





## RESEARCH ARTICLE

# Coinfection between SARS-CoV-2 and vector-borne diseases in Luanda, Angola

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## Abstract

Co-epidemics happening simultaneously can generate a burden on healthcare systems. The co-occurrence of SARS-CoV-2 with vector-borne diseases (VBD), such as malaria and dengue in resource-limited settings represents an additional challenge to the healthcare systems. Herein, we assessed the coinfection rate between SARS-CoV-2 and VBD to highlight the need to carry out an accurate diagnosis and promote timely measures for these infections in Luanda, the capital city of Angola. This was a cross-sectional study conducted with 105 subjects tested for the SARS-CoV-2 and VBD with a rapid detection test in April 2021. The participants tested positive for SARS-CoV-2 (3.80%), malaria (13.3%), and dengue (27.6%). Low odds related to testing positivity to SARS-CoV-2 or VBD were observed in participants above or equal to 40 years (odds ratio [OR]: 0.60,  $p = 0.536$ ), while higher odds were observed in male (OR: 1.44,  $p = 0.392$ ) and urbanized areas (OR: 3.78,  $p = 0.223$ ). The overall co-infection rate between SARS-CoV-2 and VBD was 11.4%. Our findings showed a coinfection between SARS-CoV-2 with malaria and dengue, which could indicate the need to integrate the screening for VBD in the SARS-CoV-2 testing algorithm and the adjustment of treatment protocols. Further studies are warranted to better elucidate the relationship between COVID-19 and VBD in Angola.

## KEYWORDS

Angola, COVID-19, malaria, dengue, coinfection, Luanda, SARS-CoV-2, vector-borne diseases

## 1 | INTRODUCTION

The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) that causes coronavirus disease 2019 (COVID-19), has become an infectious agent with a high impact on public health, causing one of the biggest outbreaks of the century.<sup>1</sup> In addition, previous studies suggest that COVID-19 caused disruptions in the health system.<sup>2</sup> and is potentially associated with a long-term burden.<sup>3</sup> The first cases of SARS-CoV-2 infection were identified in December 2019 in China and quickly spread all over the world.<sup>4</sup> Until the end of April 2021, more than 19.3 million confirmed cases and 4.1 million deaths

have been linked to the infection worldwide since the first cases emerged.<sup>5</sup> During the same period, the Angolan health authorities recorded more than 42 000 cases and 990 deaths related to the infection.<sup>5</sup>

The co-circulation of SARS-CoV-2 with vector-borne diseases (VBD) such as malaria, dengue, Zika, and chikungunya, is a big concern, as it imposes an extra burden on health systems and constitutes a major challenge from an epidemiological point of view, mainly in low- and middle-income countries (LMICs).<sup>6-9</sup> On the other hand, the similarity of symptoms in individuals infected with SARS-CoV-2 or VBD, coinfection, and cross-reaction with Flavivirus could worsen

the infectious disease management scenario, especially in tropical regions that are more affected by VBD.<sup>10–13</sup>

Although cases of malaria, dengue, Zika, and chikungunya are frequently identified in Angola,<sup>14,15</sup> with the most recent dengue fever outbreak in 2018,<sup>16</sup> there is no data on the relationship between SARS-CoV-2 and these endemic infectious diseases with active circulation in Angola. Therefore, in this study, we evaluated the coinfection rate between SARS-CoV-2 and VBD to highlight the need for integrated VBD screening with SARS-CoV-2 in Luanda, the capital city, and epicenter of the COVID-19 pandemic in Angola, to expedite the diagnosis and appropriate clinical management of the coinfecting patients.

## 2 | MATERIAL AND METHODS

### 2.1 | Study design and setting

This was a cross-sectional study performed with 105 tested for the SARS-CoV-2, malaria, and dengue in April 2021 at Instituto Nacional de Investigação em Saúde (INIS), located in Luanda. The INIS is an Angolan institute of scientific research whose main objective is to generate, develop and disseminate scientific, technological, and strategic knowledge about health and its determinants (<http://www.inis.ao/index.php/institucional/o-instituto>). The study protocol received ethical approval from the National Ethics Committee of the Ministry of Health of Angola (nr.25/2020). Even so, after clarifying the project to the participants, we request free and unwritten oral authorization from participants or their legal guardians for minors under 18 before being enrolled in the study, which was registered in the data collection questionnaire of each participant. All the information obtained was fully anonymized, used only for this project, and kept confidential.

### 2.2 | Data, sample collection, and laboratory procedure

The sociodemographic characteristics such as age, gender, and residence area of participants were collected by the research team using a structured questionnaire developed as part of this study. The questionnaire was not validated before testing on study participants. Additionally, an estimated volume of 5 ml of intravenous whole blood was collected from each participant in a tube containing ethylenediaminetetraacetic acid (EDTA). The collected samples were subjected to rapid detection tests (RDT) targeting the qualitative detection of SARS-CoV-2 (COVID-19 IgG/IgM Rapid Test Device; Abbott), *Plasmodium* (Malaria Ag P.f/P.v.; MT PromedT), and dengue virus (DENV) (SD Bioline Dengue IgG/IgM), following the instructions provided by the manufacturers. According to the manufacturer's instructions, the tests were considered positive when the two lines of any intensity appeared in the patient's control and test areas. On the other hand, tests that presented only one line in the control area and no lines in the patient area were considered negative. All invalid

results were re-tested. Participants who tested positive for immunoglobulin G (IgG) or immunoglobulin (IgM) antibodies were coded as positive, whereas participants who tested negative for either IgG or IgM were coded as negative. No external samples known to be positive for SARS-CoV-2, malaria, or dengue, were used as an external control for the rapid diagnostic kits used in this study. In addition, positive samples for dengue were subjected to molecular screening. Briefly, total viral ribonucleic acid (RNA) was manually extracted from 140 µl using the QIAamp Viral RNA kit (QIAGEN) and stored at  $-80^{\circ}\text{C}$  until further analysis. After that, the presence of DENV RNA was screened using real-time reverse-transcription polymerase chain reaction (RT-PCR) with the Applied Biosystems 7500 Fast RT-PCR System (Thermo Fisher Scientific), using the Centers for Disease Control and Prevention (CDC) Triplex real-time RT-PCR assay was used.<sup>17,18</sup> The RT-PCR was carried out using 10 µl of the RNA in a final reaction volume of 25 µl containing primers and probes targeting qualitative detection of the DENV, Zika virus (ZIKV), and chikungunya virus (CHIKV). Positive and negative control samples were included. The RT-PCR results were considered valid when positive control samples showed a cycle threshold (CT) value below 31. Therefore, specimens with CT values below 31 were considered positive while specimens with CT values equal to or above 31 were considered negative.

### 2.3 | Statistical analysis

The data were analyzed in SPSS v26 (IBM SPSS Statistics). Frequencies and percentages were presented as descriptive analyses. Mean and the standard deviation (SD) were presented to the normal distribution data.  $\chi^2$  test and logistic regression were applied to check interactions between dichotomized variables. The odds ratio (OR) with 95% confidence intervals (CIs) was also calculated. The reported *p*-values are two-tailed and deemed significant when  $p < 0.05$ .

## 3 | RESULTS

### 3.1 | Sociodemographic characteristics related to SARS-CoV-2 and vector-borne diseases

The putative sociodemographic characteristics related to SARS-CoV-2 and VBD in Luanda, are summarized in Table 1. This study included a total of 105 subjects screened for the presence of SARS-CoV-2 and VBD, such as malaria and dengue with RDT during April 2021. Age ranged from 0 to 60 years. The mean age was  $25.5 \pm 14.2$  years old. Participants under 40 years old (84.1%, 74/105), female (62.9%, 66/105), and living in peri-urbanized areas (92.4%, 97/105), were predominant in this study sample. Laboratory screening showed that 3.8% (4/105), 13.3% (14/105), and 27.6% (29/105) of participants tested positive for COVID-19 (only IgG positive), malaria (all positive to Pf and 14.3% (2/14) were Pf/Pv positive), and dengue (about 72.4% (21/29)

**TABLE 1** Sociodemographic characteristics related to SARS-CoV-2 and vector-borne diseases in Luanda, Angola

Characteristics	N (%)	SARS-CoV-2		Malaria		DENV		Univariate analysis	
		No (%)	Yes (%)	No (%)	Yes (%)	No (%)	Yes (%)	OR (95% CI)	p value <sup>a</sup>
Overall	105 (100)	101 (96.2)	4 (3.80)	91 (86.7)	14 (13.3)	76 (72.4)	29 (27.6)		
Age groups <sup>b</sup>									
<40 years	74 (84.1)	70 (94.6)	4 (5.40)	66 (89.2)	8 (10.8)	63 (85.1)	11 (14.9)	1.00	-
≥40 years	14 (15.9)	14 (100)	0 (0.0)	13 (92.9)	1 (7.10)	13 (92.9)	1 (7.10)	0.60 (0.12–2.98)	0.536
Gender									
Female	66 (62.9)	64 (97.0)	2 (3.0)	59 (89.4)	7 (10.6)	49 (74.2)	17 (25.8)	1.00	-
Male	39 (37.1)	37 (94.9)	2 (5.10)	32 (82.1)	7 (17.9)	27 (69.2)	12 (30.8)	1.44 (0.63–3.30)	0.392
Residence area									
Periurban	97 (92.4)	93 (95.9)	4 (4.10)	84 (86.6)	13 (13.4)	69 (71.1)	28 (28.9)	1.00	-
Urban	8 (7.60)	8 (100)	0 (0.0)	7 (87.5)	1 (12.5)	7 (87.5)	1 (12.5)	3.78 (0.45–32.0)	0.223

Abbreviations: DENV, dengue virus; CI, confidence interval; OR, odds ratio.

<sup>a</sup>Chi-square test ( $\chi^2$ ).<sup>b</sup>Missing value = 17.**TABLE 2** Coinfection between SARS-CoV-2 and vector-borne diseases in Luanda, Angola

Vector-borne diseases	N (%)	SARS-CoV-2 infection	
		No (%)	Yes (%)
Overall	105 (100)	101 (96.2)	4 (3.80)
Any VBD			
No	70 (66.7)	70 (100)	0 (0.0)
Yes	35 (33.3)	31 (88.6)	4 (11.4)
Malaria			
No	91 (86.7)	89 (97.8)	2 (2.20)
Yes	14 (13.3)	12 (85.7)	2 (14.3)
DENV			
No	76 (72.4)	75 (98.7)	1 (1.30)
Yes	29 (27.6)	26 (89.7)	3 (10.3)

Abbreviations: DENV, dengue virus; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; VBD, vector-borne diseases.

were IgG positive, 34.5% (10/29) were IgM positive, and 6.9% (2/29) were IgM/IgG positive), respectively. Only one (10%, 1/10) IgM positive sample for dengue tested positive for RT-PCR, whereas no sample tested positive for ZIKV and CHIKV. No statistically significant relationship was observed between sociodemographic characteristics and the reactivity rate against SARS-CoV-2, malaria, or dengue ( $p > 0.05$ ). Although not statistically significant, there was a decrease in the reactivity rate of IgG anti-SARS-CoV-2 (5.4% to not to), malaria (10.8%–7.1%), and antibodies (IgG, IgM, or IgM/IgG) against DENV (14.9%–7.1%), among the under 40 groups compared to the over 40-year-old group, respectively. In addition, there was also a decrease in the reactivity rate of IgG anti-SARS-CoV-2 (4.1% to not to), malaria (13.4%–12.5%), and anti-DENV (28.9%–12.5%), among participants from periurban areas compared to participants from an urbanized area, respectively. On the other hand, there was an increase in the reactivity rate for anti-SARS-CoV-2 (3.0%–5.1%), malaria (10.6%–17.9%), and anti-DENV (25.8%–30.8%) among female participants compared to male participants, respectively. Although not statistically significant, the likelihood of reactivity of the anti-SARS-CoV-2 or some VBD was lower in participants above or equal to 40 years (OR: 0.60 [95% CI: 0.12–2.98],  $p = 0.536$ ), while was higher in male (OR: 1.44 [95% CI: 0.63–3.30],  $p = 0.392$ ) and in participants from urbanized area (OR: 3.78 [95% CI: 0.45–32.0],  $p = 0.223$ ).

### 3.2 | Rate of coinfection between SARS-CoV-2 and vector-borne diseases

The rate of coinfection between SARS-CoV-2 and VBD is shown in Table 2. The overall co-infection rate of SARS-CoV-2 and VBD was 11.4% (4/35). The coinfection rate of IgG against SARS-CoV-2 and malaria (14.3%, 2/14) was higher compared to IgG positive against SARS-CoV-2 and dengue (10.3%, 3/29).

## 4 | DISCUSSION

The COVID-19 pandemic triggered extra pressure on public health systems, with a higher threat in endemic regions with infections transmitted by arthropod bites. Simultaneously with SARS-CoV-2, VBD also continues to spread, particularly in tropical regions.<sup>6–8</sup> Angola is endemic for malaria and arboviruses such as dengue, Zika, and chikungunya,<sup>14–16</sup> however, the possibility of cocirculation and SARS-CoV-2/VBD co-infection in hyperendemic regions with these infectious agents exists and must be monitored.<sup>9</sup> To the best of our knowledge, this is the first study assessing the coinfection between SARS-CoV-2 and VBD in Angola.

So far, no significant effort has been made to describe the coinfection situations between SARS-CoV-2 and VBD in tropical, subtropical, and temperate regions.<sup>19</sup> The overlapping incidence of COVID-19, malaria, dengue, Zika, and chikungunya could hinder the patient's timely diagnosis, treatment as well as disease prevention, in part due to some similarity of clinical symptoms.<sup>20</sup> Early clinical symptoms (e.g., fever, chills, headache, fatigue, and myalgia) and laboratory parameters (e.g., leukopenia, lymphopenia, thrombocytopenia, and elevated transaminases) could be similar in patients infected with SARS-CoV-2 as well as patients with VBD.<sup>10–13</sup> However, the overlay of SARS-CoV-2 and VBD can overwhelm health systems, which could result in incorrect diagnoses as during the onset of SARS-CoV-2 infection, malaria, and dengue share clinical and laboratory characteristics with SARS-CoV-2 which makes it difficult to distinguish which etiological agent causes the disease.<sup>6,12</sup> Also, if clinical consequences of the SARS-CoV-2 and VBD coinfection might increase the chances of severe COVID-19 and unfavorable outcomes have not been fully characterized so far and need to be addressed urgently, mainly in tropical countries. The lack of studies on the SARS-CoV-2 and VBD coinfection is not surprising given that this is a new viral infectious disease and many issues about SARS-CoV-2 infection as well as its aspects are still to be discovered.

The SARS-CoV-2 reactivity rate observed in this study (3.8%) (Table 1) was lower than that observed in a previous study (14.3%) carried out in Luanda by our research team.<sup>21</sup> This decrease in the SARS-CoV-2 positivity rate is not surprising, due to the efforts that the Ministry of Health has made to control and interrupt the transmission chain, but we cannot also exclude the possibility that there is a decrease in the number of tests due to the high cost associated with carrying out the SARS-CoV-2 test, which is not compatible with the socioeconomic distribution of the population. However, the low SARS-CoV-2 positivity rate observed in this small number of participants is only a representation and might not reflect the current scenario and prevalence of SARS-CoV-2 in Angola.

We identified about 13% for malaria and 28% for dengue in our study sample (Table 1). Overall, about 33% of the study participants had some VBD (Table 2). The rate of VBD observed in this population is not surprising, as Luanda is an endemic region with diseases transmitted by arthropod bites, such as malaria and dengue fever.<sup>15,16</sup> Furthermore, our results emphasize that there is active dissemination of VBD in Luanda, with a great possibility of transmission and/or emerging outbreaks arising from mosquito bites at a time of the SARS-CoV-2 outbreak. Currently,

the diagnosis of SARS-CoV-2 is established as soon as the RT-PCR test is positive, and often additional VBD screening is not considered. Similarly, when the patient tests positive for any VBD, SARS-CoV-2 testing may not also be required. Nevertheless, combined screening of SARS-CoV-2 and VBD in regions with active transmission of infectious diseases that could be transmitted by arthropod bite is crucial for the development of suitable therapeutic protocols to avoid the worsening of the patient's clinical condition for the coinfecting cases. In this study, about 11.4% of participants had coinfection between SARS-CoV-2 and VBD (Table 2), which could indicate that a significant proportion of patients with COVID-19 in Luanda, should benefit from differential screening to detect other infectious diseases such as malaria and dengue to expand the possibility of more effective treatment and recovery of the infected population.

In the last 20 years, the global burden of malaria has reduced, mainly with the distribution of insecticide-treated bednets, indoor spraying of residual insecticides, access to early diagnosis, and the availability of more effective antimalarial treatments.<sup>22</sup> However, as the COVID-19 pandemic has become a priority for public health agendas in so many countries around the world, it caused a devastating impact on other diseases, like malaria, which became apparent, especially in endemic regions with a high level of mortality from malaria, such as in Angola.<sup>23</sup> To reduce the impact of the COVID-19 pandemic on the increase in malaria cases and mortality, the provision of diagnoses, treatments, and other preventive interventions must be maintained while facing the COVID-19 response.<sup>24</sup> Although the malaria infection rate (13.3%) was lower compared to the dengue (27.6%) (Table 1), some aspects must be taken into account in the current context of the COVID-19 pandemic, since it appears that malaria and COVID-19 share some clinical similarities.<sup>8</sup> It is also worth mentioning that the rate of patients with SARS-CoV-2 and malaria, simultaneously (14.3%), was higher compared to patients with SARS-CoV-2 and dengue, simultaneously (10.3) (Table 2). Previous studies revealed that coinfection between SARS-CoV-2 and malaria could lead to rapid deterioration of the immune system, which can result in severe COVID-19 with unfavorable clinical outcomes.<sup>7,25</sup> Thus, considering malaria screening tests are more readily available compared to COVID-19 tests, we suggest that health professionals should consider requesting the test for malaria while screening for COVID-19, to reduce the malaria cases and mortality during the COVID-19 pandemic period, especially in hyperendemic regions, such as in non-urbanized areas, where were observed the high rates of malaria and dengue, compared to the urbanized areas (Table 1).

In countries where dengue is endemic, healthcare providers have faced challenges in distinguishing COVID-19 from dengue, as it exhibits nonspecific clinical symptoms.<sup>10</sup> Our results revealed that the coinfection rate is higher in patients with dengue (10.3%, 3/29), compared to patients without dengue (1.3%, 1/76) (Table 2). Nonetheless, future studies might explore whether there is any relationship between DENV and the possibility of simultaneous infection with SARS-CoV-2. The results of coinfection between SARS-CoV-2 and dengue observed in this study sample, disagree with a mathematical model recently published by Nicoletis et al.,<sup>26</sup> indicating that

dengue fever could protect patients against SARS-CoV-2. On the other hand, our results agree with the results observed by Teotônio et al.,<sup>13</sup> where was not observed an indication that dengue fever could protect patients from SARS-CoV-2.

Our results have severe limitations. This is a preliminary study in a small sample size that does not represent the whole population from Luanda or other regions of Angola, therefore further studies with a wide population need to be considered. Also, serological tests for dengue are known to be affected by cross-reactivity with other Flavivirus, such as Zika or yellow fever, which increases the risk of false positives results.<sup>11,12</sup> Therefore, NS1 antigen screening should be assessed, but, due to resource constraints for this project, it was not possible to do this additional screening. The coinfection rate between the SARS-CoV-2 and VBD could impair innate and adaptive immune responses, however, studies assessing clinical, hematological, and biochemical parameters of COVID-19 patients with VBD, should be considered in the future. These studies could contribute to a deeper comprehension of SARS-CoV-2 and VBD clinic evolution, the clinical outcome in the Angolan population, and accelerating COVID-19 epidemics control.

In summary, our findings showed that the VBD endemic circulation could add more pressure on health systems already overburdened during the COVID-19 pandemic period, mainly in a resource-limited setting. Thus, we suggest that patients with COVID-19-related symptoms should also be screened for VBD and vice versa, in an effort to initiate the tailoring of treatment protocols in cases of coinfection. We call on researchers to conduct further studies on VBD within the context of the COVID-19 pandemic in Angola.

## ACKNOWLEDGMENTS

The authors are grateful to all study participants. We also thank the INIS/CISA staff such as Andria Cassoma, Ana Cândido, Alda Deolinda, Lemba Félix, Luísa Dachala, Maria Avelina, Luzia Quipango, Aldina Afonso, Fátima Ferreira, Madalena Neto, Paka Azevedo for technical support and Zinga David for administrative support.

## CONFLICT OF INTERESTS

The authors declare that there are no conflict of interests.

## AUTHOR CONTRIBUTIONS

*Conceptualization, investigation, methodology, and validation:* Cruz S. Sebastião, Zoraima Neto, Jocelyne Neto de Vasconcelos, and Joana Morais. *Data curation and Formal analysis:* Cruz S. Sebastião. *Data collection and supervision:* Celestina Gaston and Joana Paula Paixão. *Writing—original draft:* Cruz S. Sebastião. *Writing—review & editing:* Cruz S. Sebastião, Euclides N. M. Sacomboio, Zoraima Neto, Jocelyne Neto de Vasconcelos, and Joana Morais. All authors approved the final manuscript for publication.


## DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available on request from the corresponding author.

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**How to cite this article:** Sebastião CS, Gaston C, Paixão JP, et al. Coinfection between SARS-CoV-2 and vector-borne diseases in Luanda, Angola. *J Med Virol.* 2022;94:366-371. <https://doi.org/10.1002/jmv.27354>