Seroprevalence of Dengue and Chikungunya antibodies among blood donors in Dar es Salaam and Zanzibar, Tanzania: a cross-sectional study

Haliya S. Shauri1*, Esther Ngadaya2, Mbazi Senkoro2, Joram J. Buza1 and Sayoki Mfinanga1,2

Abstract

Background: The potential shift of major causes of febrile illnesses from malaria to non-malarial febrile illnesses, including arboviral diseases such as chikungunya and dengue, is of concern. The last outbreaks of these infections were reported in 2018 and 2019 for chikungunya in Zanzibar and dengue in Dar es Salaam. We conducted a cross-sectional study that involved serological testing of stored blood samples from the blood banks in Temeke Referral Hospital in Dar es Salaam and the National Blood Bank Unit in Zanzibar. The samples were collected from Zanzibar and Dar es Salaam donors in May and June 2020, respectively. A total of 281 samples were included in the study, and their demographic information extracted from the registers. The samples were then transported to Muhimbili University of Health and Allied Sciences at the Microbiology Laboratory. They were subjected to an indirect ELISA to detect IgG and IgM against dengue and chikungunya viruses.

Results: Seropositive IgM samples from Dar es Salaam were 3/101 (2.97%) for chikungunya and 1/101 (0.9%) for dengue, while samples from Zanzibar were all IgM negative for both viruses. Chikungunya IgG seropositivity was significantly higher \( p \leq 0.05 \) in Dar es Salaam 21/101 (21.2%) than Zanzibar 22/180 (12.2%). There was no difference in dengue IgG seropositivity between Dar es Salaam 44/101 (43.5%) and Zanzibar 68/180 (37.8%). Similarly, dual IgG seropositivity for both dengue and chikungunya viruses were not different between Dar es Salaam 13/101 (12.9%) and Zanzibar 11/180 (6.1%).

Conclusion: Detection of IgM for dengue and chikungunya in Dar es Salaam indicates recent or ongoing transmission of the two viruses in the absence of a reported outbreak. These findings suggest the possibility of transmission of the two infections through blood transfusion. Detection of IgG antibodies for dengue and chikungunya viruses might be contributed by both; the ongoing infections and residual responses caused by preceding infections in the country. Results from blood banks may represent the tip of the iceberg. Further studies are needed to gain insight into the actual burden of the two diseases in Tanzania.

Keywords: Dengue, Chikungunya, Seroprevalence, Viruses, Tanzania
viruses may be directly transmitted through blood donation by asymptomatic donors [5]. Dengue and chikungunya viruses have similar transmission modes, same vector and pathological mechanisms, and clinical presentations [4]. The two febrile diseases are characterized by acute fever, high body temperature above 40 °C, muscle pain and headache, backache, and skin rashes [6]. Infection by any of the four dengue serotypes may be occurring with different clinical presentations and often with unpredictable clinical evaluation and outcome. Therefore, dengue case is classified as dengue fever with or without warning signs and severe dengue characterized by severe plasma leakage, severe hemorrhagic, and severe organ impairment [7]. In most cases, chikungunya is a self-limiting disease, though its complication, mainly joint pain, can persist for months or years post-infection, especially for older age [8, 9].

Arboviral disease transmission is often heterogeneous due to the vectors and host distribution and underlying social and ecological determinants [10]. The recent epidemics caused by these arboviruses has been associated with many factors, including urban expansions, population growth, and international travel and trade, which facilitate the spread of vectors and arboviruses into new niches amplification through the human-vector-human cycle [4]. Also, the areas with high temperatures and heavy rainfall followed by flooding are most favorable for mosquitoes’ growth and survival [11]. Evidence of abundances of Aedes mosquitoes has been documented in Zanzibar, where out of 200 samples, 124 (62%) were positive for immature stages of mosquitoes, of which 114 (94%) were positive for Aedes aegypti larvae and pupae [12]. In Tanzania mainland, a study conducted in the Morogoro region reported that immature Aedes mosquitoes were present in breeding sites during the rainy season (18.87%) and dry season (4.64%) [13]. Recent reports on dengue and chikungunya outbreaks show that diseases have spread in many parts globally, including Asia, the Pacific, Europe, and Africa [14–16]. Dengue outbreaks were reported in 2010, 2014, and more recently in 2018 in Tanzania [17]. The outbreak of 2019 was the worst documented dengue outbreak. Dar es Salaam was the epicenter, followed by Tanga with 6873 cases and 13 death reported [18]. The outbreak of chikungunya in Zanzibar was reported on 4th May 2018, with around 50 cases per day seen in MnaziMmoja referral Hospital [18]. Some studies have also documented chikungunya seroprevalence in different parts of Tanzania Mainland [20, 21].

Dengue cases associated with transfusions and transplantations have been reported [22, 23]. While these cases may not by themselves cause substantial public health alarm, but they may indicate possible future outbreak which may have huge public health and economic consequences. This study aimed to determine dengue and chikungunya’s seroprevalence in blood donors using the stored blood samples from Temeke referral hospital in Dar es Salaam and Zanzibar National Blood Bank in Zanzibar.

Methodology

Study site

This study was conducted in two areas of Tanzania; Dar es Salaam and Zanzibar. The archipelago of Zanzibar is a semi-autonomous region of Tanzania, situated in the Indian Ocean off the east cost of mainland Tanzania. The annual rainfall of Zanzibar is about 1600 mm in Unguja Island and 1900 mm in Pemba Island. Annual temperatures are high throughout the year, temperature range from 29 to 33 °C. Dar es Salaam is among the coastal regions of Tanzania, which lies 16 m above sea level with an average temperature of 26.1 °C/79.1 °F and annual precipitation amount to 1150 mm. These conditions in the two locations are more favorable for mosquitoes’ survival and growth. Since 2010, these areas have experienced several dengue and chikungunya cases [24].

Study design

A cross-sectional study was conducted from May to October 2020. Blood samples and demographic information were retrieved from the Temeke Referral Hospital, Dar es Salaam and the Zanzibar National Blood Bank.

IgG and IgM ELISA for detection of anti-dengue and anti-chikungunya antibodies

Serum was separated from whole blood by centrifugation and stored at − 20 °C. All anti-dengue and anti-chikungunya were detected using indirect Enzyme-Linked Immunosorbent assays ELISA (Euro immune company from Germany). All assays were performed according to the manufacturers’ procedures, and all serum samples were diluted 1 into 100 with sample diluent provided with the kits. The optical density (OD) was measured at 450 nm, and the units of antibody concentration and cut-off values calculated as described by the manufacturers. Briefly, for the Anti-dengue IgM/IgG and IgM anti-chikungunya ELISAs the diagnostic cut-off value was calculated as the average OD of negative controls + 0.300. For the IgG chikungunya ELISA, the threshold for positivity was based on the OD cut-off value of the cut-off control + 10% [25].

Statistical analysis

Data were retrieved from the computer then were compiled and analyzed using STATA v 15 software. Chi-square ($\chi^2$) was used to compare categorical data. The
association between seroprevalence and the demographic variable was done using simple logistic regression and the odds ratio (OR) with 95% confidence intervals were estimated. Prevalence differences were considered to be statistically significant if \( P \leq 0.05 \) and if the 95% confidence does not include one.

**Informed consent**

Informed consent was obtained from all subjects. All methods were carried out in accordance with declaration of Helsinki.

**Results**

**Descriptive statistics**

A total of 281 blood samples were tested, whereby 180 (64%) were from Zanzibar, and 101 (35.9%) were from Dar es Salaam. However, we could only retrieve demographic information of blood donors’ samples from Zanzibar. Out of 180 samples from Zanzibar, almost all blood samples, 171 (95%), were male donors. About 96 (53.3%) were in the age of < 30 years with a mean (SD) age of 37 (12.97) years. About 91 (50.6%) were unemployed people.

**Seroprevalence of Dengue and Chikungunya with their co-infections in Zanzibar and Dar-es-salaam**

We detected dengue IgG seropositivity in both study sites; 43.5% (44 /101) in Dar es Salaam and 37.8% (68/180) in Zanzibar prevalence was not different. However, the chikungunya IgG prevalence in Dar es Salaam 21/101 (21.2%) was significantly higher (\( P \)-value = 0.047) than in Zanzibar 22/180 (12.2%). Neither dengue nor chikungunya seropositive IgM was observed in Zanzibar, while in contrast, both chikungunya IgM 3/101 (2.97%) and dengue IgM 1/101 (0.9%) were detected in Dar es Salaam. The prevalence of dual anti-chikungunya and anti-dengue IgG antibodies in the same sample was 13/101 (12.9%) for Dar es Salaam and 11/180 (6.1%) in Zanzibar. However, the prevalence was not statistically different (\( P \)-value = 0.052). Table 1

**Risk factors for Dengue and Chikungunya**

Different risk factors were evaluated, including age, sex, marital status, and occupation association with the two diseases. However, no risk factor was associated with any of the two diseases Table 2.

**Discussion**

The study was designed to compare the seroprevalence of anti-dengue and anti-chikungunya IgM and IgG and their co-circulation in Dar es Salaam and Zanzibar. We observed dengue and chikungunya IgM seropositivity of 0.9 and 2.97%, respectively, from Dar es Salaam samples, while no IgM for dengue or chikungunya was observed in samples from Zanzibar. Therefore, there is a need for screening for these infections and continued public education/awareness of avoiding exposure to Aedes mosquitoes. Due to the lack of routine diagnosis of these diseases, dengue and chikungunya fever could be misdiagnosed as malaria and wrong prescription given, leading to adverse health effects, especially for Dengue. Complications can lead to severe dengue infection characterized by severe plasma leakage, severe hemorrhage and organ impairment [7]. Again, IgM presence indicates that dengue and Chikungunya infections are ongoing in mainland Tanzania, and may be predictive of a future epidemic with serious social and economic consequences [7, 25, 26].

The lower chikungunya and dengue IgM seropositivity in this study is similar to the result obtained from a study conducted at Kilombero district in the South-Eastern part and Bondo district, the Northern part of Tanzania [29], [30]. In another study [21], the

| Table 1  | Seroprevalence of Dengue and Chikungunya with dual infection in Zanzibar (\( N = 180 \)) and Dar-es-salaam (\( N = 101 \)) |
|----------|-------------------------------------------------------------------------------------------------|
| **Test** | **Location**                      | **Positive n (%)** | **Confidence intervals** | **\( P \)-value** |
| Dengue IgG | Zanzibar                           | 68 (37.8)          | 31.0–45.0                 | 0.342             |
|           | Dar-es-salaam                      | 44 (43.5)          | 34.0–53.0                 |                 |
| Chikungunya IgG | Zanzibar                      | 22 (12.2)          | 7.0–17                    | 0.047*            |
|           | Dar-es-salaam                      | 21 (21.2)          | 13–29                     |                 |
| Dengue IgM | Zanzibar                           | 0 (0)              | NA                       | NA               |
|           | Dar-es-salaam                      | 1 (0.9)            | – 1.0–3.0                 |                 |
| Chikungunya IgM | Zanzibar                      | 0 (0)              | NA                       | NA               |
|           | Dar-es-salaam                      | 3 (2.97)           | – 1.0–3.0                 |                 |
| Dual infection IgG | Zanzibar                      | 11 (6.1)           | 3.0–10                    | 0.052             |
|           | Dar-es-salaam                      | 13 (12.9)          | 6.0–20                    |                 |

* Chi-square test comparing prevalence between Zanzibar and Dar-es-salaam

\*\( P < 0.05 \), **\( P < 0.01 \), ***\( P < 0.001 \)
prevalence of chikungunya IgM was found to be (3.8%), which is relatively higher compared to our study. The differences could be attributed to many variables, including the type of samples used; our sample was collected from asymptomatic blood donors who could give different results if blood was taken directly from febrile patients or a random population sample. The random sample will provide an unbiased sample representing the entire population while a sample of blood donors has a high probability of being biased or unrepresentative of the population. Therefore, there is high probability for results to be different.

We observed dengue and chikungunya IgG seroprevalence in both Dar es Salaam and Zanzibar. This may be a consequence of ongoing infections as signified by IgM responses observed in this study and previous outbreaks [18], [31] since the IgG can be detected many years post-infection [32]. The anti-dengue and anti- chikungunya IgG antibodies detected in this study agree with many other studies in various parts of Tanzania [21, 27], [30], suggesting that the diseases are becoming endemic in the country.

The presence of chikungunya and dengue dual antibodies has been reported previously in Tanzania [20]. However, the reported estimates were lower than the prevalence reported in our study, which was 6.1% in Zanzibar and 12.9% in Dar es Salaam. The observed dual antibodies for chikungunya and dengue in the same individual in the study sites indicate that the two viruses are prevalent among blood donors. This may result in illness with overlapping signs and symptoms, which lead to difficulties in treatment and diagnosis.

No association was observed between seroprevalence and demographic characteristics. This might be due to a number of factors that represent the limitation of the study. To begin with, only a few demographic information was available from the blood bank register compared to what is normally collected during disease surveillance. Secondly, only samples from Zanzibar had the associated demographic data available for analysis, therefore, denying us the opportunity to compare the two locations. However, it is important to note that blood collection centers register only demographic information that is important for their purpose but not necessarily disease surveillance. Another limitation is that chikungunya and dengue viruses are not readily differentiated serologically due to cross-reactivity of their serocomplexes, so there is a need for molecular detection methods. However, the information obtained from this study will help to flag the potential danger of transmission of the two diseases through blood transfusion and also corroborate other studies suggesting that dengue and chikungunya may be endemic in Tanzania.

**Conclusion**
This is the first study to document the seroprevalence of dengue and chikungunya in blood Bank in Dar es Salaam and Zanzibar. We recommend screening for both Dengue and chikungunya viruses infection for blood donors to avoid infection via transfusion, requiring viral detection in

| Variables | Dengue IgG | Chikungunya IgG | Dual infection of Dengue and Chikungunya |
|-----------|------------|-----------------|------------------------------------------|
|           | n          | OR (95% CI)     | P-value | n          | OR (95% CI)     | P-value | n          | OR (95% CI)     | P-value |
| Age       |            |                 |         |            |                 |         |            |                 |         |
| 19–30     | 38         | Ref             |         | 11         | Ref             |         | 5          | Ref             |         |
| 31–40     | 17         | 1.6(0.78–3.65)  | 0.183   | 6          | 1.78(0.61–5.25) | 0.291   | 3          | 1.89(0.42–8.36) | 0.400   |
| 41–61     | 13         | 0.89(0.41–1.94) | 0.779   | 5          | 1.27(0.41–3.92) | 0.678   | 3          | 1.68(0.38–7.40) | 0.491   |
| Sex       |            |                 |         |            |                 |         |            |                 |         |
| Male      | 64         | Ref             |         | 22         | Ref             |         | 11         | Ref             |         |
| Female    | 4          | 1.3(0.34–5.16)  | 0.673   | 0          | NA               |         | 0          | NA               |         |
| Marital status |            |                 |         |            |                 |         |            |                 |         |
| Married   | 35         | Ref             |         | 14         | Ref             |         | 8          | Ref             |         |
| Divorced  | 1          | 0.46(0.04–4.67) | 0.517   | 0          | NA               |         | 0          | NA               |         |
| Single    | 32         | 0.74(0.41–1.37) | 0.348   | 8          | 0.47(0.18–1.2)  | 0.116   | 3          | 0.32(0.08–1.25) | 0.101   |
| Occupation |            |                 |         |            |                 |         |            |                 |         |
| Not work  | 31         | Ref             |         | 12         | Ref             |         | 7          | Ref             |         |
| Work      | 37         | 1.3(0.75–2.5)   | 0.300   | 10         | 0.83(0.34–2.04) | 0.69    | 4          | 0.56(0.15–2.0)  | 0.376    |
the form of RNA or antigen example, NS1. Also, we recommend a general population study in Dar es Salaam and Zanzibar to get a complete picture of the current disease burden in the country.

Abbreviations
ELISA: Enzyme-Linked Immunosorbents Assay; SD: Standard Deviation; KNCHREC: Kibong’oto Infectious Diseases Hospital-Nelson Mandela African Institution of Science and Technology-Centre for Educational Development in Health, Arusha; NS1: Non-Structure Protein 1; SUZA: The State University of Zanzibar; ZAHRI: Zanzibar Health Research Institute.

Acknowledgements
We gratefully acknowledge our research staff, laboratory scientists, statisticians, and hospital administration, without whom the present study would not be possible.

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Author’s contributions
HSS conceived the study, analyzed the data, and wrote the manuscript. JB, EN, SGM, and MS conceived, supervised the study procedures, and revised the manuscript. All authors read and approved the final manuscript.

Funding
This study was funded by African Development Bank (AFDB), EDCTP2 grant number RIA 2016E-1609-PANDORA-ID-NET supported under horizon 2020 (the European Union), and The State of University of Zanzibar (SUZA).

Availability of data and materials
The data used to support the findings of this study are available from the corresponding author (Shauri, Haliya) upon special request.

Declarations
Ethics approval and consent to participate
The ethical approval was obtained from the research ethics committees of the Kibong’oto Infectious Diseases Hospital-Nelson Mandela African Institution of Science and Technology-Centre for Educational Development in Health, Arusha (MDH-NM-AIST-CEDAH) – (KNCHREC) with certificate number KNCH REC0019 for collecting samples from Dar es Salaam. For Zanzibar, ethical approval was obtained from the Zanzibar Health Research Institute (ZAHRI) with certificate number ZAHRI-46. Additional permission was acquired from the respective hospitals where samples were obtained.

Informed consent
Informed consent was obtained from all subjects. All methods were carried out in accordance with declaration of Helsinki.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

Author details
1 Nelson Mandela African Institute of Science and Technology Arusha, Arusha, Tanzania.
2 National Institute for Medical Research Muhimbili, Dar es Salaam, Tanzania.

Received: 11 February 2021 Accepted: 9 August 2021 Published online: 06 September 2021

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https://doi.org/10.1186/s12879-021-05501-5
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