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DENV-2 Circulation and Host Preference Among Highly Anthropophilic, Outdoor-Biting *Aedes aegypti* in Dar es Salaam, Tanzania

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Abstract: In Tanzania, dengue outbreaks have occurred almost annually over the past decade, with each new outbreak becoming more severe. This study investigated the prevalence of dengue virus (DENV) serotypes in the wild *Aedes aegypti* and their blood sources to determine human exposure risk in Dar es Salaam, Tanzania. A two-year longitudinal survey was conducted in Ilala, Kinondoni, and Temeke districts of Dar es Salaam to sample *Ae. aegypti* mosquitoes using Biogents Sentinel trap (BGS), Prokopack aspiration and Gravid Aedes trap (GAT). Collected mosquitoes were pooled in groups of 10 and tested for DENV1-4 serotypes using reverse transcription polymerase chain reaction (RT-qPCR). Blood meal sources were identified using an enzyme-linked immunosorbent assay (ELISA). Of 854 tested pools, only DENV-2 serotype was detected in all districts (Temeke (3/371 pools), Ilala (1/206 pools) and Kinondoni (1/277-pools)). Blood meal analysis showed a strong preference for humans (81%) and mixed blood meals (17%). Out of 354 collected host seeking *Ae. aegypypti*, 78.5% were captured outdoors and 21.5% indoors. This study confirms the circulation of DENV-2 in *Ae. aegypti* populations, indicating a potential dengue outbreak risk in Tanzania. The mosquitoes' strong preference for human hosts and predominance in outdoor settings pose challenges for dengue control efforts.

Keywords: DENV; dengue fever; traps; blood feeding; longitudinal survey; serotypes; xenomonitoring

1. Introduction

Dengue fever is a significant global public health concern, responsible for hundreds and thousands cases of morbidity and mortality annually across tropical and sub-tropical regions [1]. Approximately 3.9 billion people, nearly half of the world's population are estimated to be at risk of infection [2]. The global incidence of dengue has risen dramatically in recent decades, with a record high occuring in 2023 [3,4] and 2024 [5]. Nearly 100 to 400 million new dengue cases occur each year [6], of which 90 million are manifested clinically, ranging from mild symptoms to the severe, life-threatening dengue haemorrhagic fever (DHF) and dengue shock syndrome (DSS) [6,7]. However, the vast majority of the cases are asymptomatic [8], leading to a likely underestimation of the true scale of the infection.

Dengue is now endemic in over 129 countries [9], with most cases occurring in Asia, which accounts for nearly two-third of the global burden, followed by the Americas and the African region

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[10]. In Africa, dengue cases are likely to be underreported [6] due to misdiagnosis as malaria or urinary tract infection (UTI). Even when clinically diagnosed correctly, many health systems lack sufficient diagnostic capacity to detect dengue virus (DENV) [11]. Human activities contributing to climate change, globalization, and unplanned urbanisation fuelled by rural to urban migration, accelerate the spread of dengue [12]. Additionally, it is predicted that with rapid expansion of intra- and intercontinental trade, the disease is expected to spread further, potentially tripling in the 50 years [13].

Dengue fever is caused by four antigenically distinctive virus serotypes (DENV 1-4) [14,15] which share around 65%-70% genome similarity [16,17]. The virus is an enveloped, single-stranded ribonucleic acid (ssRNA) virus belonging to the Flaviviridae family and *Flavivirus* genus [18,19]. It is transmitted from a viremic individual to another individual(s) through mosquito bites. Each serotype exhibits an independent virological characteristics, where the infection by one serotype does not confer cross immunity against the others [20]. Secondary infections with another serotype or mixed infection may potentially lead to severe forms of dengue, such as (DHF) or (DSS) [21–23]. The severity of the secondary infection is explained by the antibody-dependent enhancement (ADE) theory [24]: antibodies from a previous infection provide a long-lasting immunity against the same serotype but only temporary cross-protection against others. Thus, during a subsequent infection with a different serotype, this short-lasting immunity fails to neutralise the new serotype and forms an immune complex that facilitates viral entry into host cells, enhancing virus replication and increasing disease severity. Although all four dengue virus serotypes (DENV 1-4) circulate in Africa [25,26], DENV-2 is the most prevalent [27–29], likely due to its greater transmissibility [30,31] and greater susceptibility among local vectors [32]. These factors have important epidemiological implications.

Dengue fever is primarily transmitted through mosquito bites, with *Aedes aegypti* and *Aedes albopictus* serving as the primary and secondary mosquito vectors, respectively [33]. Both species are day bitters, which poses challenges for vector control because people are active at this time and most of mosquito interventions do not protect individuals during daytime [34]. In the absence of antiviral drugs and an effective universal vaccine [35], dengue prevention and control depends on vector control. Understanding *Ae. aegypti* host feeding preference and location is a critical aspect towards monitoring transmission and identifying potential virus reservoirs [36]. Studies show that *Ae. aegypti* primarily feed on humans [36,37], but in the presence of altenative hosts, they may also feed on other hosts [38–40]. This suggests opportunistic feeding behaviour, dependent on host availability. Dengue vectors may bite indoors or outdoors [41–44], with the mosquito endophilic resting behaviour [45] and adaptation to artificial light influencing indoor bitting [46].

In Tanzania, the first dengue incidence was reported in 1823 [25] and subsequent studies have confirmed its circulation [27,47–52]. Like other East African countries, Tanzania has all four dengue serotypes [50,53–55], which have likely driven the frequent dengue outbreaks in the country. Reports have indicated the co-circulation of multiple virus serotypes [55,56] as seen in the 2018/2019 outbreak with DENV-1 and 3 serotypes [55]. This shifting and co-circulation of dengue virus serotypes likely contribute to increasing disease severity. In the past decade, Tanzania has experienced several dengue outbreaks, with each being more severe than the former one. The deadliest dengue outbreak occurred in 2019, where about 7000 cases and 13 deaths linked to DENV-1 were reported [57,58]. In 2014, over 1,000 dengue cases and four deaths were recorded [59], with DENV-2 identified as the circulating serotype.

Despite frequent outbreaks recorded in recent years, dengue surveillance in Tanzania remains limited. In humans, most studies have reported dengue seroprevalence only during the outbreaks in Tanzania [48,60,61], leaving gaps in year-round data. Additionally, little information exists on the prevalence of dengue viruses in the mosquito population, indicating the possibility that the virus may be circulating in the mosquitoes posing a silent outbreak risk. Data for targeted control efforts including *Ae. aegypti* host preference and the location (indoors or outdoors) where these mosquitoes are most likely to feed are lacking.

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Therefore, this study aimed to determine DENV prevalence in mosquitoes to assess the risk of possible dengue outbreaks and the role of xenomonitoring for low cost and non-invasive surveillance. It also investigated the host preference and feeding location of wild *Ae. aegypti* to better understand the dengue transmission chain.

2. Materials and Methods

Study Area

The study was conducted in Dar es Salaam, Tanzania's largest economic hub (Figure 1). The city is located at 6.48'S and 39.17'E along the Indian Ocean coast with a population of nearly 5.5 million [62]. Administratively, it consists of five districts: Ilala, Kigamboni, Kinondoni, Temeke, and Ubungo. Based on previous dengue outbreaks [53,63], Ilala (1'649'912 people), Kinondoni (982'328), and Temeke (1'346'674) [62] were selected for this study.

Dar es Salaam has a tropical climate with high temperatures throughout the year and the hottest period occurring between October to February. It has one dry season and two rainy seasons, with an average annual rainfall of 1100 mm. The dry season spans from June to October while the short rainy seasons occurs from November to December, and the long rainy season extends from March to May[64].

Mosquito collection

Wild adult mosquitoes were collected from June 2022 to May 2024 using BioGents Sentinel (BGS) traps (Biogents AG, Regensburg, Germany) designated for catching host-seeking *Aedes* mosquitoes, Ovitraps (modified Biogents Gravid *Aedes* traps (BG-GAT; Biogents, Germany)) [65] for sampling gravid females, and Prokopack aspirators (John W Hock Company, Florida, USA) for collecting resting adults. Four wards were selected from each district. In each ward, 20 houses were identified, and the traps (one BGS and one GAT) were deployed in one house per day for 24-hours then the trapped mosquitoes were collected, followed by the collection of resting mosquitoes around the primeses using a Prockopack aspirator. This procedure was repeated monthly per each house for 24 months. The collected mosquitoes were morphologically identified to the species level following Wilkerson *et al* 2021 identification key[66]. Female *Ae. aegypti* were pooled in groups of 10 individuals and stored in 1.5 ml Eppendoff tubes containing RNAlater locally made at Swiss tph institute. The RNAlater was prepared by mixing 60 ml of 0.5 M EDTA with 37.5 ml of 1 M sodium citrate in 1,400 ml MilliQ water. Afterwards 1,050 g ammonium sulfate were added and the solution was filtered through a 0.2 µm filter.

Additionally, an experiment was conducted in six houses per month per district for three months to determine the abundance of host seeking *Ae. aegypti* mosquitoes indoors and outdoors. A pair of BGS traps were deployed indoors and outdoors for 24 hours, after which female mosquitoes were collected, and recorded based on their capturing location. The collected mosquitoes in this experiment were not analysed for blood meal.

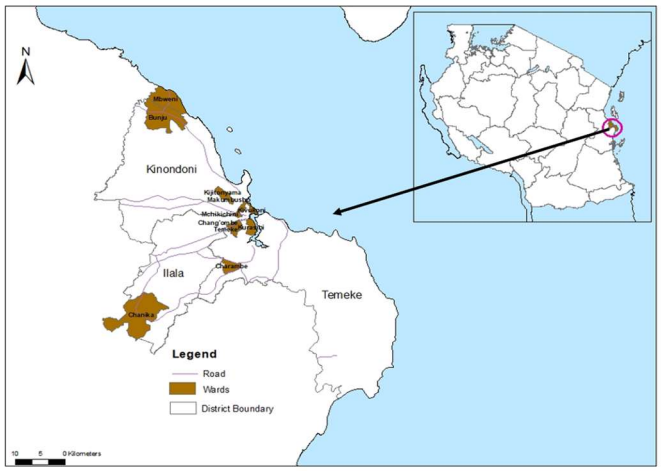

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Figure 1. Study sites of the mosquito collection. The collection of the mosquitoes was collected in three districts and four wards from each district.

RNA extraction and Dengue virus detection

RNA extraction

The extraction of RNA from mosquito pools was carried out using RNAzol® RT (Molecular research center, Cincinnati, Ohio USA) according to the manufacturer's instructions. Briefly, each pool of 10 individual mosquitoes were suspended in 200 µl of RNAzol in a 1.5 ml microcentrifuge tube, and manually ground using a sterile plastic pestle designated for grinding mosquitoes. The mixture was then centrifuged at 12,000g for 15 minutes, after which the supernatant was transferred to a new 1.5 ml microcentrifuge tube. An equal volume (200 ml) of 100% isopropanol was added to precipitate the RNA followed by incubation for 15 minutes and centrifugation at 12, 000 g for 10 minutes. The supernatant was removed and discarded. The RNA pellet was washed twice with 200 µl of 75% ethanol, centrifuged at 4,000g for 3 minutes and the ethanol was carefully removed. Finally, the RNA pellets were eluted with 50 µl of RNAse-free water and stored at -80^{0}C for molecular analysis using reverse-transcription polymerase chain reaction (RT-qPCR) [67].

Dengue virus detection

A one-step multiplex RT-qPCR [68] was performed using the CFX96 Bio-rad PCR machine (Bio-Rad Laboratories Inc. Hecules, California USA). The primers and probes used in the assay were adapted with modification from Balingit et all [69] (Table 1). The reaction was performed in 25 µl reaction volumes using the Luna® Universal Probe One-Step RT-qPCR Kit (New England Biolabd, Ipswich, Massachusetts, USA) consisting of 5 µl RNA template, 10 µl of Luna Universal Probe One-Step Reaction Mix (2X), 1 µl of Luna WarmStart RT Enzyme Mix (20X), 0.8 µl each of forward and reverse primers (10 µM), and 0.4 µl of probes (10 µM). Each sample was analysed in duplicates. The RT-qPCR cycling conditions were as follows: reverse transcription at 50˚C for 30min, initialization at 95˚C for 2min, followed by 45 cycles of denaturation at 95˚C for 15sec, and annealing/extension at 60˚C for 1min. RNAse-free water was used as a template for the negative control. Samples with average cycle threshold (Ct) higher than 37 were considered negative for either DENV serotype.

Blood meal source

Blood-fed *Aedes* mosquito samples collected over two sampling years and preserved in 1.5 ml Eppendorf microcentrifuge tubes containing locally made RNAlater were selected and tested for polyclonal anti-IgG antibodies targeting vertebrates commonly found in the study area, including humans, dogs, chickens and bovines, using an enzyme-linked immunosorbent assay (ELISA) as described by Beier *et al* [70]. Briefly, the abdomen of each mosquito was separated from the rest of the body parts and triturated in 1x phosphate buffered saline (PBS) using a handheld motorised micro-pestle (DWK Life Sciences, Faust Laborbedrf AG,Schaffhausen, Switzerlan) made for grinding mosquitoes. A 96-well ELISA plate (Greiner Bio-One Microlon™, Monroe, North Carolina, USA) was coated with 50 µl of Mab solution at 4 µg/ml and incubated for 30 minutes. After incubation, the contents were aspirated, and the excess liquid was removed by tapping the plate on a tissue paper. The wells were then filled with 250 µl of blocking buffer (BB) and incubated for 1 hour. Following this, the buffer was drained, and 45 µl of BB was dispensed into each well. Next, 5 µl of each sample was loaded into the wells containing the 45 µl of BB and incubated for 2 hours at room temperature. Same procedures were followed for positive and negative controls. After incubation, the plate contents were aspirated, and the wells were washed three times with 250 µl of washing buffer (PBS + Tween 20). A 50 µl aliquot of the appropriate conjugate solution was then added to each well and incubated for 30 minutes at room temperature. The conjugate was removed by washing the wells four times with 250 µl of washing buffer (PBS+Tween 20). Finally, 100 µl of substrate solution (2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid, ABTS) was added to each well, followed by a 30-minute incubation at room temperature.

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Table 1. Primers and Probes used for DENV serotyping from *Aedes aegypti* mosquito samples.

DENV serotype detected	Primer and probes	Nucleotide sequence (5′ → 3′)	Fluorophore and 3′ Quencher
DENV-1	DEN-1 forward	CAAAAGGAAGTCGTGCAATA	FAM
	DEN-1 reverse	CTGAGTGAATTCTCTCTACTGAACC	
	DEN-1 probe	CATGTGGTTGGGAGCACGC	
DENV-2	DEN-2 forward	CAGGCTATGGCACTGTCAC	HEX
	DEN-2 reverse	CCATTTGCAGCAACACCATC	
	DEN-2 probe	CTCTCCGAGAACGGGCCTCGACTTCAA	
DENV-3	DEN-3 forward	GGACTGGACACACGCACTCA	CY5
	DEN-3 reverse	CATGTCTCTACCTTCTCGACTTGTCT	
	DEN-3 probe	ACCTGGATGTCGGCTGAAGGAGCTTG	
DENV-4	DEN-4 forward	TTGTCCTAATGATGCTGGTCG	CY5.5
	DEN-4 reverse	TCCACCTGAGACTCCTTCCA	
	DEN-4 probe	TTCCTACTCCTACGCATCGCATTCCG	CY5/BHQ3

Data analysis

All data obtained were analysed using STATA package version 16 (Stata corp, College Station, TX).

DENV infection rate in mosquitoes

The infection rate was calculated by determining the proportion of DENV-positive mosquitoes among those tested by qRT-PCR. Usually, the minimum infection rate (MIR) and maximum infection rate (MaxIR) are computed as follows:

$$MIR = \left(\frac{x}{k}\right) * 1{,}000$$

$$\text{MaxIR} = \left(\frac{x*m}{k}\right) * 1{,}000$$

However, since MIR tends to underestimate and MaxIR overestimates infection rates, both are imprecise. Therefore, the Maximum Likelihood Estimate (MLE) with 95% confidance interval was used to provide a more accurate estimate.

$$\text{MLE} = -\frac{1}{m}\ln\left(1-\frac{x}{n}\right) * 1{,}000$$

where,

k = total number of mosquitoes tested

x = Number of positive pools

m = Number of mosquitoes per pool (assuming equal pool size)

n=total number of pools tested

Bloodmeal preference

A descriptive analysis was performed to compare the percentage of blood-fed mosquitoes across different hosts. The anthropophagy percentage was defined as the proportion of mosquitoes with human blood meals across all districts.

Host seeking preference

A descriptive analysis was performed to compare the proportion of host seeking mosquitoes collected indoors and outdoors. A negative binomial regression model was employed to determine if there was a statistically significant difference in host-seeking mosquitoes collected indoors versus those collected outdoors. The fix terms in the model were location (indoors vs outdoors), district and ward, while day and household were included as random effects. The models estimated the mean incidence rate ratios (IRR) and 95% confidence intervals around the means.

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3. Results

DENV serotypes 1-4 prevalence

A total of 854 pools, with 10 mosquitoes per pool, were tested for DENV. Of these pools, 371 were from Temeke, 206 from Ilala and 277 from Kinondoni district (Table 2). DENV serotype 2 (DENV-2) was detected in all the three districts, with Temeke having the highest infection rate 0.81 per 1000 mosquitoes (Table 2). This likely indicates that the population in Temeke district is at a higher risk of contracting dengue fever compared to those in Ilala and Kinondoni districts. The viruses were detected in all years of mosquito sampling (Table 2). Indicating that the viruses are existing in the study area and not introduced.

Table 2. DENV serotype detected from pooled *Aedes aegypti* mosquitoes in Dar es Salaam.

Districts	Mosquito samples				
	Pools tested	Positive	DENV-serotype	*Detection year*	*Infection rate per 1000*
Temeke	371	3	DENV-2	2023 & 2024	0.81 (0.18, 2.39)
Ilala	206	1	DENV-2	2024	0.49 (0.012, 2.80)
Kinondoni	277	1	DENV-2	2023	0.39 (0.009, 2.20)
Total	**854**	**5**	**DENV-2**	**2023 & 2024**	**0.41 (0.013, 1.10)**

Host preference

A total of 298 mosquito samples were tested for the origin of their blood meals from human, dog, chicken, and cow. Of these, 68.8% tested positive for either one or more blood meal sources (hosts) in the ELISA test, while 31.2% samples showed no reaction.

Aedes aegypti showed a strong preference for human blood, with approximately 166 mosquitoes (81.0%) feeding on humans, followed by chicken (1.5%) and dog (0.5%) (Figure 2). About 17% of the mosquitoes had taken a mixed blood meals from human and other hosts while none had fed on cow (Figure 2). The majority of blood-fed mosquitoes (69.3%) were collected using the Prockopack aspirator, followed by BGs trap 27.8% and GAT traps 2.9% (Table 3).

Table 3. Blood-fed *Aedes aegypti* mosquitoes collected by trap type.

Traps	BGS trap	Prokopack aspirator	GAT
Blood fed *Aedes aegypti*	57	142	6
Percentage blood fed	27.8	69.3	2.9

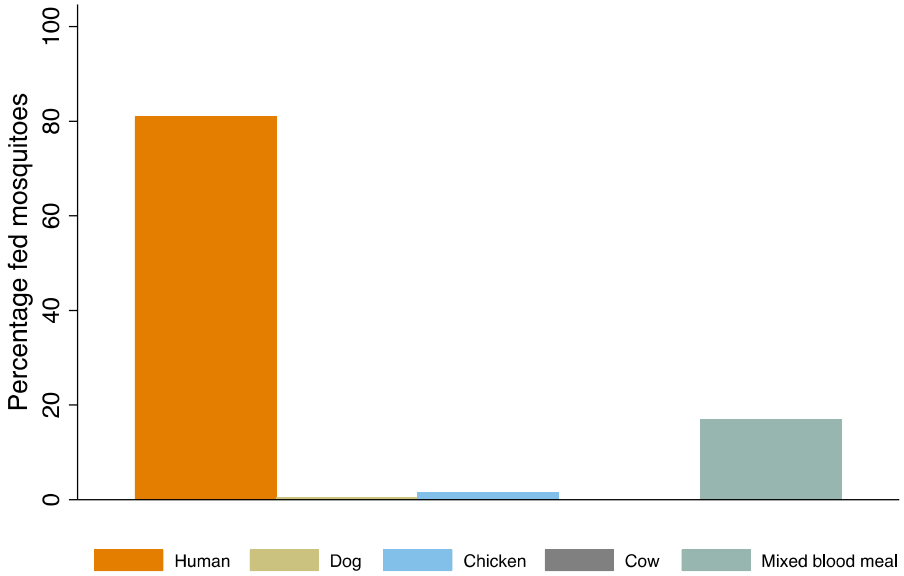

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Figure 2. Host-feeding preference of *Aedes aegypti*. *Mixed blood meal refers to blood meals from human and other hosts.

Host seeking Aedes aegypti mosquitoes

Using BGS traps, 354 female *Ae. aegypti* mosquitoes were collected from both indoor and outdoor locations. More than three quarters 78.5% (n=278) were caught outdoors, while 21.5% were collected indoors. The outdoors mosquito count was 4.33 higher than the indoor count (95%CI: [2.38-7.89], p-value<0.001) (Table 4). Among the three districts, Temeke had the higher count than Ilala and were statistically significant (Table 4).

Table 4. Percentage and incidence rate ratio (IRR) of host-seeking *Aedes aegypti* collected indoors and outdoors.

	N	n(%)	IRR (95%CI)	P-value
Collection location				
Indoors	54	76 (21.5)	1	-
Outdoors	54	278 (78.5)	4.33 (2.38-7.89)	<0.001
Districts				
Ilala	18	42 (11.9)	1	-
Kinondoni	18	135 (38.1)	4.24 (1.98-9.06)	<0.001
Temeke	18	177 (50.0)	5.03 (2.39-10.58)	<0.001

Legend: N= collection days, n=number of mosquitoes, IRR=Incidance rate ratio, 95%CI=95% confidence interval.

4. Discussion

Understanding pathogens circulation in vectors is crucial for disease control. This study reports the presence of DENV-2 circulating in mosquitoes from Dar es Salaam city throughout the survey period, suggesting ongoing virus circulation rather than a new introduction. The virus serotype reported in this study is the same as the one detected in 2014 outbreak [53], highlighting the possibility that the virus has been persistent in the ecosystem since then. However, whole genome sequencing would be required to elucidate whether this is the case or if a new introduction of a different DENV-2 genotype occured.

All four DENV serotypes (DENV 1-4) have circulated in Tanzania [50], with different serotypes predominating in each outbreak. This shifting pattern may explain the increasing number of dengue cases and deaths during subsequent outbreaks [58], a trend also observed in West Africa [71] and other endemic regions [4]. In 2019, WHO reported a dramatic increase in dengue cases across several countries in Africa, particularly in the sub-Saharan region [72]. This rise reflects a broader global increase in dengue in all global WHO regions [6]. Despite the growing evidence, African countries including Tanzania, lack comprehensive data on the exact magnitude of dengue virus distribution due to limited epidemiological, entomological and virological surveillance, since dengue is a neglected tropical disease and African vector control efforts focus mainly on malaria.

Genotyping studies have shown a relatedness of dengue virus genotypes detected in East Africa, particularly Tanzania [53,59] and Kenya [73,74], to virus genotypes from Asian countries such as India and Singapore [55] as well as China [59]. This indicates that the viruses are being imported from the East via international travel and trade [59]. Africa's rapid population growth and urbanisation will likely further accelerate virus spread. By 2050, nearly 60% of the continent's population is expected to live in cities [75]. Increased human mobility and urbanisation will be inevitable, therefore, deliberate dengue monitoring efforts are needed. In this context, routine screening at national and international entry points could be implemented to reduce introductions of new virus genotypes, although this may be cost prohibitive.

This study identified Temeke, Ilala and Kinondoni districts as areas with dengue infected-mosquitoes, suggesting that these are priority areas for dengue xenomonitoring. The infected mosquitoes indicate a potential risk of dengue outbreaks in these areas, necessitating proactive *Aedes*

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mosquito surveillance. Additionally, it emphasizes the need for the government authorities to implement dengue control measures such as larval source reduction, targeted insecticide spraying, and educating citizens on larval control and the importance of seeking health care in case of potential dengue like symptoms. Furthermore, it highlights the need for the implementation of the International Health Regulations 2005 (IHR) to reduce further risk of transmiting viruses to other areas [76]. The IHR is a WHO legal framework for managing public health events and emergencies that have the potential to cross borders.

In Tanzania, dengue prevalence often exceeds 10% in human samples [48,59,60,77]. This study reports a minimal 0.08% dengue mosquito infection rate, lower than what was reported by Mboera *et al* [53] in 2014 during an outbreak. However, in that study, they collected larvae instead of adults, which might has overestimated the infection rates, given they do not represent the host-seeking population, and the samples could have been biased if siblings from the same transovarilly-infected egg batch were sampled. The findings from the present study align with those of Chilongola *et al.* [78] and Joseph *et al*. [79] in East Africa, Mojica *et al*. [80] in Nicaragua and Ecuador in Latin America, and Maneerattanasak *et al.* [81] in Southeast and South Asia. Similarly, this is consistent with reports on other female arthropod-transmitted diseases such as malaria [82,83], where *Plasmodium* infection rates in *Anopheles* mosquitoes are typically very low even in high endemic areas.

This study has demonstrated that, *Ae. aegypti* from Dar es Salaam are highly anthropophagic, with nearly more than 80% feeding on humans. This behaviour significantly increases dengue transmission risk, as human-mosquito contact is a key driver of virus spread [84]. The findings of this study are consistent with research from West Africa (Senegal and Burkina Faso) [41,85,86] as well as India [87], South East Asia (Thailand) [36], Australia [88], Latin America (Brazil, Equador and Peru) [89] and North America (USA) [90].

Aedes aegypti aegypti (*Aaa*) and *Aedes aegypti formosus (Aaf)* are the *Ae. aegypti* subspecies commonly found in Africa[91]. *Aaa* is considered an urban mosquito primarily responsible for urban dengue and yellow fever transmission [92]. *Aaf* inhabits peri-urban environments and serves as an agent for sylvatic dengue as well as yellow fever transmission [92,93], is less competent for dengue [94] and less anthropophilic [95]. In this study, we were unable to distinguish between the two subspecies. Given the lack of comprehensive research on their coexistence in the country, our findings emphasize the need for genomic studies to accurately characterize them.

As reported in other studies [40,41,85], human-animal mixed blood meals were frequently observed in this study, highlighting the feeding flexibility of *Ae. aegypti*. This mosquito species is known for transmitting dengue virus and other viruses, including yellow fever virus (YFV), Chikungunya virus (CHIKV) and Zika virus (ZIKV), all of which pose a substantial public health burden. While the ability of *Ae. aegypti* mosquitoes to feed on multiple animal species has implications for virus transmission, the role of animals as reservoirs for DENV, CHIKV and ZIKV in urban settings remains unlikely. While YFV does have non-human primates that serve as reservoir hosts [96], saving as a potential virus spillover source. Studies have described systems demonstrating the potential co-infection of DENV serotypes in an individual [97,98]. With all four DENV serotypes circulating in the country [50], it is likely that the mosquitoes' ability to feed on multiple human hosts could essentially lead to individuals being infected with more than one virus serotype. This co-infection may result in complications, including dengue haemorrhagic fever (DHF) and dengue shock syndrome (DSS).

Moreover, the location where mosquitoes seek blood meals from hosts and rest after feeding has significant implications for the application of interventions to control mosquito vectors. This study demonstrated that more than three quarters of *Ae. aegypti* preferentially seek blood meals outdoors, highlighting a challenge in controlling this vector because the majority of mosquito control interventions, particularly those developed for malaria control, are applied indoors, targeting indoor host seeking and resting mosquitoes. This finding is similar to studies of *Ae. aegypti* in Burkina Faso [99,100] and Ghana [101]. This indicates that larval source reduction [102] might be the most likely intervention to be successful at the community level. In addition, the release of Wolbachia has shown

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excellent efficacy in preventing dengue when deployed at a city scale [103]. Further work is ongoing to measure the resting behaviour and susceptibility of the Dar es Salaam population of *Ae. aegypti* to insecticides used for mosquito control.

Study Limitations

To fully understand the indoor and outdoor ecology of *Ae. aegypti*, traps targeting host-seeking, resting and oviposition behaviours need to be deployed concurrently both indoors and outdoors. However, we were unable to collect indoor mosquitoes using GATs because of the smell of the infusion, and with Prokopack aspirators due to house entry restrictions, as most of the time the residents were absent. As a result, we collected mosquitoes with only BGS, a trap type designated for collecting host-seeking mosquitoes. Therefore, we recommend that for future studies, the indoor and outdoor mosquito collection should also include resting collections because the use of traps with a lure may bias the collections towards human-fed mosquitoes [89]. Additionally, blood meal analysis for host preference was performed on only four hosts (humans, dog, chicken and cow). However, we found that 93 samples did not react suggesting that the mosquitoes may have contained blood meals from hosts not included in the analysis. In Kenya, *Ae aegypti* has been found to feed on goats, rats and cats [104], which were also present in the present study site, but not tested for. Therefore, future studies should include a broader range of potential hosts.

5. Conclusions

This study confirms the circulation of DENV-2 in the mosquito population in Dar es Salaam, highlighting the risk of a potential dengue outbreak in Tanzania. Dar es Salaam is one of Africa's major metropolitan cities, with a population of nearly six million. It serves as the economic hub of Tanzania, so it experiences a significant influx of local and international travellers. The presence of DENV-2 in mosquitoes as well as the strong human feeding preference of *Ae. aegypti* indicates the potential risk of DENV transmission to humans. These findings emphasise the need for enhanced surveillance and targeted proactive vector control measures including removal of breeding sites to mitigate dengue outbreaks.

6. Patents

Author Contributions: Conceptualisation: FSCT and SJM; Data curation: FSCT, OD, MH, and HM; Formal analysis: FSCT; Investigation: FSCT, OD, MH, HM, SH, LDB, JM, JJM, NSL and TGM; Methodology: FSCT, PM and SJM; Supervision; SJM; Writing original draft: FSCT; Visualization: SJM, PM, SH and LMH; Writing review and editing SJM, PM, SH and LMH; Funding acquisition: SJM. All authors read and approved the final manuscript draft.

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Abbreviations

BGS	Biogent sentinel trap
GAT	Gravid *Aedes* trap
DENV	Dengue virus
DHF	Dengue haemorrhagic fever
DSS	Dengue shock syndrome
ITN	Insecticide treated net
IRS	Indoor residual spray
DF	Dengue fever
YF	Yellow fever
CHIK	Chikungunya
ZIK	Zika
IHR	International health regulations
WHO	World health organisation
IRR	Incidence rate ratio
OR	Odds ratio
ELISA	Enzyme-linked immunosorbent assay.
qRT-PCR	Quantitative reverse transcriptase polymerase chain reaction
MIR	Mosquito infection rate

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