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# Simultaneous Seroprevalence of *Toxoplasma gondii*, Rubella Virus and Human Immunodeficiency Virus (HIV) Infections Among Pregnant Women in Owendo, Gabon

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## ABSTRACT

***Background:*** *Infections caused by Toxoplasma gondii (T. gondii), HIV, and rubella virus are major causes of adverse pregnancy outcomes in sub-Saharan Africa. Data from Gabon remain limited. This study aimed to determine the simultaneous seroprevalence of these pathogens among pregnant women in Owendo, Gabon.*

***Methods:*** *A prospective descriptive study was conducted from October 2022 to May 2024 at the Owendo University Teaching Hospital. Pregnant women in their first trimester were screened for HIV (Vidas® Duo Ultra HIV5) and for T. gondii and rubella IgG/IgM antibodies (Vidas® bioMérieux ELFA). Sociodemographic data and serological results were analysed using Statview 5.0, with significance set at $p < 0.05$.*

***Results:*** *Among 1,705 participants (median age 25 [20–31] years), HIV prevalence was 3.45% (46/1,334), mainly in women aged 25–35 years (p=0.003). T. gondii IgG seroprevalence was 63.5% (658/1,036), while IgM positivity was 1.9% (18/927). Rubella IgG antibodies were detected in 85.7% (786/917), with 2.3% (21/917) IgM positivity. Combined immunity to both Toxoplasma and Rubella was observed in 53.2% (475/893), whereas 6.7% (60/893) were non-immune.*

***Conclusion:*** *More than one-third of pregnant women in Owendo remain susceptible to at least one major congenital infection. These findings highlight the need for integrated antenatal screening, improved vaccination coverage, and targeted health education to reduce preventable perinatal morbidity and mortality in Central Africa.*

## Keywords

Gabon, HIV infection, Pregnancy, Rubella, Toxoplasmosis.

## Introduction

Infections caused by HIV, *Toxoplasma gondii* (*T. gondii*), and the rubella virus remain major public health concerns, particularly among pregnant women, owing to the risk of in utero transmission and congenital abnormalities that may affect the fetus. These complications include spontaneous abortion, congenital toxoplasmosis, and congenital rubella syndrome [1-4].

*T. gondii* is a cosmopolitan protozoan parasite. The severity of toxoplasmosis is non- immune pregnant women, those fetuses are at increased risk of congenital transmission [5]. Transplacental

infection may result in a wide range of fetal manifestations, from spontaneous abortion and intrauterine death to severe meningoencephalitis. Although, congenital infection can cause serious disease in neonates, most infected infants are asymptomatic at birth [6]. Transmission occurs mainly through the ingestion of food or water contaminated with oocysts shed by cats, or through consumption of undercooked meat containing tissue cysts [7].

HIV is primarily transmitted through sexual contact, blood exposure, and mother-to-child transmission during pregnancy, childbirth, or breastfeeding. Rubella, caused by the rubella virus, is spread via respiratory droplets and mainly affects children and young adults. Clinically, it presents with fever and rash [8-10]. When these infections occur during the first trimester of pregnancy, they are associated with adverse pregnancy outcomes, including preterm delivery, low birth weight, miscarriage, intrauterine fetal death, stillbirth, or congenital malformations such as congenital rubella syndrome [2-4,11,12].

To prevent such fetal complications, the World Health Organization (WHO) recommends systematic screening for HIV, toxoplasmosis, and rubella in all pregnant women. In France, the annual number of congenital toxoplasmosis cases remains around 250 [13]. Despite the availability of a safe and cost-effective vaccine, 17,865 cases of rubella were reported across 78 countries in 2022 [11]. Furthermore, the WHO estimates that 1.4 million pregnant women in developing countries are living with HIV, of whom 90% reside in Africa [14].

In Gabon, data on the seroepidemiology of these congenital infections in the general population are limited. Information regarding the seroprevalence of HIV, toxoplasmosis, and rubella among pregnant women, as well as the associated burden of congenital syndromes, remains scarce and insufficiently documented.

This study therefore aimed to determine, for the first time, the simultaneous seroprevalence of HIV, *Toxoplasma gondii*, and rubella among pregnant women in Owendo. It specifically assessed IgG and IgM antibodies directed against *T. gondii* and the rubella virus to evaluate the level of protective immunity and potential risks in this population attending the Owendo University Teaching Hospital, the only public facility in the city providing free antenatal care.

## Patients and Methods

### Study Design and Period

A prospective descriptive study was conducted from October 2022 to May 2024 at the Owendo University Teaching Hospital. Located in the southern part of Libreville, the capital of Gabon, the municipality of Owendo has undergone substantial economic, demographic, and social transformations over the past two decades, leading to its administrative recognition as a separate commune. It lies within the Komo-Mondah Department in the Estuaire Province, approximately 20 km southwest of Libreville. The city comprises two districts and hosts two public health facilities: the Owendo University Teaching Hospital and the Professor Daniel Gahouma Institute of Infectious Diseases. In 2010, the local population was estimated at approximately 70,000 inhabitants. The Owendo University Teaching Hospital serves as the commune's only mother-and-child reference centre, thereby providing valuable epidemiological data on maternal and congenital infections. Until the recent opening of the Institute of Infectious Diseases, it remained the only hospital in the area.

### Study Population

The study population included pregnant women attending their first antenatal visit at the Owendo University Teaching Hospital during the study period. Only women in their first trimester of pregnancy were eligible for inclusion. Those in their second or third trimester, as well as women who declined to participate, were excluded from the study.

### Recruitment and Blood Sampling

After obtaining verbal, informed, voluntary consent, each pregnant woman attending her routine antenatal assessment and agreeing to the anonymous use of her results was enrolled. A 5 mL sample of venous blood was collected into plain Vacutainer® tubes. Sociodemographic data were recorded for each participant. Blood samples were transported in refrigerated cool boxes to the UMRAIP laboratory, where were separated by centrifugation at 3,000 rpm for 5 minutes.

### Serological Analyses

Quantitative detection of specific IgG and IgM antibodies against the rubella virus and *Toxoplasma gondii* was performed using the Vidas® (bioMérieux) automated system. This platform employs a sandwich immunoassay coupled with enzyme-linked fluorescent assay (ELFA) technology, following the manufacturer's instructions. Results were interpreted according to the predefined threshold values (Table 1), allowing classification as negative, equivocal, or positive.

**Table 1:** Interpretation of *Toxoplasma gondii* and rubella immunoglobulin M (IgM) and immunoglobulin G (IgG) antibody results.

| Interpretation | IgG | IgM |
|---|---|---|
| **Toxoplamosis** | | |
| Negative | < 4 IU/mL | < 0,55 |
| Indetermined | [4;8[ IU/mL | [0,55; 0,65] |
| Positive | ≥ 8 IU/mL | > 0,65 |
| **Rubella** | | |
| Negative | < 10 IU/mL | < 0,80 |
| Indetermined | [10;15[IU/mL | [0.80 ;1.2[ |
| Positive | ≥ 15 IU/mL | ≥ 1.2 |

### HIV Screening

HIV screening was performed using the combined detection method for anti-HIV-1 (group O) and anti-HIV-2 antibodies, together with the p24 antigen, on the Vidas® Duo Ultra HIV5 platform. Tests were conducted on serum or plasma samples

collected in tubes containing either lithium heparin or EDTA, according to the standard protocol.

### Operational Definitions

- Past exposure to rubella virus or *Toxoplasma gondii*: defined as the presence of rubella- or toxoplasma-specific IgG antibodies, indicating protective immunity acquired either through natural infection or vaccination.
- Recent infection with rubella virus or *T. gondii*: defined as the presence of specific IgM antibodies.

### Statistical Analysis

Serological data collected between 2022 and 2024 were extracted from the medical microbiology laboratory records. The recorded information included the date of testing, patient age, and the results of HIV, toxoplasmosis, and rubella screening (IgG and IgM). For each participant, only the first available result was retained to avoid duplication.

Descriptive statistics were computed to determine proportions and their corresponding 95% confidence intervals (95% CI). Hypothesis testing was used to assess potential associations, with statistical significance set at $p<0.05$. All analyses were performed using Statview software, version 5.0.

### Ethical Considerations

This study was conducted as part of routine hospital activities and adhered to the ethical principles of the Declaration of Helsinki. For confidentiality, each participant was automatically assigned an anonymous identification code generated by the laboratory software.

Written informed consent was obtained from all pregnant women prior to inclusion in the study. Sociodemographic data were extracted from the participants' medical records following authorisation from the administration of the Owendo University Teaching Hospital.

## Results

### Characteristics of the Study Population

A total of 1,705 pregnant women were enrolled between October 2022 and May 2024. The median age of participants was 25 years (interquartile range [IQR]: 20–31 years), and the median gestational age at inclusion was 12 weeks (IQR: 10–15 weeks). The majority of women (82.5%, n=1,406/1,705) were between 18 and 35 years of age (Table 2).

### Seroprevalence of Toxoplasmosis

Among the 1,036 women tested for *Toxoplasma gondii* antibodies, 63.5% (n=658) were IgG-positive, indicating prior exposure or acquired immunity, while 36.5% (n=378) were seronegative. Toxoplasma-specific IgM antibodies, reflecting recent infection, were detected in 1.9% of women (n=18/927). The proportion of IgG-positive cases was higher among women aged over 35 years (67.9%; n=53/78), and all IgM-positive samples were found within this same age group (Table 3). Among IgM-positive participants, 88.9% also had detectable IgG antibodies whereas 11.1% were IgG-negative, consistent with very early seroconversion.

**Table 2**: General characteristics of the study population.

| Variables | N (1705) | % |
|---|---|---|
| **Age** | | |
| < 18 years | 129 | 7,6 |
| 18-25 years | 660 | 38,7 |
| 26-35 years | 746 | 43,7 |
| > 35 years | 170 | 10,0 |
| **Number of samples analysed (N=1337)** | | |
| HIV | 1334 | 78,2 |
| Toxoplasmosis | 1040 | 61,0 |
| Rubella | 922 | 54,1 |

### Seroprevalence of Rubella

Of the 917 women tested for rubella, 85.7% (n=786) were IgG-positive, consistent with protective immunity, while 14.3% (n=131) lacked rubella IgG antibodies. Seropositivity was highest among women aged over 36 years (93.2%; n= 60/63). Rubella-specific IgM antibodies, suggesting recent infection, were detected in 2.3% (n=21/917) of participants, all of whom were younger than 35 years (Table 3). Among these, 81.0% were also IgG-positive, whereas 19.0% showed isolated IgM positivity, indicating probable primary infection.

**Table 3**: Seroprevalence of toxoplasmosis and rubella among pregnant women according to age group.

| | IgG + / IgM - | | IgG - / IgM- | | IgM + with or not IgG | |
|---|---|---|---|---|---|---|
| | n | % | N | % | n | % |
| **Toxoplasmosis** | | | | | | |
| < 18 years | 79/122 | 64,8 | 43/122 | 35,2 | 0/122 | 0 |
| 18-25 years | 238/401 | 59,4 | 154/401 | 38,4 | 9/401 | 2,2 |
| 26-35 years | 272/435 | 62,5 | 156/435 | 35,9 | 7/435 | 1,6 |
| > 36 years | 53/78 | 67,9 | 23/78 | 29,5 | 2/78 | 2,6 |
| **Rubella** | | | | | | |
| < 18 years | 85/115 | 73,9 | 25/115 | 21,7 | 5/115 | 4,4 |
| 18-25 years | 303/364 | 83,2 | 50/364 | 13,7 | 11/364 | 3,1 |
| 26-35 years | 321/375 | 85,6 | 49/375 | 13,1 | 5/375 | 1,3 |
| > 36 years | 60/63 | 93,2 | 3/63 | 4,8 | 0/63 | 0 |

### Seroprevalence of HIV

HIV testing was performed in 1,334 women, representing 78.2% of the total cohort. The overall HIV seroprevalence was 3.45% (n=46/1,334), with the highest rate observed among women aged 25–35 years. This age-related variation was statistically significant ($p$=0.003). HIV-1 accounted for almost all infections (98%, n = 49/50), while a single participant presented with mixed HIV-1 + HIV-2 infection.

### Age-Related Serological Patterns

As shown in Table 3, the seroprevalence of *Toxoplasma gondii* infection increased modestly with age—from 64.8% among adolescents (< 18 years) to 67.9% in women aged > 36 years—

while IgM positivity remained rare (< 3%) across all groups. Roughly one-third of participants were seronegative, indicating persistent susceptibility during pregnancy.

In contrast, rubella immunity (IgG+/IgM-) increased more markedly with age, from 73.9% in adolescents to 93.2% among women > 36 years; whereas susceptibility (IgG-/IgM-) declined from 21.7% to 4.8%. IgM positivity for rubella was infrequent (≤ 4.4%) and absent among the oldest age group.

### Co-infections

Only one woman, belonging to the 25-35-year age group, presented with HIV–rubella co-infection. No cases of HIV-toxoplasmosis co-infection were detected. All women who tested positive for HIV were IgG-positive and IgM-negative for toxoplasmosis, indicating prior exposure with no evidence of recent infection.

Among the 893 women simultaneously screened for both *Toxoplasma gondii* and rubella, 53.2% (n = 475) were immune to both infections (IgG-positive for toxoplasmosis and rubella), whereas 6.7% (n = 60) showed no immunity to either pathogen (IgG-negative for both). Concurrent IgM positivity for both agents was observed in a single participant (Figure 1).

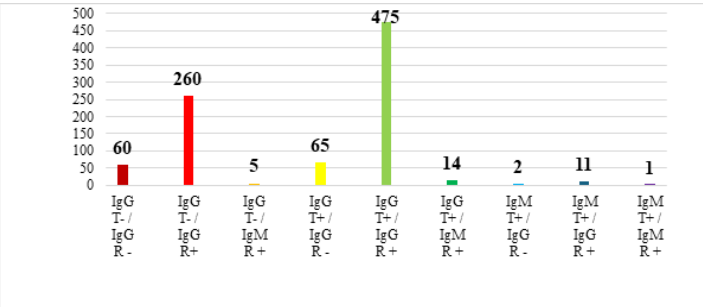


**Figure 1:** Simultaneous seroprevalence of toxoplasmosis and rubella among pregnant women.

## Discussion

Infections caused by *Toxoplasma gondii*, HIV, and the rubella virus constitute a triple infectious burden with significant implications for maternal and neonatal health in Central Africa. This study conducted in Owendo (Gabon) reveals non-negligible prevalences of these three pathogens among pregnant women, underlining the need for integrated and regionally informed interventions based on current epidemiological data.

The observed HIV seroprevalence of 3.45% in this cohort is consistent with national estimates reported by the *Programme National de Lutte contre le Sida* and comparable to those of neighbouring countries such as Cameroon (4.7%), Congo-Brazzaville (3.8%), and the Central African Republic, where rates can reach up to 15% in certain rural or medically underserved areas [15,16]. The clustering of infections among women aged 25–35 years, as demonstrated in this study, reflects an accumulation of risk during the most sexually active period, in line with findings from Bouaré et al. in Mali and Mishra et al. on the social dynamics of HIV in sub-Saharan Africa [17,18]. These results reinforce the urgency of scaling up early HIV testing, rapid initiation of antiretroviral therapy, and prevention of mother-to-child transmission (PMTCT), which remain suboptimal in some peri-urban areas despite WHO's goal of eliminating paediatric HIV by 2030 [19].

Regarding *Toxoplasma gondii*, the high IgG seroprevalence observed in Owendo (63.5%) is consistent with rates reported in Libreville, Lambaréné, Congo, and the Democratic Republic of the Congo, where prevalence typically ranges between 50% and 75%, depending on hygiene conditions, living standards, and dietary habits [4,20,21]. This high seropositivity reflects past exposure in a hot and humid tropical environment favourable to oocyst survival. The detection of IgM antibodies in 1.9% of cases indicates that recent infections still occur during pregnancy, posing a potential risk of congenital toxoplasmosis. The lack of IgG avidity testing constitutes a diagnostic limitation, preventing precise dating of recent infections. Frequently reported risk factors include the consumption of undercooked meat, handling of unwashed raw vegetables, contact with infected cats, and poor food or domestic hygiene [7,22]. Despite the potential severity of congenital forms, toxoplasmosis screening remains absent from routine antenatal care in many Central African countries. Pilot studies in Kinshasa and Douala have demonstrated the feasibility and acceptability of combined rapid testing during antenatal consultations, paving the way for more effective preventive management [23,24].

For rubella, the IgG seroprevalence of 85.7% observed in this study indicates relatively widespread immunity, acquired either through vaccination or prior natural infection. Nevertheless, 14.3% of pregnant women remained seronegative, representing a non-negligible risk for congenital rubella syndrome (CRS) in the event of infection during pregnancy. This immunity level remains below the 95% threshold required to interrupt viral transmission among women of reproductive age, as recommended by the WHO [8]. The results exceed those reported in certain regions of Cameroon (71%) and Congo (75%) but remain lower than levels observed in countries with well-established vaccination programmes [16,25]. The 2.3% IgM positivity in this cohort indicates ongoing viral circulation, most likely drove by clusters of suboptimal vaccine coverage in peri-urban settings. In Gabon, rubella vaccination was introduced relatively late into the Expanded Programme on Immunisation (EPI), and cohorts of women born before vaccine introduction remain vulnerable. A targeted postpartum vaccination strategy for seronegative women could rapidly reduce CRS incidence, as successfully implemented in East Africa [26-28].

The age-related increase in *Toxoplasma gondii* and rubella IgG seropositivity observed in this study reflects the progressive acquisition of immunity through cumulative exposure. Comparable trends have been reported among pregnant women in Nigeria, where *T. gondii* seroprevalence ranged from 56% to 74% depending on age, dietary habits, and cat contact [29]. Similarly, the steady increase in rubella IgG positivity, from 74%

among adolescents to over 90% in older women, mirrors findings from East Africa, where partial vaccination coverage coexists with natural immunity [30]. A systematic review confirmed wide heterogeneity in rubella immunity across sub-Saharan Africa, reflecting incomplete vaccine rollout and limited coverage among women of reproductive age [31]. These findings underscore the need to strengthen preconception and antenatal screening, together with the implementation of rubella immunisation programmes, as recommended by the WHO (2020) [32]. Improved health education on food hygiene and safe contact with animals could further reduce the risk of *T. gondii* infection during pregnancy.

The findings of this study call for an integrated approach to perinatal infection prevention, particularly through the routine implementation of combined antenatal testing for HIV, toxoplasmosis, rubella, and syphilis. Such a "one-stop testing" model would optimise human and logistical resources while ensuring comprehensive coverage of infectious risks. Concurrently, community health education must be strengthened to improve women's understanding of transmission routes, risk behaviours, and the importance of regular antenatal follow-up. Community health workers and midwives can play a pivotal role in awareness and case monitoring. Reinforcing the EPI, particularly regarding rubella, remains essential, alongside the establishment of harmonised regional surveillance systems to document trends in maternal and child health indicators related to these infections.

This single-centre, cross-sectional study may not fully represent pregnant women across Gabon. The absence of IgG avidity testing limited the dating of recent *Toxoplasma gondii* infections, and no longitudinal follow-up was performed to confirm seroconversion or assess neonatal outcomes. Sociodemographic variables such as parity, education, and vaccination history were unavailable. Finally, reliance on serology alone may have led to slight misclassification of recent infections due to low antibody titres or cross-reactivity.

Despite these limitations, this work provides the first simultaneous assessment of HIV, *Toxoplasma gondii*, and rubella infections among pregnant women in Gabon, generating essential baseline data for public health planning. By demonstrating the feasibility of integrated serological surveillance within routine antenatal care, it offers a practical model for one-stop screening applicable to similar resource-limited settings. The results contribute directly to national and regional efforts to prevent congenital infections and support WHO targets for the elimination of mother-to-child transmission.

## Conclusion

Overall, the results show that in Owendo, more than one in three pregnant women is exposed to an infection potentially transmissible to the fetus. Although the situation has improved compared with previous decades, it remains concerning. There is a pressing need to intensify integrated screening, expand vaccination coverage, and strengthen health education, particularly in peri-urban settings. Better coordination between programmes targeting HIV, parasitic diseases, and vaccine-preventable viral infections and reproductive health systems will be crucial to reducing preventable neonatal morbidity and mortality in this region.

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