OPEN ACCESS

Saudi Journal of Pathology and Microbiology
Abbreviated Key Title: Saudi J Pathol Microbiol
ISSN 2518-3362 (Print) | ISSN 2518-3370 (Online)
Scholars Middle East Publishers, Dubai, United Arab Emirates
Journal homepage: https://saudijournals.com

Original Research Article

Seroprevalence of Arboviruses in Travelers in Morocco: Focus on Dengue and Zika

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DOI: https://doi.org/10.36348/sjpm.2024.v09i10.002 | **Received:** 08.09.2024 | **Accepted:** 14.10.2024 | **Published:** 17.10.2024

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Abstract

Dengue and Zika viruses belong to the arbovirus family and are emerging infectious diseases transmitted by Aedes mosquitoes, prevalent in tropical and subtropical regions. Their proliferation is facilitated by factors such as adaptability, climate change, and globalization. Despite surveillance efforts by the World Health Organization (WHO), global monitoring remains limited. This study aimed to ascertain the seroprevalence of these diseases among travelers returning from endemic regions. A prospective, cross-sectional study was conducted on 2023 targeted travelers in the Democratic Republic of Congo and the Central African Republic. Participants were selected based on specific criteria over a six-month period. Blood samples were assessed in our laboratory using the VirClia® automated chemiluminescence immunoassay to detect Dengue and Zika IgG and IgM antibodies. The analysis involved interpretation of the results using a specific formula. The study included a young, asymptomatic cohort, averaging 30.8 years of age. Travelers were categorized according to age, sex, and country of residence. Dengue virus seroprevalence was 18.6%, predominantly IgG. The prevalence of ZIKV infection was 12.9%. The seroprevalence of these arboviruses varies according to sociodemographic factors. Epidemiological studies play a crucial role in assessing the prevalence of vector-borne diseases and informing public health strategies to mitigate their global impact.

Keywords : Seroprevalence, Arboviruses, Emerging infectious diseases, Dengue, Zika, Travelers.

I. INTRODUCTION

Arboviruses represent a group of emerging viral diseases, including Dengue (DENV), Zika (ZIKV), Chikungunya (CHIKV), Yellow Fever (YFV), and other viruses. They are transmitted by mosquitoes of the genus *Aedes*, such as *Aedes aegypti* and *Aedes albopictus*. These diseases are widespread in tropical and subtropical regions and pose a major challenge to public health [1].

According to the World Health Organization (WHO), approximately 390 million Dengue virus infections occur annually. About 3.9 billion people are at risk of infection with this flavivirus [2]. Currently, 89 countries and territories have reported evidence of Zika infections. WHO closely monitors the spread of these diseases and recommends prevention, diagnosis, and control measures. However, global surveillance remains limited [3].

The rapid spread of these mosquitoes worldwide is attributed to their remarkable adaptability, and ability to reproduce in various stagnant water containers. This phenomenon is exacerbated by globalization, international travel, and climate change, favoring their geographical expansion [1].

Dengue fever, caused by (DENV), can cause various symptoms. From mild fever to potentially life-threatening illness. Severe Dengue fever is characterized by severe bleeding with a risk of hemorrhagic shock and organ failure [2].

As for (ZIKV), it raised global concerns owing to its neurological complications. For example, Guillain-Barré syndrome in adults and cases of microcephaly in newborns contract during pregnancy [3].

The objective of this study was to determine the seroprevalence of (DENV) and (ZIKV) in travelers returning from endemic areas. By targeting a young and active population, we highlighted the potential risk of introducing new viral diseases into the country.

II. MATERIAL AND METHODS

This was a prospective, cross-sectional descriptive study of travelers who visited the Democratic Republic of Congo (DRC) in Bunia and the Central African Republic (CAR) in Bangui in 2023. The inclusion criteria were based on Moroccan nationality for individuals aged 18 years or older. Those who resided in these two countries for at least six months. Travelers of foreign nationality and/or under 18 years of age were excluded from the study.

Blood samples were collected in serum separator tubes without anticoagulant. The samples were centrifuged to isolate the serum samples. These sera were then transported in isothermal coolers to the Bacteriology-Virology laboratory at the HMA in Marrakech. The samples were stored at -20°C.

The immunoassay method used was chemiluminescence (CLIA) on a VirClia® automated analyzer (Vircell). Unitary tests for Dengue (DENGUE IgG and abDENGUE IgM) and Zika (ZIKA IgG and ZIKA IgM) antibodies were used for qualitative detection. The results were expressed in relative light units (RLU). These were interpreted by comparing the amount of light emitted by the sample to that of a standard sample. The index was calculated according to the following formula: Index = (RLU patient sample/RLU standard sample). An index greater than 1.1 was considered positive, less than 0.9 negative, and between 0.9 and 1.1 equivocal.

Data were collected from the exploitation forms and imported into an Excel spreadsheet for analysis. The statistical study was mainly based on descriptive statistics rather than analytical statistics, without using inferential statistical tests or similar methods.

Consent has been obtained from all participants in this study. The Ethics and Research Committee of the Avicenna Military Hospital of Marrakesh has issued approval May 15, 2024. We certify that the research project described below has received the necessary ethical approvals for human research, as required by the Avicenne Military Hospital of Marrakech. All authors affirm that this study did not include any animal subjects or tissues. Additionally, no financial support was provided by any organization for the submitted work, and there are no financial relationships with organizations that could have a vested interest in it. Furthermore, there are no other relationships or activities that could be perceived as influencing the submitted work.

III. RESULTS

1. Sociodemographic characteristics:

This study involved a population aged 23-48 years. The mean age was 30.8 years old. The travelers were divided into four age groups. The most represented groupe was aged 25-34 years. This was followed by 35-44 years, 18-24 years, and finally 45-54 years. Prevalence rates were 47.1% (n = 33), 28.6% (n = 20), 21.4% (n = 15), and 2.9% (n = 2), respectively. The sex breakdown showed a clear male predominance, with 91.4% (n = 64), compared to only 8.6% (n = 6) of women. Over a six-month period, the study population was divided into two equivalent groups: half of the travelers stayed in Bunia, DRC, while the other half resided in Bangui, CAR. None of the participants showed any clinical symptoms during the study (Table 1).

Table 1: Sociodemographic characteristics and seroprevalence status of travelers returning from endemic areas, Democratic Republic of Congo and Central African Republic, 2023 (n = 70)

Socio demographic characteristics	Population T = 70		Dengue				Zika			
			Seropositives T = 13		Seronegatives T = 57		Seropositives T = 9		Seronegatives T = 61	
	n	%	n	%	n	%	n	%	n	%
Age groups										
18-24	15	21,4	3	20,0	12	80,0	1	6,7	14	93,3
25-34	33	47,1	8	24,2	25	75,8	2	6,1	31	93,9
35-44	20	28,6	2	10,0	18	90,0	5	25,0	15	75,0
45-54	2	2,9	0	0,0	2	100,0	1	50,0	1	50,0
Gender										
Male	64	91,4	12	18,8	52	81,2	9	14,1	55	85,9
Female	6	8,6	1	16,7	5	83,3	0	0,0	6	100,0
Country of stay										
DRC (Bunia)	35	50	8	22,9	27	77,1	1	2,9	34	97,1
CAR (Bangui)	35	50	5	14,3	30	85,7	8	22,9	27	77,1
Overall seroprevalence			Dengue				Zika			

	n	%	n	%
IgG (+) IgM (-)	11	15,8	5	7,2
IgG (+) IgM (+)	1	1,4	3	4,3
IgG (-) IgM (+)	1	1,4	1	1,4
IgG (-) IgM (-)	57	81,4	61	87,1
CAR: Central African Republic; DRC: Democratic Republic of Congo; n : Sample size; % : Corresponding percentages; T : Total				

2. Dengue

The overall seroprevalence of Dengue virus among the tested travelers was 18.6% (n = 13), while 81.4% (n = 57) were negative (Figure 1). Seventy serum samples were tested for anti-DENGUE IgG and abDENGUE IgM antibodies. Of ther travelers, 15.8% (n = 11) were positive for IgG alone, 1.4% (n = 1) were positive for IgM only, and both for IgM and IgG (Figure 2). The mean index of positive antibodies was 1.75 (standard deviation (SD): 0.22) for IgG and 1.34 (SD: 0.26) for IgM. The Dengue seroprevalence varied according to the sociodemographic characteristics of the study population. Individuals aged 25-34 years had the highest seropositivity, 24.2% (n = 8). This was followed by those aged 18-24 and 35-44 years age groups, 20.0% (n = 3) and 10.0% (n = 2), respectively. No seropositivity was observed in the 45-54 years age group. In terms of sex, 18,8% (n = 12) of seropositive men far outnumbered seropositive women, 16,7% (n = 1). Finally, for place of stay, the seroprevalence was 22.9% (n = 8) for travelers in the DRC versus 14.3% (n = 5) for those in the CAR (Table 1).

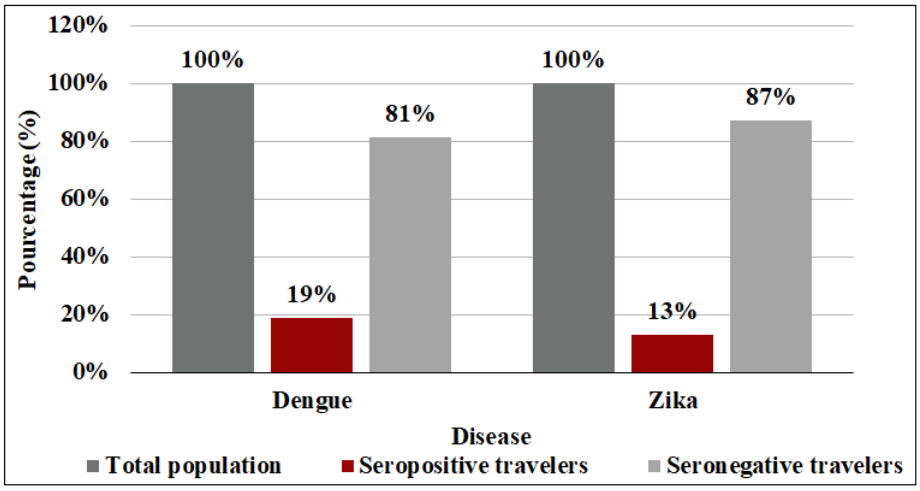

Figure 1: Global seroprevalence of Dengue and Zika among travelers, Democratic Republic of Congo and Central African Republic, 2023 (n = 70)

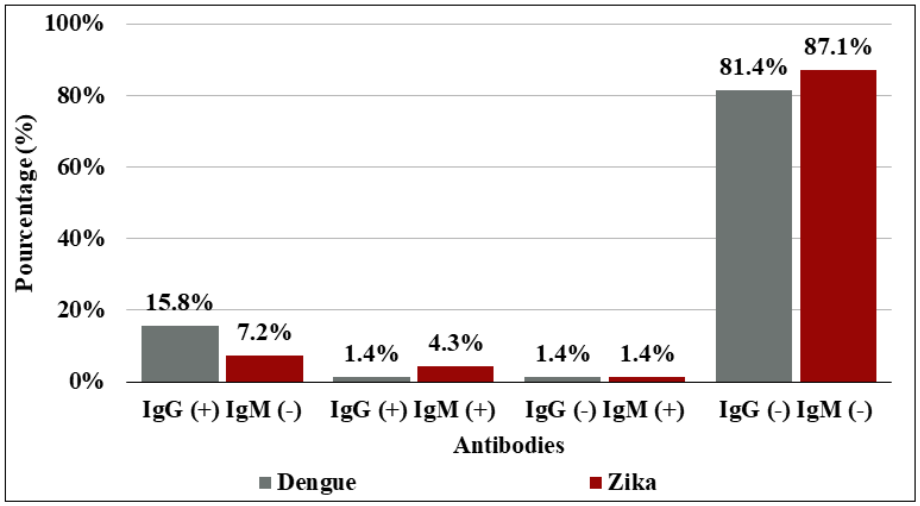

Figure 2: Distribution of the population according to the seroprevalence of anti-Dengue and anti-Zika antibodies, Democratic Republic of Congo and Central African Republic, 2023 (n = 70)

3. Zika

For Zika virus, the overall seroprevalence increased to 12.9% (n = 9), while 87.1% (n = 61) of the cases were negative (Figure 1). Of the sera tested for anti-ZIKV IgG and IgM antibodies, five travelers were seropositive for IgG only (7.2%). Only one individual was seropositive for IgM alone (1,4%). Unlike Dengue, three individuals were seropositive for both IgG and IgM (4.3%) (Figure 2). The mean index of positive antibodies was high for IgG and IgM, reaching 2.26 (SD: 0.29) and 2.34 (SD: 0.31), respectively. Zika seroprevalence in the age groups of 25-34 years and 18-24 years was similar, ranging from 6.1% (n = 2) to 6.7% (n = 1). However, it varied considerably between 25.0% (n = 5) and 50.0% (n = 1) for the other two groups aged 35-44 and 45-54 years, respectively. Seropositive male travelers represented 14.1% (n = 9) of the cases, while all female travelers were seronegative. For the country of stay, the seroprevalence was higher among travelers who stayed in the CAR than the DRC, 22.9% (n = 8) versus 2.9% (n = 1) (Table 1).

IV. DISCUSSION

Arboviruses, which are often neglected, have significantly affected many individuals in the tropical and subtropical regions worldwide. Thus, they pose a major public health challenge [1]. Our study focused on the seroprevalence of Dengue and Zika viruses in travelers returning from endemic areas. We analyzed these findings based on national and international data. To highlight trends over time and address current and future challenges in terms of diagnosis and prevention.

1. What is the situation in Morocco? Current situation of Dengue and Zika vectors in Morocco

Morocco has historically not experienced endemic transmission of arboviruses, such as Dengue and Zika. However, the country remains at risk owing to the possibility of imported cases. Similar to the presence of vector mosquitoes, *Aedes aegypti* and *Aedes albopictus* [4].

A study conducted in 2016 by Bennouna *et al.*, reported the presence of *Aedes albopictus* in Rabat. However, there is no evidence of its establishment [5]. In response, the Ministry of Health implemented preventive measures aligned with the WHO recommendations. Strengthening epidemiological and entomological surveillance systems. The aim was to detect imported cases from affected countries at an early stage and raise awareness among healthcare professionals and the public [4].

As part of these efforts, an entomological survey was carried out in Rabat from April to November 2017 and in Casablanca from May to November of the same year. During the mosquito development season in Morocco, particularly in *Aedes albopictus*. The results revealed the presence of five mosquito species in Rabat, including one *Aedes* species. The first *Aedes albopictus* larvae and nymphs were discovered on May 30, 2017. Following the control measures by the Communal Hygiene Office, the numbers decreased. However, reappearances were again observed in August and September, primarily on Al Kortobia Street. The presence of *Aedes albopictus* was confined to the Al Kortobia Street and Youth and Sports Delegations. Awareness campaigns effectively reduce their presence [4].

No *Aedes albopictus* were observed in Casablanca during the study period. Rare-positive larval traps are associated with *Culex pipiens*, a mosquito species that is not known to transmit these arboviruses [4].

In 2019, Amraoui *et al.*, conducted a study on the potential of *Aedes albopictus* to cause the emergence of arboviruses in Morocco. They experimentally infected a field-collected strain of *Aedes albopictus* in Morocco with (DENV), (ZIKV), (CHIKV) and (YFV) viruses. The results showed that these species were highly capable of transmitting all four arboviruses. Detection of the latter in mosquito saliva was possible on the third day of (CHIKV). On the fourteenth day for (DENV) and (YFV), but on the twenty-first for (ZIKV) after infection [6].

Additionally, the Intergovernmental Panel on Climate Change (IPCC) has identified Morocco as a country with a significant potential for global warming. These findings have substantial implications for various infectious diseases [7]. Furthermore, in 2023, tourism in Morocco reached a historical high. Approximately 14.5 million tourists welcomed, with a remarkable increase in higher rates in 2022 and 2019 [8]. These findings suggest that local transmission of arboviruses by the newly introduced *Aedes albopictus* in Morocco is a plausible scenario [7, 8].

2. Dengue

The only study on Dengue in Morocco was conducted by Bajjou *et al.*, in 2016. It involves patients returning from sub-Saharan Africa and Southeast Asia, with an estimated overall prevalence of 9.5% (n = 21) [9]. In our study, Dengue antibody screening performed in our laboratory using chemiluminescence revealed that 18.6% (n = 13) of 70 samples were seropositive. This variation in prevalence may be attributed to the diagnostic method used. Bajjou *et al.*, employed real-time polymerase chain reaction (RT-PCR) to offer higher specificity and better suitability for early diagnosis [9]. However, our study used serology. A suitable late-stage diagnostic tool, but less specific, can produce false-positive results due to antibody cross-reactivity with other flavivirus family viruses, notably (ZIKV) [9]. We compared our results with those reported in international literature. We observed similar rates to

those reported in a survey by Riddell and Babiker in East London (26.1%, n = 44) and Pierro *et al.*, in Italy (20.4%, n = 83) [10, 11]. However, a lower rate was observed in a study conducted in Hungary in 2023, by Nagy *et al.*, Where 9.8% (n = 75) of the 758 samples tested positive for Dengue [12]. In contrast, an earlier study by Norman *et al.*, involving 861 symptomatic travelers in Spain reported a Dengue positivity rate of 53.0% (n = 456) [13]. Similarly, Huhtamo *et al.*, found an estimated rate of 31.2% (n = 5) among 16 individuals who stayed in Madeira, Portugal. During the Dengue epidemic of 2012 [14].

In our study, the seroprevalence of Dengue according to the age group was higher. It reached 24.2% (n = 8) in individuals aged 25-34 years. However, these results should be interpreted with caution due to the limited sample size. This pattern raises questions regarding factors that influence the prevalence of seropositivity by age. This challenges the commonly accepted notion that the risk of Dengue infection increases with age. This notion is supported by the studies conducted by Egger *et al.*, (2007) and Huang *et al.*, (2023) [15, 16].

A clear male predominance was observed in our study population, with a higher number of seropositive cases in males than in females. This finding is consistent with that of a study conducted in Pakistan in 2022 by Khan *et al.*, [17]. In addition, there are six other Asian countries, including the Lao People's Democratic Republic, Philippines, Singapore, Sri Lanka, and Cambodia [18]. This trend may be explained by the fact that men tend to remove shirts during the warmer months. This exposes them to mosquito bites and causes transmission of arboviruses [17, 18].

According to the country of stay, the difference in seroprevalence observed between the DRC (n = 8, 22.9%) and CAR (n = 5, 14.3%) was probably attributable to a more favorable climate. Bangui's hot and humid climate offers optimal conditions for the mosquito vectors *Aedes aegypti* and *Aedes albopictus.* In addition, the dense urban environment of Bangui promotes mosquito breeding in stagnant waters and abandoned containers. Facilitating viral transmission through mosquito bites [19]. Studies conducted in Pakistan and Europe have shown that the incidence of this disease is influenced by various climatic factors. Higher transmission rates have been observed in the temperature range of 28-32°C [20,21].

The first confirmed case of Dengue fever In the DRC in 1960 revealed a widespread exposure. Most of the population have antibodies against the virus. Three serotypes (DENV-1 to DENV-3) have been recorded, with DENV-1 being the most predominant [19, 22, 6]. Four studies were conducted on the DRC between 1998 and 2015. They used different phenotypic and molecular diagnostic methods (enzyme-linked immunosorbent assay, rapid diagnostic tests, and RT-PCR) to assess Dengue prevalence. The reported prevalence rates varied from 2.7% (n = 111) to 8.8% (n = 45) [19]. Additionally, the co-circulation of the Dengue virus and other arboviruses was documented during the 2012 epidemic in Kinshasa [19, 22].

In the CAR, the risk of Dengue was considered "sporadic/uncertain" by the WHO. However, limited resources and ongoing conflicts hamper surveillance and data collection. This lack of information raises questions regarding the actual prevalence and frequency of epidemics and the unique challenges posed by Dengue in this country [2].

3. Zika

According to a recent meta-analysis by Villarroel *et al.*, in 2024, 84 studies from 49 countries were published between 2000 and 2023. In total, 63864 individuals were included. The global seroprevalence of ZIKV was 21.0% (n = 13411). In Africa, the overall seroprevalence in the 18 countries was 8.4%. However, higher rates were recorded in Gabon and Burkina Faso. Seroprevalence was correlated with epidemics in 13 countries in the Americas (39.9%). The global seroprevalence in the Western Pacific, including Indonesia, was similar to that observed in our study. In addition to the Eastern Mediterranean, notably in Honduras, Mexico, Colombia, and Nicaragua. However, autochthonous transmission has not been reported [23].

This systematic review highlights the significant diversity in the seroprevalence of Zika virus worldwide, correlating with the results of the present study (12.9%, n = 9). These disparities can be attributed to methodological discrepancies. These include enzyme-linked immunosorbent assays (ELISA), lateral flow immunoassays (LFA), indirect immunofluorescence (IFI), colorimetric tests, hemagglutination inhibition assays (HIA), and various neutralization tests [23]. By contrast, our approach is based on chemiluminescence for serodiagnosis. These heterogeneities may also result from the choice of the population and sample size. In addition, they may reflect varying degrees of mosquito exposure during the study period. Variations in the number of studies per country are also a limitation, and may influence seroprevalence upward or downwards [23].

Compared with Dengue, the seroprevalence of the Zika virus by age group showed significant variability in our study. The rates ranged from 50% (n = 1) in individuals aged 45-54 years, to rates oscillating between 25.0% (n = 5) and 6.1% (n = 2) in younger individuals. These observations are consistent with those of a study conducted in 2022 in the general population. Who were living in the outlying forest areas of Peninsular Malaysia (Perak and Pahang) and East

Malaysia (Sabah). In that study, Khoo *et al.*, observed higher seroprevalence in age groups over 45 years, reaching 64.6% (n = 89) [24]. However, these results differ from those of a study conducted in Pakistan in the year 2024. Chen S *et al.*, found that individuals under 40 years were the most affected. The seroprevalence of people simultaneously exposed to several arboviruses, including Zika virus, was 67.57% (n = 25). High neutralizing activity among outdoor workers [25]. These results suggest that certain human activities or environments may increase the likelihood of exposure to arboviruses [24, 25].

The study population was characterized by a clear male predominance (n = 64, 91.4%). A higher rate of seropositivity was observed in men (n = 9, 14.1%) than in women (n = 0, 0.0%). This finding mirrors the high proportion of men (n = 33, 89.19%) reported by Chen *et al.*, [25]. Another study conducted in Thailand in 2024 by Kitro *et al.*, found that most participants were male (n = 254, 83.1%). The length of stay in that study was longer than that in ours (six years versus six months) [26]. However, these results diverge from those of the meta-analysis by Villarroel *et al.*, in which women represented a higher proportion of all participants [23]. Similarly, seroprevalence was higher in women (n = 488, 69.1%) according to a study by Khoo *et al.*, [24]. In contrast, no significant difference in ZIKV seroprevalence between sexes was observed in the subpopulations of a study conducted in Salvador, northeast Brazil [27].

Initially discovered in Uganda in a rhesus macaque in 1947, the Zika virus caused sporadic outbreaks in humans in Africa and Asia between the 1960s and the 1980s. However, since 2007, Zika epidemics have been recorded in Africa, the Americas, Asia, and the Pacific. Environmental and socioeconomic factors significantly influenced the distribution and epidemiology of Zika and other arboviruses. This is particularly true in low-income countries with tropical or subtropical climates [28].

In CAR and DRC, 22.9% (n = 8) and 2.9% (n = 1) of returning travelers, respectively, were seropositive. Although the Zika virus status in CAR is still poorly understood, no major epidemics have been reported to date. Similarly, in the DRC, the situation remains largely unexplored. This is because of the favorable mosquito-friendly climate and population mobility. However, there is a potential risk for transmission in these countries. Continuous surveillance and increased research are necessary to better understand and control the spread of Zika [28, 3].

Finally, excluding certain groups of travelers and the limited length of stay may have caused selection bias. Confounding bias may arise from uncontrolled factors that may have affected the study results. For example, there are differences in mosquito protection behaviors between the sexes. Climatic and environmental differences between the areas where participants stayed could also bias the results regarding the prevalence of Dengue and Zika. The use of a single serological diagnostic method to detect anti-DENV and anti-ZIKV antibodies could lead to variable results, depending on the sensitivity and specificity of the different methods, causing potential information bias. These biases could be a limitation of this study. Therefore, it is recommended that further larger and long-term studies be conducted, including symptomatic and asymptomatic populations of all ages using a variety of direct and indirect diagnostic methodologies.

V. CONCLUSION

In endemic countries, particularly Africa, epidemiological studies are essential to assess the prevalence of Dengue and Zika. The objective is to develop more sensitive and specific diagnostic tools. These studies may include serological surveys to detect the presence of antibodies in the exposed individuals. In addition, research has been conducted on potential viral vectors.

Despite the low overall prevalence of mosquito-borne diseases in Morocco. Environmental changes, including climatic, economic, and social factors, play crucial roles in the importation and transmission of arboviruses. Therefore, the introduction of species such as *Aedes albopictus* could potentially lead to new public health challenges. It is imperative to assess and control this risk by strengthening the surveillance of emerging arboviruses in the country. To mitigate these risks, increased and targeted awareness among health care professionals and travelers is paramount. The key preventive measures are both collective and individual. Media awareness, vector control efforts, and protection against mosquito bites.

ACKNOWLEDGEMENTS

We express our gratitude to our Professors and all those who contributed to this work, in particular the team of the Bacteriology-Virology and Molecular Biology Laboratory, The Avicenna Military Hospital, Marrakesh. As well as that of the Bacteriology-Virology Laboratory, Oued Eddahab Military Hospital, Agadir and the Internal Medicine Department, Avicenne Military Hospital, Marrakesh.

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