# Seroepidemiology Study of Cytomegalovirus and Rubella in Pregnant Women in Luanda, Angola: Geospatial Distribution and its Association with Socio-Demographic and Clinical-Obstetric Determinants

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## Abstract

**Background:** The main objective was to study the seroprevalence of anti-CMV and anti-Rubella antibodies in pregnant women of Luanda (Angola), identify the primary maternal infection during gestation and to evaluate the socio-demographic risk factors associated with CMV and Rubella virus infections.

**Methods:** A prospective cross-sectional study was conducted from August 2016 to May 2017. Blood samples were collected and specific anti-CMV and anti-Rubella antibodies (IgG and IgM) were quantified by electrochemiluminescence (COBAS e411). Demographic and clinical data were collected by standardized questionnaire. Bivariate and multivariate logistic regression analysis was used to quantify the effect of clinical and obstetric risk factors on virus seroprevalence. The level of statistical significance was set as $p<0.05$, and Odds Ratio (OR) and 95% Confidence Intervals (95% CI) were computed.

**Results:** The 396 pregnant women participated in the study aged from 15 to 47. Of the participants, 382 (96.5%) had anti-CMV IgG antibodies, 8 (2.0%) had anti-CMV IgG and IgM antibodies and 6 (1.5%) were seronegative. For Rubella virus, 347 (87.6%) were positive for anti-IgG, 4 (1.0%) positive for anti-IgG and IgM, and 45 (11.4%) were seronegative. The mean age of CMV positivity was 28.4 (SD ± 6.2) and for Rubella virus was 28.6 (SD ± 6.1). The multivariate logistic regression analysis has shown a significant association between Rubella virus infection and pregnant women without child (OR 2.673; CI: 1.026 - 7.007) and suffering spontaneous abortion (OR 3.232; CI: 1.192 - 7.952). In contrast, the level of schooling, residence, occupation, marital status, number of children in the household, basic sanitation, gestational age, history of miscarriages and hepatitis B were not significantly associated with the Rubella virus infection.

**Conclusions**: Overall, this study showed that there is a high seroprevalence of anti-CMV and anti-Rubella antibodies in pregnant women in Luanda. Therefore, it is important a rapid and accurate diagnosis of CMV and Rubella infection in pregnant women to prevent congenital infections. Rubella vaccination should be offered to women non-immune to Rubella. Overall, it would be important to implement national screening for CMV, Rubella and other diseases linked to maternal and child health.


## Background

Several infections caused by microorganisms can be transmitted to the fetus during pregnancy, causing fetal death or sequelae to the newborn: Toxoplasmosis, Other (syphilis, varicella-zoster, parvovirus B19), Rubella, Cytomegalovirus (CMV) and Herpes infections as known by TORCH infections [1]. The agents of the TORCH infections, lead to the development of a maternal infection and can pass in the uterine circulation at any gestational age [1]. On the other hand, the infection can lead to spontaneous abortions, sterility, congenital malformations and intrauterine fetal loss. Prenatal surveillance is important for the prevention of vertical transmission.

The Angolan National Health Development Plan (2012–2025) does not include the control of diseases such as Rubella and Cytomegalovirus. The absence of early diagnosis and the consequent lack of treatment of maternal infections can considerably increase the rates of perinatal morbidity and mortality.

CMV or Herpesvirus 5 is a member of the Herpesviridae family, being the largest virus [2]. Man is the only reservoir for CMV and the virus transmission occurs after direct or indirect contact with saliva, oropharyngeal secretions, endocervical secretions, urine, sperm, breast milk, tears, blood products or organs, and by vertical transmission [3]. However, CMV infection is less contagious than that of other members of the same family of Varicella Zoster Virus [2]. The main infected cells are the endothelial cells and the leukocytes, and then the hematogenous propagation is carried out in the organism. Like the other members of the Herpesviridae family, CMV arrest in body (latent phase) after a primary infection. The acquired immunity is not completely protective and secondary infections may occur during the latent phase [4]. The pregnant women are contaminated by patients who excrete large numbers of viruses, including children, immunosuppressed patients and transplanted patients. Vertical transmission is transplacental hematogenous and occurs during maternal viraemia. The transmission rate varies with the type of maternal infection: between 30 and 50% on primary infections and 0.1 and 3% on secondary infections (re-infections or reactivations of a latent infection) [2, 4]. The gestational age may also play an important role in the vertical transmission rate, increasing with gestational age: 36% in the first trimester, 44% in the second and 77.6% in the last trimester. However, the consequences of CMV infection are more severe when maternal infection occurs before 20 weeks [2, 4]. Also, CMV is considered a common opportunistic infection among individuals infected with the human immunodeficiency virus (HIV), a major source of serious viral complications among organ transplant recipients and a major cause of hearing loss, vision loss and mental retardation in children with congenital infection [5].

Rubella is caused by a virus of the Togaviridae family and genus Rubivirus. The most common form of virus transmission is through direct contact with respiratory secretions droplets of infected persons [6]. It is a benign disease characterized by a macular rash accompanied by low fever, joint pain, pharyngitis and cervical adenopathies [6], however, it becomes severe to fetus when it occurs during pregnancy [7]. The teratogenic properties of the rubella virus were first discovered in Australia in 1941 by Gregg who associated the occurrence of rubella during pregnancy with the presence of congenital cataracts [8]. Maternal infection with the rubella virus during the first trimester is often associated with fetal death, miscarriage or adverse neonatal outcome, including heart problems, cataracts and deafness, known as congenital rubella syndrome (CRS), which has a higher neonatal morbidity [9]. This severity of fetal infection is related to the period of organogenesis, due to the high virus tropism for fetal tissues [10]. Some defects were later reported as consequence of maternal infections in the second trimester [9]. In order to avoid vertical transmission, seronegative women should be detected during the preconception period and their pregnancies be planned after immunization [11].

In Angola, there are no reports of CMV and Rubella studies. Therefore, the main objective of this work was to study the seroprevalence of anti-CMV and anti-Rubella antibodies in pregnant women attending at Lucrécia Paim Maternity Hospital (LPMH) of Luanda and to provide a detailed analysis of the

geographical distribution. In addition, we aimed to identify the maternal infection and to evaluate the risk factors associated with CMV and Rubella infection.

## Methods

# Ethical Considerations

The present study has been approved by the Research Ethics Committee of LPMH through the National Institute of Public Health of the Republic of Angola (nº 301019) (S1 File).

Participant individuals provided a written signed informed consent prior to sample collection and for participants younger than 18 years, informed consent was provided by parents or guardians after a detailed explanation of the objectives of the work.

# Study Population

The study population was constituted by pregnant women monitored for routine prenatal assessment at LPMH, a reference maternity in Angola, located in Luanda. The referred maternity is a a tertiary-level public health institution specialized in maternal and child health care, teaching and research. The health institution offers outpatient and inpatient services, has 400 beds for hospitalization video laparoscopy, hysteroscopy, milk bank, pathological anatomy, assisted reproductive services, genetics and mammography. Consequently, the women attended in the LPMH come from all over the country, however mainly from Luanda, the capital of Angola.

The study included women aged from 15 to 47 years, who had a pregnancy proven by ultrasonography and laboratory tests. For the obstetric follow-up we counted on the collaboration of the medical and nursing team of the department of obstetrics of the LPMH.

# Sociodemographic, clinical and housing characteristics of the pregnant women

A standardized questionnaires in face-to-face interviews were used to obtain the socio-demographic, clinical, and housing characteristics from the pregnant women (S2 File). Socio-demographic items included age, residence, occupation, schooling level, and socio-economic status. Clinical characteristics included gestation age, number of pregnancies, history of abortion, the frequency of miscarriages, child born premature, number of children with disabilities and with mental retardation, presence of any underlying disease, long-term fever, history of hepatitis B and HIV. The housing characteristics like type of flooring, destination of faeces, the number of children in the household and crowding were also inclued. The questionnaire was written in Portuguese, the official language in the Republic of Angola, developed and revised accordingly.

# Blood Sample Collection and Laboratory Procedures

A cross-sectional study was conducted from August 2016 to May 2017 and a total of 396 pregnant women were included in the survey.

Blood samples was collected and serum samples were obtained after centrifugation. These samples were immediately transferred (properly packaged in dry ice) to the Clinical Pathology Service of Clínica Sagrada Esperança (Luanda) and kept at -80 °C until serological analysis.

The quantification of anti-CMV and anti-Rubella IgG and IgM antibodies was done by Electrochemiluminescence (ECL) using commercially available kits for COBAS e411 (Roche, Sistemas de Diagnósticos Lda), according to the guidelines of the manufacturer. The anti-CMV IgG and IgM levels were expressed as IU/mL and COI (Cut-off index), respectively: values < 0.5 IU/mL of anti-CMV IgG antibody are considered negative and values ≥ 1.0 IU/mL are considered positive; values < 0.7 COI of anti-CMV IgM antibody are considered negative and values ≥ 1.0 are considered positive.

The anti-Rubella IgG and IgM levels were expressed as IU/mL and COI, respectively: values < 10.0 IU / mL of anti-Rubella IgG antibody are considered negative and values ≥ 10.0 IU / mL are considered positive; values < 0,8 COI of anti-Rubella IgM antibody are considered negative and values ≥ 1.0 are considered positive.

# Geospatial analysis

The address of pregnant women was collected during the interview allowing the identification of the residence place. This information was converted into geographic coordinates (latitude and longitude) through the www.google.pt/maps/. The spatial distribution of pregnant women was assessed through a Kernel Density Function that allowed the estimation of the intensity of events across a surface.

# Stastical Analysis

The data entry was carried out using Excel software and analysed using Statistical Package for the Social Sciences (SPSS) version 20. The exploratory analysis of the categorical variables and quantitative variables are presented as percentages (mean ± SD). Bivariate and multivariate logistic regression were developed to assess the effect of different risk factors on rubella virus seroprevalence. The level of statistical significance was set as $p < 0.05$, and Odds Ratio (OR) and 95% Confidence Intervals (95% CI) were computed.

## Results

Between August 2016 and May 2017, a total of 396 pregnant women were tested for anti-CMV and anti-Rubella antibodies. The distribution of IgG and/or IgM antibodies to CMV and rubella of seropositive and seronegative pregnant women is summarized in Table 1. The majority of women had previous

exposure to the CMV virus: 382 (96.5%) were positive for anti-CMV IgG; 8 (2.0%) were positive for anti-CMV IgM and IgG; and 6 (1.5%) were seronegative for anti-CMV antibodies. For Rubella virus, 347 (87.6%) were positive for anti-Rubella IgG, 4 (1.0%) were positive for anti-Rubella IgM and IgG and 45 (11.4%) were seronegative (Table 1). Although the majority of women are immune to rubella virus, a significant number are susceptible to the primary infection.

Table 1
**Profile of antibodies IgG and IgM anti-CMV and anti-Rubella virus on pregnant women from Luanda (Angola).**

| Immune response | Number | Percentage (%) | Interpretation |
|---|---|---|---|
| Anti-CMV | | | |
| IgG(+) IgM (-) | 382 | 96.5 | Previous Exposure |
| IgG(+) IgM (+) | 8 | 2.0 | Active (Primary/Latent) infection |
| IgG(-) IgM (-) | 6 | 1.6 | Susceptible to primary infection |
| IgG(-) IgM (+) | 0- | 0- | Recent primary infection |
| *Total positive* | 390 | 98.5 | Overall prevalence |
| *Total negative* | 6 | 1.5 | Overall prevalence |
| Anti-Rubella | | | |
| IgG(+) IgM (-) | 347 | 87.6 | Immune |
| IgG(+) IgM (+) | 4 | 1.0 | Active (Primary/Latent) infection |
| IgG(-) IgM (-) | 45 | 11.4 | Susceptible (to primary infection) |
| IgG(-) IgM (+) | 0 | 0 | Recent primary infection |
| *Total positive* | 351 | 88.6 | Overall prevalence |
| *Total negative* | 45 | 11.4 | Overall prevalence |

The characteristics of the individual pregnant women and details on home conditions and food habits were collected using a structured questionnaire (S3 File).

The ages of the pregnant women ranged from 15 to 47 years with an average of 28.4 ± 6.2 (Mean ± SD); pregnant women between 26 and 35 years were the majority of participants (n = 207) (Table 2). Regarding education level, 3 (0.8%) are illiterate, 149 (37.6) have basic education, 200 (50.5%) have a high school education and 44 (11.1%) have higher education. In relation to residence, 180 (45.5%) live in the municipality of Luanda, 87 (22.0%) in the municipality of Belas, 86 (21.7%) in the municipality of Viana, 29 (7.3%) in the municipality of Cazenga and 14 (3.5% ) in the municipality of Cacuaco. Regarding occupancy,150 (37.9%) women work on public administration services, 64 (16.2%) are students, 63 (15.9%) are domestic, 49 (12,4%) are street vendors, 39 (9.8%) are store employees and 31 (7.8%) work in the catering area. The majority of pregnant women (270; 68.2%), are single and 126 (31.8%) are married. The mean of gestational age was 14.5 ± 6.8 (Mean ± SD) weeks and 63.9% of the pregnant women had more than one children (with mean birth rate of 1.8 ± 0.7 (Mean ± SD)).

Table 2

**Sociodemographic characteristics of seropositive and seronegative pregnant women to CMV and Rubella virus and respective prevalences.**

| Characteristics | CMV | | | | Rubella vírus | | | | Total |
|---|---|---|---|---|---|---|---|---|---|
| | Positive n (%) | Seroprevalence (%) | Negative n (%) | Seroprevalence (%) | Positive n (%) | Seroprevalence (%) | Negative n (%) | Seroprevalence (%) | |
| **Age group (years)** | | | | | | | | | |
| ≤ 19 | 23 (5.9) | 100.0 | 0 (00.0) | 0 (00.0) | 18 (5.1) | 78.3 | 5 (11.1) | 21.7 | 23 (5.8) |
| 20–25 | 110 (28.2) | 99.1 | 1 (16.7) | 0.9 | 97 (27.6) | 87.4 | 14 (31.1) | 12.6 | 111(28.0) |
| 26–35 | 202 (51.8) | 97.6 | 5 (83.3) | 2.4 | 185 (52.7) | 89.4 | 22 (48.9) | 10.6 | 207(52.3) |
| 36–47 | 55 (14.1) | 100.0 | 0 (00.0) | 0 (00.0) | 51 (14.6) | 92.7 | 4 (8.9) | 7.3 | 55 (13.9) |
| **Education** | | | | | | | | | |
| Illiterate | 3 (0.8) | 100.0 | 0 (00.0) | | 3 (0.9) | 100.0 | 0 (00.0) | 0 (00.0) | 3 (0.8) |
| Elementary School | 148 (37.9) | 99.3 | 1 (16.6) | 0.7 | 136 (38.7) | 91.3 | 13 (28.9) | 8.7 | 149 (37.6) |
| High school | 196 (50.3) | 98.0 | 4 (66.7) | 2.0 | 172 (49.0) | 86.0 | 28 (62.2) | 14.0 | 200 (50.5) |
| Higher education | 43 (11.0) | 97.3 | 1 (16.7) | 2.7 | 40 (11.4) | 90.9 | 4 (8.9) | 9.1 | 44 (11.1) |
| **Residence** | | | | | | | | | |
| Belas | 85 (21.8) | 97.7 | 2 (33.3) | 2.3 | 76 (21.7) | 87.4 | 11 (24.2) | 12.6 | 87 (22.0) |
| Cacuaco | 13 (3.3) | 92.9 | 1 (16.7) | 7.1 | 13 (3.7) | 92.9 | 1 (2.2) | 7.1 | 14 (3.5) |
| Viana | 85 (21.8) | 98.8 | 1 (16.7) | 1.2 | 76 (21.7) | 88.4 | 10 (22.2) | 11.6 | 86 (21.7) |
| Cazenga | 29 (7.5) | 100.0 | 0 (00.0) | 0.0 | 25 (7.1) | 86.2 | 4 (9.0) | 13.8 | 29 (7.3) |
| Launda | 178 (45.6) | 98.9 | 2 (33.3) | 1.1 | 161 (45.8) | 89.4 | 19 (42.2) | 10.6 | 180 (45.5) |
| **Employment** | | | | | | | | | |
| Homemakers | 62 (15.9) | 98.4 | 1 (16.7) | 1.6 | 57 (16.2) | 90.5 | 6 (13.3) | 9.5 | 63 (15.9) |
| Public administration service | 147 (37.7) | 98.0 | 3 (50.0) | 2.0 | 131 (37.3) | 87.3 | 19 (42.2) | 12.7 | 150 (37.9) |
| Student | 62 (15.9) | 96.9 | 2 (33.3) | 3.1 | 56 (16.0) | 87.5 | 8 (17.8) | 12.5 | 64 (16.2) |
| Restaurant waitress | 31 (7.9) | 100.0 | 0 (00.0) | 0 (00.0) | 26 (7.4) | 83.9 | 5 (11.1) | 16.1 | 31 (7.8) |
| Street vendor | 49 (12.6) | 100.0 | 0 (00.0) | 0 (00.0) | 45 (12.8) | 91.8 | 4 (8.9) | 8.2 | 49 (12.4) |
| Shop assistants | 39 (10.0) | 100.0 | 0 (00.0) | 0 (00.0) | 36 (10.3) | 92.3 | 3 (6.7) | 7.7 | 39 (9.8) |
| **Marital status** | | | | | | | | | |
| Married | 126 (32.3) | 100.0 | 0 (00.0) | 0 (00.0) | 110 (31.3) | 87.3 | 16 (35.6) | 12.7 | 126 (31.8) |
| Single | 264 (67.7) | 97.8 | 6 (100.0) | 2.2 | 241 (68.7) | 89.3 | 29 (64.4) | 10.7 | 270 (68.2) |
| **Gestational age** | | | | | | | | | |
| 1st Trimester | 199 (51.0) | 98.5 | 3 (50.0) | 1.5 | 181 (51.6) | 89.6 | 21 (46.7) | 10.4 | 202 (51.0) |

| Characteristics | CMV | | | | Rubella vírus | | | | Total |
|---|---|---|---|---|---|---|---|---|---|
| | Positive n (%) | Seroprevalence (%) | Negative n (%) | Seroprevalence (%) | Positive n (%) | Seroprevalence (%) | Negative n (%) | Seroprevalence (%) | |
| 2nd Trimester | 152 (39.0) | 98.1 | 3 (50.0) | 1.9 | 135 (38.4) | 87.1 | 20 (44.4) | 12.9 | 155 (39.1) |
| 3rd Trimester | 39 (10.0) | 100.0 | 0 (00.0) | 0 (00.0) | 35 (10.0) | 89.7 | 4 (8.9) | 10.3 | 39 (9.9) |
| **Number of births** | | | | | | | | | |
| 0 | 84 (21.5) | 97.7 | 2 (33.3) | 2.3 | 71 (20.2) | 82.6 | 15 (33.3) | 17.4 | 86 (21.7) |
| 1 | 118 (30.3) | 99.2 | 1 (16.7) | 0.8 | 103 (29.3) | 86.6 | 16 (35.5) | 13.4 | 119 (30.0) |
| 66 (16.9) | 97.1 | 2 (33.3) | 2.9 | 61 (17.4) | 89.7 | 7 (15.6) | 10.3 | 68 (17.2) | |
| ≥ 3 | 122 (31.3) | 99.2 | 1 (16.7) | 0.8 | 116 (33.1) | 94.3 | 7 (15.6) | 5.7 | 123 (31.1) |
| **Spontaneous abortion** | | | | | | | | | |
| Yes | 27 (6.9) | 100.0 | 0 (00.0) | 0 (00.0) | 20 (5.7) | 74.1 | 7 (15.6) | 25.9 | 27 (6.8) |
| No | 363 (93.1) | 98.4 | 6 (100.0) | 1.6 | 331 (94.3) | 89.7 | 38 (84.4) | 10.3 | 369 (93.2) |
| **History of miscarriages** | | | | | | | | | |
| No | 219 (56.2) | 98.2 | 4 (66.7) | 1.8 | 190 (54.1) | 73.2 | 33 (73.3) | 26.8 | 223 (56.3) |
| Yes | 171 (43.8) | 98.8 | 2 (33.3) | 1.2 | 161 (45.9) | 93.1 | 12 (26.7) | 6.9 | 173 (43.7) |
| **Children at home** | | | | | | | | | |
| Yes | 386 (99.0) | 98.5 | 6 (100) | 1.5 | 348 (99.1) | 88.8 | 44 (97.8) | 11.2 | 392 (99.0) |
| No | 4 (1.0) | 100.0 | 0 (00.0) | 0 (00.0) | 3 (0.9) | 75.0 | 1 (2.2) | 25.0 | 4 (1.0) |
| **Number of children in the household** | | | | | | | | | |
| 0 | 4 (1.0) | 100.0 | 0 (00.0) | 0 (00.0) | 3 (0.9) | 75.0 | 1 (2.2) | 25.0 | 4 (1.0) |
| 1 | 137 (35.1) | 98.6 | 2 (33.3) | 1.4 | 118 (33.6) | 84.9 | 21 (46.7) | 15.1 | 139 (35.1) |
| 2 | 152 (39.0) | 98.1 | 3 (50.0) | 1.9 | 141 (40.2) | 91.0 | 14 (31.1) | 9.0 | 155 (39.1) |
| ≥ 3 | 97 (24.9) | 99.0 | 1 (16.7) | 1.0 | 89 (25.3) | 90.8 | 9 (20.0) | 9.2 | 98 (24.8) |
| **Hepatitis B infection** | | | | | | | | | |
| No | 265 (67.9) | 98.5 | 4 (66.7) | 1.5 | 237 (67.5) | 88.1 | 32 (71.1) | 11.9 | 269 (67.9) |
| Yes | 125 (32.1) | 98.4 | 2 (33.3) | 1.6 | 114 (32.5) | 89.8 | 13 (28.9) | 10.2 | 127 (32.1) |
| **HIV status** | | | | | | | | | |
| No | 332 (85.1) | 98.5 | 5 (83.3) | 1.5 | 294 (83.8) | 87.2 | 43 (95.6) | 12.8 | 337 (85.1) |
| Yes | 58 (14.9) | 98.3 | 1 (16.7) | 1.7 | 57 (16.2) | 96.6 | 2 (4.4) | 3.4 | 59 (14.9) |

| Characteristics | CMV | | | | Rubella vírus | | | | Total |
|---|---|---|---|---|---|---|---|---|---|
| | Positive n (%) | Seroprevalence (%) | Negative n (%) | Seroprevalence (%) | Positive n (%) | Seroprevalence (%) | Negative n (%) | Seroprevalence (%) | |
| **Awareness of CMV and Rubella** | | | | | | | | | |
| Does not know anything about the disease | 351 (90.0) | 98.9 | 4 (66.7) | 1.1 | 314 (89.5) | 88.5 | 41 (91.1) | 11.5 | 355 (89.6) |
| Heard speak, but do not know anything about it | 34 (8.7) | 94.4 | 2 (33.3) | 5.6 | 33 (9.4) | 91.7 | 3 (6.7) | 8.3 | 36 (9.1) |
| Know anything about the disease | 5 (1.3) | 100.0 | 0 (00.0) | 0 (00.0) | 4 (1.1) | 80.0 | 1 (2.2) | 20.0 | 5 (1.3) |
| **Pre-natal consultation was performed in all pregnancies** | | | | | | | | | |
| Yes | 381 (97.7) | 98.4 | 6 (100.0) | 1.6 | 342 (97.4) | 88.4 | 45 (100.0) | 11.6 | 387 (97.7) |
| No | 9 (2.3) | 100 | 0 (00.0) | 0 (00.0) | 9 (2.6) | 100.0 | 0 (00.0) | 0 (00.0) | 9 (2.3) |
| **Access to basic sanitation** | | | | | | | | | |
| Yes | 251 (64.4) | 98.4 | 4 (66.7) | 1.6 | 224 (63.8) | 87.8 | 31 (68.9) | 12.2 | 255 (64.4) |
| No | 139 (35.6) | 98.6 | 2 (33.3) | 1.4 | 127 (36.2) | 90.1 | 14 (31.1) | 9.9 | 141 (35.6) |
| **Total** | **390** | | **6** | | **351** | | **45** | | **396** |

More than half of the participants (64.4%) reported having basic sanitation at home and 141 (35.6%) did not have (Table 2). Most of the pregnant women, 355 (89.6%) reported lack of knowledge of the virus diseases under study, 36 (9.1%) had hear of CMV and Rubella but they do not know anything about it and 5 (1.3%) pregnant women reported knowing something about the diseases under study.

The frequency of CMV infection was higher among pregnant women in the first trimester (199; 51.0%), followed by pregnant women in the second (152; 39.0%) and in the third trimester (39; 10.0%). The frequency of CMV infection in relation to the pregnant women age was 5.9% (n = 23) in the group of ≤ 19 years, 28.2% (n = 110) in the group of 20–25 years, 51.2% (n = 202) in the group of 26–35, and 14.1% (n = 55) in the group of 36–47 years (Table 2). The mean age of CMV positivity was 28.0 ± 7.1 (Mean ± SD). Regarding the obstetric history, the frequency of CMV infection was similar in pregnant women regardless of the month of gestation and parity (number of births or miscarriages). Among the pregnant women, 173 (43.7%) had a history of abortion and 223 (56.3 %) never had an miscarriage and there was no child death in the postpartum period (Table 2).

The frequency for Rubella virus was also higher among pregnant women in the first trimester (181; 51.6%) followed by pregnant women in the second trimester (135; 38.4%) and in the third trimester (35;10.0%) (Table 2). The frequence of infection in relation to the age of pregnant women was 5.1% (n = 18) in the group of ≤ 19 years, 27.6% (n = 97) in the group of 20–25 years, 52.7% (n = 185) in the group of 26–35 years and 14.6% (n = 51) in the group of 36–47 years (Table 2). The mean age of rubella virus positivity was 28.6 ± 6.2 (Mean ± SD).

We also study the frequence of hepatitis B and human immunodeficiency virus (HIV) in pregnant women and their association with Rubella and CMV virus infections. (Table 2). In relation to Hepatitis B, 269 pregnant women (67.9%) presented a negative result and 127 (32.1%) a positive result, of which 125 (98.4%) had a positive result to CMV infection (122 with previous exposure and 3 an active primary infection), and 2 (1.6%) presented susceptibility to primary CMV infection (Table 2). Also of the 127 pregnant women positive for hepatitis B, 114 (89.8.%) presented a positive result to Rubella virus (112 had prior exposure to the virus and 2 presented active primary infection), and 13 (28.9 %) were susceptible to primary infection. In relation to HIV, 337 (85.1%) pregnant women had negative serology, and 59 (14.9%) had positive results, of which 58 (98.3%) with previous exposure to CMV, and 1 (6.7 %) with susceptibility to CMV infection (Table 2). On the other hand, of the 59 pregnant women with HIV positive serology, 57 (96.6%) had a positive result to rubella virus (56 with previous exposure to the virus and 1 with active virus infection), and 2 (4.4%) with susceptibility to the infection.

The seroprevalence of Rubella infection in Angolan pregnant women according to independent categorical variables evaluated in this study are summarized in Table 3. In the bivariate logistic regression analysis, the variables of number of births (OR 2.478; CI: 1.144–5.374), history of miscarriages

(OR 2.062; CI: 1.069–4.194), and spontaneous abortions occurred during the study (OR 3.048; CI: 1.135–7.394), were considered an independent predictor of IgG seropositivity against rubella among pregnant women (Table 3). Other analyzed factors such as maternal age, gestacional age, residence, occupation, educational status, awareness of Rubella, access to basic sanitation, hepatitis B, were not associated with seropositivity on surveyed population. The multivariate logistic regression analysis (adjusted to age) confirm a significant increased risk for rubella in women without children (OR 2.673; CI: 1.026–7.007) and suffering spontaneous abortion (OR 3.232; CI: 1.192–7.952).

Table 3
Binomial logistic regression models for the final analysis of risk factors associate for seropositivity of IgG anti-rubella antibodies in 396 pregnant woman in Luanda province, Angola.

| Variable | OR (95% IC) | p-value | OR (95% IC) | p-value |
|---|---|---|---|---|
| | Unadjusted | | Adjusted by age | |
| **Age** | | | | |
| ≤ 25 years old (ref) | | | | |
| 25–29 years old | 0.962 (0.439–2.059) | 0.923 | | |
| ≥ 30 years old | 0.609 (0.286–1.272) | 0.190 | | |
| **Education** | | | | |
| Low (up to elementary school) | 0.619 (0.304–1.196) | 0.167 | 0.615 (0.300–1.197) | 0.166 |
| High (high school or higher education) (ref) | | | | |
| **Employment** | | | | |
| Public administration service | 1.510 (0.704–3.395) | 0.299 | 1.761 (0.792–4.121) | 0.175 |
| Homemakers | 1.033 (0.340–2.863) | 0.951 | 1.102 (0.361–3.079) | 0.856 |
| Student | 1.402 (0.516–3.664) | 0.492 | 1.064 (0.368–3.001) | 0.905 |
| Street vendor, saleslady and restaurant waitress (ref) | | | | |
| **Marital status** | | | | |
| Single (ref) | | | | |
| Married | 1.347 (0.696–2.543) | 0.363 | 1.390 (0.715–2.639) | 0.319 |
| **Gestational age** | | | | |
| 1st trimester (ref) | | | | |
| 2nd and 3rdtrimestre | 1.346 (0.722–2.536) | 0.351 | 1.377 (0.737–2.604) | 0.317 |
| **Number of births** | | | | |
| 0 | 2.478 (1.144–5.374) | 0.0203* | 2.673 (1.026–7.007) | 0.0439* |
| 1 | 1.692 (0.789–3.627) | 0.1724 | 1.694 (0.748–3.825) | 0.2020 |
| 2 or 3 (ref) | | | | |
| **Children at home** | | | | |
| 0 or 1 | 1.818 (0.969–3.404) | 0.0606 | 1.689 (0.840–3.394) | 0.139 |
| 2 or more (ref) | | | | |
| **Spontaneous abortion** | | | | |
| Yes | 3.048 (1.135–7.394) | 0.018* | 3.232 (1.192–7.952) | 0.0139* |
| No (ref) | | | | |
| **History of miscarriages** | | | | |
| Yes (ref) | | | | |
| No | 2.062 (1.069–4.194) | 0.0364* | 2.048 (0.957–4.508) | 0.0676 |
| **Hepatitis B** | | | | |
| Positive | 0.844 (0.413–1.636) | 0.627 | 0.839 (0.410–1.629) | 0.617 |
| Negative (ref) | | | | |
| **Access to basic sanitation** | | | | |
| Yes (ref) | | | | |
| No | 0.892 (0.452–1.697) | 0.735 | 0.867 (0.437–1.653) | 0.672 |

OR: Odds Ratio, CI: Confidence Interval * Statistically significant ($p < 0.05$)

The geospatial distribution of pregnant women seropositive for CMV and Rubella antibodies in Luanda can be observed in Fig. 1. Based on participants' place of residence we observed a marked geographical pattern, with a high incidence density near Lucrecia Paim Maternity. The geographical distribution of pregnant women with and without antibodies to CMV and Rubella was similar, and we observed a statistically significant spatial dependency.

## Discussion

The present study was performed to investigate the seroprevalence of CMV and rubella infection in pregnant women in the northern Angola city of Luanda. We found an overall seroprevalence rate for CMV infection of 98.5% and for Rubella infection about 88.6%. This is the first study conducted in Angola to evaluate the rates of CMV and Rubella infection in pregnant women. In a general way, there is a lack of knowledge about the epidemiology of CMV and Rubella infection in Angola. In this context, we cannot compare our seroprevalence results with others in pregnant women in Angola.

CMV infection is endemic in almost all the world, occurring throughout the year without seasonal variations. The rates of seropositivity in the population vary greatly according to geographical, ethnic and socioeconomic factors. The prevalence of CMV-specific antibodies increases with age and in the less favored socioeconomic groups of developed and developing countries [12, 13]. Other known contributing factors for CMV infection including education, sexual promiscuity, and blood transfusion [14, 15]. Also the contact with children is considered a risk factor, once the young children stands out as sources of CMV infection in pregnant women [16]

Our results of the seroprevalence of CMV antibodies in pregnant women were similar to previous studies in other African countries: 92% in Nigeria [16], 97.5% in Sudan [17], 96.3% in Tunisia [18], 87% in Gambia [19], 88.5% in Ethiopia [20], and 86% in Kenya [21]. Moreover, our data were also similar with results report in other world countries with values between 92.6% and 100%: Iran [22], Palestine [23], China [24], Brazil [25], Turkey [26], Nigeria [27] and Cuba [28]. In contrast, our prevalence was higher than that reported in developed countries: 42.3% in Germany [29], 46.8% in France, 49% in the United Kingdom [30], 54% in Norway [31], 56.3% in Finland [32], 58.7% in France [33], 65.9% in Italy [34], 66% in Japan [35], 62.4% in Poland [12] and 70.0% in the United States [36].

In the present study, all women who were CMV-IgM positive were also seropositive for IgG. In most of these cases there is a need to perform IgG avidity test as an alternative to provide the status of acute infection [37, 38]. However, in our study it was not possible define what proportion of these cases represented primary infection or reactivation because we we didn't have acess to IgG avidity test.

In USA a recent analysis of CMV IgM seroprevalence in women at reproductive age also found that 97.5 % of IgM seropositive women were CMV IgG seropositive [36]. Other study performed in Pakistan, showed that 95.3 % of individuals who were IgM seropositive were equally seropositive for IgG [39]. In countries with a high prevalence of CMV infection, such as Korea and Turkey, IgG avidity testing have shown that none of the women with a proflile of IgM and IgG positive had evidence of a primary infection [26, 37]. As such, we could suggest that the great majority of seropositive cases in our study represent viral recurrent reinfection or reactivation rather than primary infection. The high IgG seropositivity is alarming which calls for the need to screen these women for potential active infections. Further studies on the impact of CMV on poor pregnancy outcomes are highly recommended in the developing countries [40].

The profile of CMV-IgM negative/CMV-IgG positive in pregnant women (96.5%) indicates that the great majority of infections probably occurred during childhood or adolescence. Our results showed that all age groups are equally likely to be infected with CMV, being the prevalence high at all ages (15–47 years old).

Several studies showed that low socioeconomic status was found to predict CMV IgG seropositivity [12]. The majority Angolan population is of low socioeconomic level. Although we did not have data about the socioeconomic level (through the material deprivation index; MDI) of the studied population, it should be pointed out that this study was carried out in a public maternity hospital where the majority of the attendants are women of low family income. Moreover, the majority of population in the city of Luanda reside in highly populated squatters with close contacts which favors transmission of airborne diseases.The womens participating in the present study resides in the urban area and there were no differences in CMV prevalence in relation to a specific area of residence. The high prevalence also could be explained by poor hygienic conditions that can to perpetuate the cycle of CMV transmission in the developing countries [40]. However, in the present study we not found stastically association between basic sanitation and CMV seropositivity.

In the present study, HIV infected women were more likely to be CMV IgG seropositive than HIV negative women. WHO recommends that all the pregnant women should be advise and tested for HIV at the first prenatal visit. There is a need to improve prenatal services in our setting to ensure that all women are counseled and tested for HIV.

CMV is a virus that has a great potential to proliferate in humans for several reasons. The infection is usually subclinical allowing that infected individuals remain active and thus maintain the possibility of transmission to other susceptible individuals. Moreover, the CMV is not eradicated from the host after the primary infection, remaining in the body for the rest of its life [13], and occasionally may be reactivated (endogenous infection). In addition, the host although infected do not acquire immunity and can undergo further infection by different strains of the virus (exogenous reinfection) [41]. Another explanation for the easy spread of CMV is that its excretion may persist for an extended period of time. For example, it is known that in the case of congenital infections, viral excretion by children may occur for years, increasing the likelihood of transmission to other individuals [42, 43]. On the other hand, some studies have observed that closed environments with many children are sites that facilitate the spread of the virus. In all these cases the transmission occurs mainly through contact with urine or saliva of infected children [44]. We analyzed the influence of the contact of pregnat women with

children through the number of births and children at home. The women with children at home had a greater seroprevalence of CMV infection than those without children at home.

An additional finding of our study was that the majority of pregnant women with IgG and IgM positive were in the first trimester of gestation, the period of highest risk to the fetus in case of virus transmission [45]. It is reasonable to hypothesize that some infections would have been avoided had these women been informed at an earlier stage of pregnancy. Ideally, all women should be tested for CMV antibody and informed before pregnancy. Indeed, in a population of women receiving fertility treatment, preconception screening and counseling, seems to have a risk reduced to CMV infection in pregnancy [46]. Moreover, preconception testing would also reduce problems arising from the detection and interpretation of CMV-specific IgM antibody in pregnant woman [47, 48].

CMV stands out as the major cause of congenital infection, reaching rates between 0.2 and 2.6% of the total number of births worldwide, being responsible for cases of neonatal mortality and morbidity [49]. Fetal CMV infection occurs in approximately 40% of cases of maternal primary infection [50]. Therefore, it would be beneficial to inform pregnant women about the need for follow-up to detect prenatal infection and to plan appropriate intervention such as the use of drugs to control infection and / or prevent fetus infection [20].

The epidemiological importance of Rubella virus is related to the Congenital Rubella Syndrome (CRS) that affects the fetus or the newborn due to the infection contracted by the mother during pregnancy. The overall seroprevalence of rubella among pregnant women in the present study was 88.6%. Similar results has been reported from other African countries such as Ethiopia (89%) [51], Senegal (90.1%) [52], Namibia (85.0%) [53] Burkina Faso (95%) [54] and Zimbabwe (92%) [55]. Also in other countries of the world the prevalence is high: 88.1% and 93.5% in Turkey [56, 57]; 87% in United States of America (USA); 98% in Spain; and 96.3% Iran [58, 59]. In contrast, the seroprevalence in this study is higher than reports from Democratic Republic of Congo (58.97%) [60], Sudan (65%) [61] and Nigeria (68%) [62]. These variations might be due to the difference in the endemicity of the virus, the sample size of the studies, and the laboratory methods used.

The profile of IgM and IgG immunoglobulins is important to characterizing infection in a given area [63]. The presence of only IgM or both IgM and IgG antibodies at the same time indicates an acute/recent rubella virus infection. However, the presence of IgG antibody in the absence of IgM is a seromarker of immunity against rubella virus [64]. The absence of both IgM and IgG antibodies indicates susceptibility to acquiring rubella infection.

In the present study, both rubella-specific IgM and IgG antibodies were analyzed among pregnant women to determine acute/recent infections and the levels of immunity against rubella virus infection in Luanda, Angola. Despite the general very high seroprevalence of rubella infection, 11.4 % of the pregnant woman were seronegative. The susceptibility rate in among adult women could result in outbreaks of CRS [51, 65]. Therefore, attention must be paid to the susceptible group of women in this study in order to reduce the risk of CRS in their future pregnancies.

Based on the previous recommendations of the US National Committee for Clinical Laboratory Standards (NCCLS) [66] and international agreements and guidelines [67], in the absence of IgM, the pregnant women who had rubella IgG levels $\geq$ 10 IU/ml were classified as immune and those with IgG levels < 10 IU/ml were classified as susceptible. In the present study, 87.6% of the pregnant women had IgG levels of > 10 IU/ml (Tabela 1). None of these pregnant women had a previous history of rubella vaccination and they were immune from rubella infections. This might be due to the endemicity of the virus in the study area that sustained previous infections of the participants before conception or during their childhood, as rubella infection is common among children and teenagers in some countries [51, 68].

The prevalence of rubella IgG (87.6%) in this study was greater than reported in Burkina Faso (77%) [54], in Niger (53%) [69] and southern India (65%) [70]. However, the IgG positivity rate was lower than that found in studies conducted in other countries like Nigeria (97.9%) [71], Cameroon (88.6%) [72], Turkey (96.1%) [73], Italy (85.8%) [74] and Mexico (97.1%) [75]. These variations in rubella IgG positivity in different countries might be due to the difference in the endemicity of the rubella virus and the presence or absence of rubella vaccination in their immunization programs.

In the present study, 1.0 % of the total pregnant women had both rubella IgM and IgG antibodies. The rubella virus re-infection following natural immunity is very rare [51]. Therefore, the pregnant women might be in stages of primary rubella infection. Since these pregnant women were in the first and second trimester of pregnancy, they might have acquired the infection during the pregnancy and subsequently developed IgG antibodies within 30 days of infection [76]. This indicates that these pregnant women might not be immune before pregnancies and the fetuses can not be excluded from rubella-associated risks.

Although there are no data of CRS in Angola, the newborns from women infected with rubella during early pregnancy might acquire a congenital rubella infection and be born with rubella-associated congenital anomalies or CRS. Therefore, the screening of women of child-bearing age before conception or during pregnancy might be crucial to reduce the consequences of acute rubella infection during pregnancy [51].

In the multivariate logistic regression analysis, a statistically significant association was found between rubella IgG positivity with spontaneous abortions during the study; all pregnant women who had a spontaneus abortions were seropositive for anti-rubella-IgG. The rubella was considered as an etiologic agent for miscarriages in many countries [57, 77]. Therefore, more attention should placed on those pregnant women who had recent or acute infections due to the teratogenic nature of the virus [78]. Moreover, pregnant women who have a previous bad obstetric history may be more vulnerable to acquiring acute rubella infections [79]. Although the mechanism is not clear and further studies are needed, a similar finding has also been reported in other studies [77, 80].

No statistically significant difference was found between anti-Rubella antibodies positivity and socio-demographic characteristics of the pregnant women. A similar finding was also reported in Namibia [53], Southern Ethiopia [81], and Nigeria [82].

All the pregnant participants in the present study live in urban settings. The high population density in urban areas might increase the contact rate and pregnant women without protective levels of rubella immunity might acquire the infections [51]. A finding was reported in the pre-vaccine era in other countries [83, 84, 85].

To reduce the circulation of the Rubella virus, vaccination is essential and is the only way to prevent the disease [57, 86]. Rubella is commonly mistaken for other diseases because symptoms such as sore throats and headaches are common to other infections, making it difficult to diagnose [57]. Although not serious, rubella is particularly dangerous in the congenital form. In this case, it may leave irreversible sequels in the fetus as glaucoma, cataract, cardiac malformation, delayed growth, deafness and others. Therefore, prevention should be focused [86].

The WHO [65] suggests the following strategies for the prevention of Rubella: (i) Provide right to protection to school-age women and / or girls (ii) Vaccinate to provide indirect protection by reducing the transmission of rubella virus infection (iii) a combination of these approaches. The rubella vaccine was included in the Angolan national vaccination plan in April 2018 an initial stage only covered children up to 14 years of age [87].

The prevalence of CMV and Rubella infection can be attributed to low socioeconomic status and poor hygienic. Currently, about 36% of the population lives below the poverty line and with limited access to basic public services (water, sanitation, energy, health, education and housing). In the education sector, Angola is considered by UNESCO as a low educational development index country, ranking 111th out of 120 countries in the UNESCO Education for All 2012, with a value of 0.685 and a gender parity index of 0.734 [88].

There is no way to know how many cases of CMV and Rubella are identified each year in Angola. Thus, there aren´t effective intervention to control CMV and Rubella infection in the country. In the case of CMV, preventive measures including changes in hygiene behavior of seronegative pregnant women should be implemmented as well as routine maternal screening for primary infection. Moreover, treatment with hyperimmune human immunoglobulin and the administration of aciclovir or its derivative valaciclovir should be considered once do not have teratogenic side effects when administered in the early stages of pregnancy [89, 90].

## Conclusion

Overall, this study showed that there is a high seroprevalence of anti-CMV and anti -Rubella antibodies in pregnant women in Luanda. Therefore, it is important improve rapid and accurate diagnosis of CMV and Rubella infection in pregnant women to prevent major complications such as congenital infections. It would be also important to implement national screening on CMV, rubella, and other diseases linked to maternal and child health. This study showed that although most pregnant women were immune to rubella in Luanda, a considerable number are still not immune. Therefore, rubella vaccination should be offered to women with rubella-specific susceptibility to prevent further complications such as congenital infections, reduce the incidence of birth defects, and preserve maternal and child health.

## Abbreviations

**95% CI** 95% Confidence Intervals

**COI** Cut-off índex

**CRS** Congenital Rubella Syndrome

**ECL** Electrochemiluminescence

**HIV** human immunodeficiency virus

**LPMH** Lucrécia Paim Maternity Hospital

**MDI** material deprivation index

**NCCLS** National Committee for Clinical Laboratory Standards

**OR** Odds Ratio

**SPSS** Statistical Package for the Social Sciences

**TORCH** Toxoplasmosis, Other (syphilis, varicella-zoster, parvovirus B19), Rubella, Cytomegalovirus and Herpes

**UNESCO** United Nations Educational, Scientific and Cultural Organization

**WHO** World Health Organization

## Declarations

### Ethics approval and consent to participate

The present study has been approved by the Research Ethics Committee of Lucrécia Paim Maternity Hospital (LPMH) through the National Institute of Public Health of the Republic of Angola (nº 301019; S1 File). Participating individuals provided a written signed informed consent prior to sample collection and for participants younger than 18 years, informed consent was provided by parents or guardians after a detailed explanation of the objectives of the work.

### Consent to publish

Not Applicable'

### Availability of data and materials

Authors declare that all data are fully available without restriction.

### Competing Interests

The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

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### Authors' contributions

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## Figures

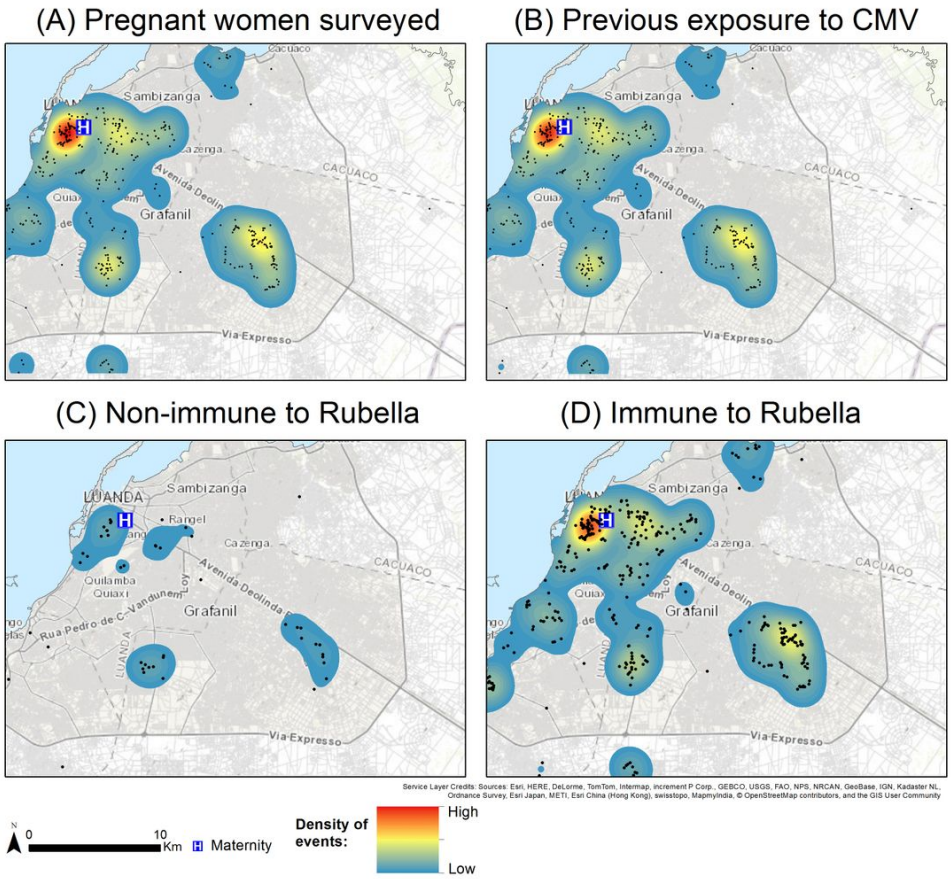


**Figure 1**

Geographical distribution and Gaussian kernel density surface map of pregnant women (A) with anti-CMV antibodies (B), non-immune to Rubella virus (C) and immune to Rubella virus (D) in Luanda, Angola. Note: The designations employed and the presentation of the material on this map do not imply the expression of any opinion whatsoever on the part of Research Square concerning the legal status of any country, territory, city or area or of its authorities, or concerning the delimitation of its frontiers or boundaries. This map has been provided by the authors.

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