attitudes, and practices regarding rubella and its vaccine in order to optimize adherence to the program (many women in our cohort were unaware of rubella, often confusing it with measles).

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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Abbreviations

CI	Confidence Interval
CPN	Prenatal Consultation
CRS	Congenital Rubella Syndrome
DRC	Democratic Republic of the Congo
GAVI	Global Alliance for Vaccines and Immunization
HIV	Human Immunodeficiency Virus
IgG	Immunoglobulin G
IgM	Immunoglobulin M
INRB	National Institute for Biomedical Research
IQR	Interquartile Range
KHC	Kingasani Hospital Center
MMR	Measles-Mumps-Rubella
OR	Odds Ratio
RBG	Rub IgG
RCV	Rubella-Containing Vaccine
UNICEF	United Nations Children's Fund
WHO	World Health Organization