



## RESEARCH ARTICLE

# HIV, hepatitis B virus, hepatitis C virus, and syphilis among pregnant women attending antenatal care in Luanda, Angola: Seroprevalence and risk factors

Cruz S. Sebastião<sup>1,2,3,4</sup> | Zoraima Neto<sup>1</sup> | Domingos Jandondo<sup>1</sup> |  
Marinela Mirandela<sup>1</sup> | Joana Morais<sup>1,2,5</sup> | Miguel Brito<sup>2,6</sup> 

<sup>1</sup>Molecular Biology Laboratory, Instituto Nacional de Investigação em Saúde, Luanda, Angola

<sup>2</sup>Department of Laboratory, Centro de Investigação em Saúde de Angola, Luanda, Angola

<sup>3</sup>Instituto Superior de Ciências da Saúde, Universidade Agostinho Neto, Luanda, Angola

<sup>4</sup>Laboratory of Immunobiology and Pathogenesis of CEDOC, NOVA Medical School, Faculdade de Ciências Médicas, Universidade NOVA de Lisboa, Lisboa, Portugal

<sup>5</sup>Faculdade de Medicina, Universidade Agostinho Neto, Luanda, Angola

<sup>6</sup>Health and Technology Research Center, Escola Superior de Tecnologia da Saúde de Lisboa, Instituto Politécnico de Lisboa, Lisboa, Portugal

## Correspondence

Miguel Brito, Health and Technology Research Center, Escola Superior de Tecnologia da Saúde de Lisboa, Instituto Politécnico de Lisboa, Av. D. João II, Lote 4.69.01, 1990-096 Lisboa, Portugal.  
Email: [miguel.brito@estesl.ipl.pt](mailto:miguel.brito@estesl.ipl.pt)

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## Abstract

Infectious diseases during pregnancy remain a public health concern, especially in a resource-limited setting. The study aimed to determine the seroprevalence and determinants of HIV and co-infection with hepatitis B virus (HBV), hepatitis C virus (HCV), and syphilis among pregnant women attending antenatal care in Luanda, the capital city of Angola. A cross-sectional study was conducted with 1612 pregnant women screened for HIV during antenatal care. HIV-reactive were also screened for the HBV, HCV, and syphilis using immunoassay kits. A logistic regression model, adjusted odds ratios (AOR) and their 95% confidence interval (CI) were calculated with a level of significance set at 5%. The overall seroprevalence of HIV was 2.6%. About 13% of HIV-positive pregnant women were coinfecting. From which, 7.5% were reactive to HBV and 5% to syphilis. There was no reactivity to HCV. Pregnant women younger aged than 25 years were significantly protected from HIV-infection (AOR, 0.43 [95% CI, 0.20-0.91],  $P = .026$ ). The co-infection was 1.3 times (AOR, 0.04-41.0) in younger aged than 25 years, 7.0 times (AOR, 0.50-99.2) to residents in urbanized areas, and 1.4 times (AOR, 0.10-20.9) in pregnant women with a high educational level. In conclusion, infectious diseases are a public health burden among pregnant women in Luanda. However, include an integrated antenatal screening mainly in urbanized areas is crucial to reduce the spread of infectious diseases in different communities of Angola.

## KEYWORDS

Angola, hepatitis B virus, hepatitis C virus, HIV infection, Luanda, pregnant women, syphilis

## 1 | INTRODUCTION

Infectious diseases during pregnancy remain a public health concern especially in low- and middle-income countries (LMIC).<sup>1</sup> Among the infections with importance in the morbidity and mortality rates, the human immunodeficiency virus (HIV), hepatitis B virus (HBV), hepatitis C virus (HCV), and syphilis can be highlighted. Globally, an estimated 37.9 million people were living with HIV infection worldwide,<sup>2</sup> 257 million and 71 million people with HBV and HCV infection,

respectively,<sup>3</sup> and 6 million new cases of syphilis are detected each year.<sup>4</sup> In a resource-limited setting, the screening of these infections during pregnancy may reduce the risk of vertical transmission to less than 5%, however, without intervention, the vertical transmission can be about 15% to 45%.<sup>5</sup> A previous study conducted in Luanda showed that the prevalence of HIV, HBV, and syphilis among pregnant women is 4.5%, 8.1%, and 5.4%, respectively.<sup>6</sup>

Infectious diseases during pregnancy play a significant role in maternal mortality rates and increase the risk of mortality among

neonates during the first weeks of life. However, understanding the factors related to infectious diseases would be beneficial for any program to prevent the spread of infections. Previous studies have reported that the lack of formal education, inadequate access to health care and poverty increase the risk of spread infectious diseases mainly in LMICs.<sup>7</sup> Limited information regarding determinants of the spread of infectious diseases is available among pregnant women from Luanda. Thus, the current study was conducted to estimate the seroprevalence and determinants related to infectious diseases in pregnant women attending antenatal care in Luanda, the capital city of Angola. This seroepidemiological study is critical in understanding and to strengthen the control of infectious diseases in the different communities of Angola.

## 2 | MATERIALS AND METHODS

### 2.1 | Study design and setting

The current study was part of a cross-sectional study carried out with 1612 pregnant women at all stages of pregnancy attending antenatal care at the Lucrecia Paim Maternity hospital during the months of April to June 2018, in Luanda, the capital city of Angola. This is a public hospital and reference center which provides health care for pregnant women and newborns from all provinces of Angola. The hospital team registered and codified of participant's identities and providing follow up care. The research team used a structured closed-ended questionnaire to obtain information regarding personal sociodemographic characteristics (age, local of residence, level of education, and occupation).

### 2.2 | Sample collection, molecular, and serological testing

The capillary puncture was performed and an estimated volume of 50  $\mu$ L of the blood samples was collected with capillary tubes in each participant. An anti-HIV rapid test was performed at the health facility using the Determine HIV 1/2 Rapid Test (Alere, Japan). The rapid HIV test was considered positive when two lines of any intensity appeared in the control and patient areas, while tests with only one line in the control area and no lines in the patient area were considered negative. All tests that did not reveal any lines or did not reveal the line in the control area were considered invalid and the test was repeated with a new sample. The HIV-reactive pregnant women were retested with the rapid HIV test Unigold HIV (Trinity Biotech, Ireland), according to the national guidelines for HIV testing in Angola.<sup>8</sup> Pregnant women were considered HIV-positive when they presented independent positive results for both Determine and Unigold rapid HIV tests. There were no discordant results. A volume of 5 mL of intravenous whole blood was collected in a tube containing ethylenediaminetetraacetic acid (EDTA) in all HIV-positive pregnant women. The tubes containing the blood samples were centrifuged,

the plasma was aliquoted and stored at  $-80^{\circ}\text{C}$  until further analysis. Plasma samples were thawed and HIV ribonucleic acid (RNA) was manually extracted from 140  $\mu$ L of plasma with the QIAamp Viral RNA kit (QIAGEN, Germany), according to the instructions provided by the manufacturer. The HIV-infection was further confirmed by nested polymerase chain reaction (PCR) using the protocol described previously.<sup>9</sup> Commercially available immunoassay rapid test kits were used to screen the presence of the HBV (Rapid Labs, UK), HCV (Rapid Labs), and Syphilis (Labmann). Briefly, a volume of 50  $\mu$ L of plasma sample was used for each rapid test targeting the HBV, HCV, and Syphilis. Following the manufacturer's instructions, the rapid tests were considered positive when two lines appeared in the control and patient areas, while tests with only one line in the control area were considered negative. All tests that did not reveal any lines or did not reveal the line in the control area have been invalidated and the test repeated. No external controls (known as positive and negative) were included. The laboratory procedures, molecular and serological testing were performed at the molecular biology laboratory of the Instituto Nacional de Investigação em Saúde (INIS), in Luanda, Angola.

### 2.3 | Statistical analysis

The data were coded, entered, and analyzed in the Statistical Package for the Social Sciences (SPSS) version 25 (IBM SPSS Statistics). The frequencies and percentages were part of the descriptive analysis. Mean and the standard deviation (SD) were recorded to the data normally distributed. All variables were categorized and dichotomized to check potential interactions on the occurrence of infection. A univariate and multivariate logistic regression model was performed with all independent variables. The goodness of fit was based on the Hosmer-Lemeshow test. Odds ratio (OR) and their 95% confidence intervals (CIs) were calculated. The reported p-value are two-tailed and deemed statistically significant when  $P < .05$ .

### 2.4 | Ethical considerations

HIV test is offered in all pregnant women as part of the routine tests in antenatal screening public health programs. Ethical approval for this study was obtained from the Ethics Committee of Angola (reference number 13/2018) and the general directorate from Lucrecia Paim Maternity (reference number 083/GDG/MLP/2018). Moreover, verbal and written consent was secured from each pregnant woman, their parent or legal guardian after the objectives of the study were explained to them. The information from the participants and the clinical sample collected was used only for the stated objectives and kept confidential. Consent to participate was secured from all participants, their parent or legal guardian before being enrolled in the study, and agreed with the publication of the findings.

### 3 | RESULTS

#### 3.1 | Sociodemographic characteristics

A total of 1612 pregnant women took part in this study. The general background of pregnant women is displayed in Table 1. Age ranged from 12 to 45 years. The mean age was  $27 \pm 7$  years old. A greater number of the pregnant women (61.8%) were in the age group  $\geq 25$  years, living in a rural area (60.1%), with a high educational level (57.3%), and employed (54.5%). There were 102 (6.3%) pregnant women in their first trimester of gestational, 176 (10.9%) in their second trimester, and 1334 (82.8%) in their third trimester.

#### 3.2 | Seroprevalence and determinants of HIV and co-infection

Forty-two pregnant women out of 1612 enrolled in this study were reactive against HIV antibodies, which represented an overall seroprevalence of 2.6%. Although no significant outcome was observed, multivariate analysis showed marginally increase in likelihood of contracting HIV infection in pregnant women residents in an urbanized area (AOR, 1.41 [95% CI, 0.76-2.60],  $P = .276$ ), with a high educational level (AOR, 1.01 [95% CI, 0.53-1.95],  $P = .969$ ), and employed (AOR, 1.03 [95% CI, 0.54-1.97],  $P = .933$ ). On the other hand, both univariate and multivariate analyses showed a significant protective factor from HIV-infection in pregnant women younger aged than 25 years ( $P < .05$ ) (Table 1).

The screened and respective determinants of co-infection were evaluated in 40/42 HIV-positive pregnant women. Due to the lack of screening kits, 2/42 HIV-positive pregnant women were not tested.

Overall, 5/40 (12.5%) HIV-positive pregnant women were coinfecting. From which, 3/40 (7.5%) were reactive to HBV and 2/40 (5%) were reactive to syphilis. None of these screened HIV-positive pregnant women were reactive to HCV and there was no triple infection. There was no significant outcome regarding sociodemographic characteristics and co-infection, but the likelihood to be coinfecting in pregnant women younger aged than 25 years was 1.3 times (AOR, 0.04-41.0), residents in urbanized areas were 7.0 times (AOR, 0.50-99.2), and with a high educational level was 1.4 times (AOR, 0.10-20.9) (Table 2).

### 4 | DISCUSSION

HIV testing in pregnant women is a good indicator of the epidemic in the general population and has been used to predict the prevalence of young children.<sup>10</sup> Since the first case of HIV infection was reported in 1985 in Angola, the infection has spread rapidly to the general population from urbanized to rural areas.<sup>11</sup> Although there are studies with a comparatively high seroprevalence in pregnant women, as in Ethiopia (5.5%),<sup>12</sup> Tanzania (5.6%),<sup>13</sup> and Cameroon (6%),<sup>14</sup> the HIV prevalence in this study was slightly lower. In contrast, it is higher than reports in pregnant women from India (0.88%),<sup>15</sup> and Brazil (0.09%).<sup>16</sup> The variation in HIV prevalence across studies might be attributed to the socio-cultural differences, sexual behavior, and surveillance methodologies during antenatal care. Although our findings do not represent the general population from Luanda, it may partly reflect the effect of effective awareness national programs on the control of HIV infection in Luanda imposed in health services since 2004.<sup>11</sup>

Evidence supported that infectious diseases are associated with income since in LMICs there is less access to preventive information

**TABLE 1** Seroprevalence and determinants of HIV infection among pregnant women attending antenatal care in Luanda, Angola, 2018

Characteristics	(%)	HIV prevalence		Univariate analysis		Multivariate analysis <sup>a</sup>	
		Negative (%)	Positive (%)	OR (95% CI)	P-value	AOR (95% CI)	P-value
Overall	1612 (100)	1570 (97.4)	42 (2.6)				
Age groups							
<25 y	615 (38.2)	606 (98.5)	9 (1.5)	0.43 (0.21-0.91)	.028*	0.43 (0.20-0.91)	.026*
$\geq 25$ y	997 (61.8)	964 (96.7)	33 (3.3)	1.00	...	1.00	...
Place of residence							
Rural	969 (60.1)	947 (97.7)	22 (2.3)	1.00	...	1.00	...
Urban	643 (39.9)	623 (96.9)	20 (3.1)	1.38 (0.75-2.55)	.302	1.41 (0.76 - 2.60)	.276
Education							
Low	688 (42.7)	670 (97.4)	18 (2.6)	1.00	...	1.00	...
High	924 (57.3)	900 (97.4)	24 (2.6)	0.99 (0.53-1.84)	.981	1.01 (0.53-1.95)	.969
Occupation							
Unemployed	733 (45.5)	714 (97.4)	19 (2.6)	1.00	...	1.00	...
Employed	879 (54.5)	856 (97.4)	23 (2.6)	1.01 (0.55-1.87)	.975	1.03 (0.54-1.97)	.933

Abbreviations: AOR, adjusted odds ratio; CI, confidence interval; OR, odds ratio.

<sup>a</sup>Adjusted for all the independent variables listed.

\*Variables were significant ( $P < .05$ ).

**TABLE 2** Determinants of co-infection among HIV-infected pregnant women attending antenatal care in Luanda, Angola, 2018 (N = 40)

Characteristics	HBV		Syphilis		Univariate analysis		Multivariate analysis <sup>a</sup>	
	No (%)	Yes (%)	No (%)	Yes (%)	OR (95% CI)	P-value	AOR (95% CI)	P-value
Overall	37 (92.5)	3 (7.5)	38 (95.0)	2 (5.0)				
Age groups								
<25 y	7 (87.5)	1 (12.5)	8 (100)	0 (0.0)	1.00 (0.96-0.4)	1.000	1.33 (0.04-41.0)	.868
≥25 y	30 (93.8)	2 (6.3)	30 (93.8)	2 (6.3)	1.00	...	1.00	...
Place of residence								
Rural	19 (95.0)	1 (5.0)	20 (100)	0 (0.0)	1.00	...	1.00	...
Urban	18 (90.0)	2 (10.0)	18 (90.0)	2 (10.0)	4.75 (0.48-46.9)	.182	7.01 (0.50-99.2)	.150
Education								
Low	15 (88.2)	2 (11.8)	16 (94.1)	1 (5.9)	1.00	...	1.00	...
High	22 (95.7)	1 (4.3)	22 (95.7)	1 (4.3)	0.44 (0.07-3.01)	.406	1.44 (0.10-20.9)	.790
Occupation								
Unemployed	15 (83.3)	3 (16.7)	16 (88.9)	2 (11.1)	1.00	...	1.00	...
Employed	22 (100)	0 (0.0)	22 (100)	0 (0.0)	0.0 (0.0-0.0)	.998	0.0 (0.0-0.0)	.998

Abbreviations: AOR, adjusted odds ratio; CI, confidence interval; HBV, hepatitis B virus; OR, odds ratio.

<sup>a</sup>Adjusted for all the independent variables listed.

and healthcare.<sup>1</sup> Moreover, the inequality of resource distribution in the population unemployed and with low educational levels lead to risky sexual behavior and an increase in infectious diseases. In our multivariate analysis, there is evidence that the overall HIV prevalence in pregnant women from Luanda is related to advanced aged, local of residence, educational levels, and occupation. Moreover, favorable results to HIV infection were observed in pregnant women younger aged than 25 years (AOR, 0.43,  $P = .026$ ) (Table 1). In contrast, pregnant women older-aged than 24 years from Cameroon,<sup>17</sup> South Africa,<sup>18</sup> and Rwanda,<sup>19</sup> were more likely to be infected with HIV. Increased HIV prevalence in adult women may have been exacerbated by low education, low access to health care, and unemployment. We found that pregnant women resident in urbanized areas, with a low educational level, and unemployed are less likely to visit an antenatal hospital, making these a vulnerable group for infectious diseases (Table 1). Consistent with our results, high HIV prevalence was reported in pregnant women from urbanized areas in the Democratic Republic of the Congo,<sup>20</sup> and Tanzania,<sup>13</sup> but different results were reported in Rwanda,<sup>19</sup> Cameroon,<sup>17</sup> and Ethiopia.<sup>21</sup> In contrast with our results, low HIV prevalence was observed in pregnant women with a high educational level in the USA,<sup>22</sup> and China.<sup>23</sup>

The high prevalence of HIV/HBV co-infection observed is not surprising since the HIV/HBV co-infection in sub-Saharan Africa is estimated at 6% to 25%.<sup>24</sup> However, a high HIV/HBV co-infection in pregnant women suggests a potential source for the spread of viral infections in Luanda. Our findings are consistent with those reported in HIV-positive pregnant women from Cameroon (7.7%),<sup>25</sup> higher than to reported in Nigeria (0.5%),<sup>26</sup> Botswana (3.1%),<sup>27</sup> Rwanda (4.1%),<sup>19</sup> and Sudan (5.6%),<sup>28</sup> but is lower than that reported in Ethiopia (12.1%),<sup>12</sup> and Ghana (14.9%).<sup>29</sup> The proportion of HIV/HCV co-infection observed in our cohort was compared to reported in

southern (3.3%) and north (42.3%) of sub-Saharan Africa,<sup>30</sup> and European countries (12.3%).<sup>31</sup> Most of the time, high HIV/HBV co-infection is attributed to the fact they share mutual routes of transmission. The low prevalence of HCV in our study may be explained by the fact these infections were evaluated exclusively in HIV-infected pregnant women or by the effectiveness of serological rapid diagnostic tests. On the other hand, the prevalence of syphilis was high to that reported in HIV-positive pregnant women from Uganda (0.52%)<sup>32</sup> and Tanzania (0.9%).<sup>33</sup>

Although there was no evidence to suggest a significant association, it is worth noting that most cases of co-infections were observed in pregnant women older-aged than 24 years, from urbanized areas, with low educational and unemployed (Table 2). The high concentration of key populations such as sex workers, injecting drug users and men who have sex with men in urbanized settings may explain the high risk of the spread of infectious diseases in urbanized areas compared to the rural areas. Our findings suggest that efforts to prevent the spread of infectious diseases should begin in urbanized areas and expand into rural areas. Additionally, HBV vaccination strategies and screen of syphilis should be widely applied regardless of HIV prevention strategies.

This study has some potential limitations. First, the study was cross-sectional which limited the ability to examine causal relationships and not entirely represent the whole population in Luanda or pregnant women in other regions from Angola. Besides that, out of forty-two HIV-positive pregnant women, two were not screened for co-infection due to the lack of serologic tests at the time of the research, and the lower number of coinfecting pregnant women limit a robust analysis. Second, HBV, HCV, and syphilis infection have been evaluated only in HIV-positive pregnant women. Third, a possible underestimation of the prevalence of the

evaluated markers should be considered since rapid diagnostic tests less sensitive than enzyme-linked immunosorbent assay (ELISA) or PCR were used to screen HBV, HCV, and syphilis. Fourth, HIV-RNA viral load and detection of other important markers of HBV, HCV, and syphilis were not determined because of the lack of laboratory setup. Despite these shortcomings, our findings provide important data on the epidemiology of infectious diseases in pregnant women from Luanda. Thus, further studies using ELISA or PCR tests for screening infectious diseases should be conducted in the larger population of different groups and communities of Angola. Besides, further studies on demographic and behavioral risk factors (eg, clinical/medical related factors) that influence the emergence and spread of infectious diseases in pregnant women should be carried out in Angola.

In summary, this study showed that HIV, HBV, and syphilis infections are important public health issues in pregnant women from Luanda that need to be addressed, while HCV infection does not seem to be the public health burden particularly among women living with HIV infection. Older-aged, urbanized areas, low educational levels, and unemployed were vulnerable groups. Our findings suggest that is crucial an integrated screened during antenatal care to avoid maternal transmission and the eventual adverse effects on neonates in Luanda. Moreover, health education about the routes of transmission and prevention should be given to reduce the spread of infectious diseases, particularly in pregnant women from urbanized settings.

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#### CONFLICT OF INTERESTS

The authors declare that there are no conflict of interests.

#### AUTHOR CONTRIBUTIONS

CSS, JM, and MB had full access to all of the data in the study. CSS, JM, and MB take responsibility for the study concept, design, integrity of the data, and the accuracy of the data analysis. CSS performed serological and molecular screening. CSS, DJ, and MM contributed to mobilization, data collection, and technical support. JM, ZN, and MB contributed to administrative support, supervised data collection, and fieldwork. CSS drafted the original draft. CSS,

MB, and JM were responsible for statistical analysis and interpretation. CSS, MB, JM, and ZN conducted a critical revision of the manuscript for intellectual content. All authors read and approved the final manuscript for publication.

#### ORCID

Miguel Brito  <http://orcid.org/0000-0001-6394-658X>

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