



Dengue Vector Distribution and their Infection Status in Selected Regions in Tanzania

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Abstract

Dengue fever is an important arboviral infection globally. Tanzania has experienced multiple dengue outbreaks since 2010 leading to mortalities and public distress. Dengue is caused by viruses which are transmitted to humans by vector mosquitoes. Nevertheless, the abundance, distribution and extent of viral infections of these vectors are poorly understood. The aim of this study was to characterize dengue vectors and determine their virus infection rates. Adult mosquitoes were collected from selected regions in Tanzania including Dar es Salaam, Tanga, Coast, and Arusha regions using CO₂ baited mosquito magnet traps between December 2018 to February 2019, additional samples were collected during dengue epidemic in June 2019. Samples were fixed in RNA later and preserved at -20 °C for virus detection using Reverse Transcriptase – Polymerase Chain Reaction (RT-PCR). A total of 1530 mosquitoes were collected and morphologically identified as: *Aedes* (75%), *Culex* (13%), *Anopheles* (10%) genera and 2% other mosquitoes. All *Aedes* mosquitoes were identified as *Aedes aegypti* of which 46% were from Dar es Salaam, 31% Tanga, 21% Coast and 2% from Arusha. The abundance of *Ae. aegypti* significantly differed across sampling locations ($p = 4.6425E-09$), and the virus infection rate was 2%. The presence of these vectors and the detection of dengue viruses is an indication of ongoing arboviral activities necessitating intensification of vector control programmes.

Keywords: *Aedes aegypti*, dengue vectors, arboviruses, Tanzania

Introduction

Dengue fever is an important arboviral infection that causes public health burdens in tropical and subtropical regions of the world. Dengue fever global incidence has increased dramatically in the recent past and more than half of the world's population is at risk of contracting the disease, up to 400 million dengue cases occur each year (Guha-Sapir and Schimmer 2005). The risks of severe cases and deaths from dengue fever have increased tremendously and the associated

economic burdens are a strong impediment especially in developing countries. Tanzania has experienced re-occurring dengue outbreaks in 2010, 2012, 2013, 2014 and the recent one occurred in the year 2018 through 2019 resulting in economic and public health distress. The 2018/2019 dengue epidemic was the worst, in which 1,222 people were diagnosed with dengue fever; this total exceeded the previous 2014 outbreak in which a total of 961 cases were confirmed. For all the previous dengue outbreaks, Dar es

Salaam city has been the epicenter of the epidemics (Mirembe 2019).

The reasons for emergence and re-emergence of dengue and other arboviral infections are multifactorial and some are unknown but mosquitoes have been incriminated for the transmission of arboviruses. Due to proximity with humans, mosquito vectors spread many pathogens causing infections between people and other animals. Globally, mosquitoes in the genus *Aedes* such as *Aedes aegypti* and *Aedes albopictus* have been proved to be responsible for transmission of several globally important arboviruses such as dengue, rift valley fever, zika, chikungunya, Nile encephalitis virus, yellow fever and dengue haemorrhagic fever (Braack et al. 2018, Failloux et al. 2002, Kasili et al. 2009). Our previous studies in Dar es Salaam (Mathias et al. 2017, Philbert and Ijumba 2013) recorded high density of *Ae. aegypti* in artificial containers around human habitations. These vectors have also been reported in other parts of the country (Bisimwa et al. 2016). Other species in the *Aedes* genera are also capable of transmitting these viruses, for example during the yellow fever outbreaks in East Africa, *Ae. simpsoni* species was incriminated for transmission of yellow fever viruses (Failloux et al. 2002). Apart from mosquitoes in the *Aedes* genera, arboviruses have also been isolated from several other species such as *Anopheles* spp and *Culex* spp. Some of these secondary vector species are also present in Tanzania. The presence of these vectors is an indication of ongoing arboviral activities that lead to high seroprevalence of chikungunya and dengue fevers reported from other parts of the country (Kajeguka et al. 2016).

Apart from dengue fever, Tanzania has had in the past multiple epidemics of other arboviral infections also vectored by *Ae. aegypti* mosquitoes such as yellow fever and rift valley fever (Bisimwa et al. 2016). Other

studies have attempted to detect arboviruses from mosquito vectors following previous arboviral infections outbreaks in the country (Bisimwa et al. 2016, Bisimwa et al. 2018, Mboera et al. 2016). Specifically, Bisimwa et al. (2016) detected only chikungunya viruses in mosquito vectors, although the team screened for screened for Chikungunya, dengue, yellow fever and rift valley fever viruses. Moreover, Mboera et al. (2016) reported the viral infection rate of 8% dengue viruses in *Ae. aegypti* mosquitoes following the 2014 dengue outbreak in Dar es Salaam. In addition, Bisimwa et al. (2018) reported 47.6% and 30% infection rates for dengue and chikungunya viruses, respectively from *Ae. aegypti* mosquitoes collected in Kagera, Mwanza, Morogoro and Mbeya regions. Nevertheless, the extents of viral infections in vector mosquitoes in other parts of the country have not been well established. Currently, there is no treatment and/or vaccine for dengue fever, and the problem is further compounded by lack of proper diagnostic procedure of the disease (Bouloy and Weber 2010), therefore, vector control remains the most viable approach. Vector control has led to the decrease of malaria prevalence in various countries (O'Meara et al. 2010), it is therefore a promising approach to pursue. Nonetheless, for effective control of vectors, it is essential that potential arboviruses vectors present in the region are identified for surveillance to monitor their species composition, species abundance and their viral infection status to establish the hot spots for target control should an outbreak occur. The aim of this study therefore, was to characterize dengue vectors, establish their viral infection rates through virus detection in regions where arboviral epidemics have occurred in the recent past in Tanzania. The findings are important for management of dengue infections and other vector borne diseases in the country.

Materials and Methods

Study area and design

A cross-sectional study was conducted to collect flood water mosquito species (*Aedines*) from regions that had reported dengue fever and other arboviral infections epidemics in the recent past. The selected regions included Dar es Salaam, Tanga, Coast, and Arusha. In each region, there were three locations for adult mosquito collection as indicated in Figure 1. The sampling locations were: Dar es Salaam (Sinza,

University of Dar es Salaam [UDSM] and Tegeta), Coast (Vikindu, Vianzi and Mkuranga), Tanga (Bombo Hospital, Nguvumali and Muheza), Arusha (Njiro, Moshono, and Mto wa Mbu). Sampling was carried out between December 2018 and February 2019. For Dar es Salaam region, mosquito sampling was again carried out in June 2019 during the dengue epidemic. Recording was done using mosquito sampling forms and each sampling site was georeferenced using GPS.



Figure 1: Map of Tanzania showing mosquito sampling locations.

Mosquito sampling

The collection of adult mosquitoes was done using the CO₂ baited mosquito magnet traps. Traps were fixed outdoor, in the morning at 06.00 am and were removed in the evening at 07.00 pm for three consecutive days in each sampling location. From the trap, adult mosquitoes were siphoned using aspirators, placed in paper cups and maintained on 10% glucose soaked in water until in the evening. The aspirated mosquitoes were euthanized using chloroform. Using the published morphological keys (Darsie and Samanidou-Voyadjoglou 1997, Gillies and De Meillon 1968), specimens were sorted according to their respective species (*Aedes* mosquitoes) and genera (other mosquitoes) under the stereomicroscope. The mosquitoes were pooled into groups of 10 individuals according to their sex, species and or genera, place of collection and placed in a well labelled 1.5 ml eppendorf tube. The mosquitoes were preserved in RNA later (Ambion, Inc., Texas, US), maintained under liquid nitrogen and later transported to the Tanzania Veterinary Agency Laboratory, Tanga and kept in the freezer at -20 °C for later detection of the viruses using RT-PCR.

RNA extraction from mosquitoes

Mosquitoes were pooled, each pool contained ten mosquitoes collected from the same sampling location and of the same species mainly *Aedes aegypti*. Two pools of the samples were *Culex quinquefasciatus* mosquitoes collected from Tanga. For each pool, samples were ground in 1.5 ml eppendorf tube using a micro-pestle before RNA was extracted using Direct-zol RNA minPrep kit (ZYMO research, USA) following manufacturer's instructions. Briefly, the crushed samples were homogenized using bashing beads from the kit and the zymo-spin column I was loaded to make a volume of 700 µl. The mixture was centrifuged for 1 min at 13000 rpm (Eppendorf AG 22331 Hamburg, Germany)

at room temperature to remove debris and the supernatant was transferred to a new tube. The supernatant was diluted using equal volume of 95% ethanol, transferred into a zymo-spin column II and centrifuged again for 1 min at 13000 rpm (Eppendorf AG 22331 Hamburg, Germany) at room temperature. The suspensions were digested with 5 µl DNase I, 75 µl DNA digestion buffer in the presence of 400 µl RNA wash buffer and incubated at room temperature for 15 minutes. Afterwards, 400 µl Direct-zol RNA pre-wash was added to the column and centrifuged for 2 min at 8000 rpm at room temperature. The flow was discarded followed by addition of 700 µl RNA wash buffer, centrifuged again for 2 minutes at 8000 rpm at room temperature. The content was then transferred to the RNase free tube and eluted by adding 50 µl RNase-free water and centrifuged. The extracted RNA was immediately stored at -70 °C for subsequent RT-PCR reactions.

Reverse Transcriptase – Polymerase Chain Reaction (RT-PCR) followed by Real Time PCR

The quantitative detection of dengue viruses specific RNA was done using Real Star RT-PCR Kit (Altona Diagnostic GmbH Hamburg, Germany) 3.0 in AriaMax real time PCR system (Agilent Technologies, USA). In the reaction mixture, each of the 30 µl of PCR consisted of 5 µl of master A, 15 µl of the master B and 10 µl of RNA extract. The PCR thermal cycling conditions were reverse transcribed at 55 °C for 20 minutes, followed by 95 °C for 2 minutes of denaturation and then cycling stage of 45 cycles at 95 °C for 15 seconds, 55 °C for 45 seconds and 72 °C for 15 seconds. The detection and amplification of dengue virus was performed using primers D1(TCA ATA TGC TGA AAC GCG CGA GAA ACC G) and D2 (TTG CAC CAA CAG TCA ATG TCT TCA GGT TC) as previously described in Bisimwa et al. (2018). Results were interpreted as positive when quantitative cycle (Ct / Cq)

value obtained was less than 36 and the internal controls were positive. If the sample showed no amplification signal in the detection system but the internal control is positive, the sample was considered negative. Experiments for RNA extraction and virus detection were conducted at Tanzania Veterinary Agency Laboratory, Tanga branch.

Data analysis

Entomological and molecular data were summarized in descriptive statistics and presented in graphs and tables. The mosquito abundances collected from different sampling sites were determined by calculating the relative density in which $RD = NA/N \times 100$; where RD = relative density, NA = number of all specimens of each species collected from each location, and N = the number of specimens of all species collected. The comparison of mosquito abundance from different sites was computed by χ^2 test, the software used is R 3.6.1 at 95% Confidence Interval (CI).

Results

A total of 1530 adult mosquitoes were collected from Dar es Salaam, Coast, Tanga, and Arusha regions. The composition of the mosquitoes collected by their generic names is presented in Figure 2.

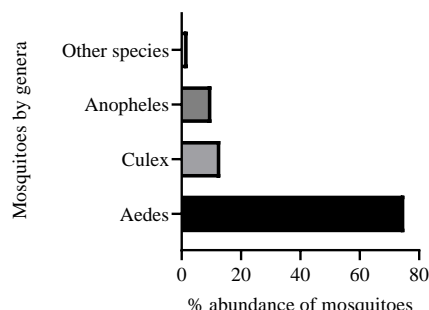


Figure 2: Percentage composition of mosquitoes by their generic names.

Mosquitoes in the *Aedes* genera constituted 75% of the total collection (Figure 2). Other genera include *Culex* and *Anopheles* with 13% and 10%, respectively. Nevertheless, the focus of this study was on major dengue vectors; therefore, only the mosquitoes in the *Aedes* and *Culex* genera were identified to species level. A total of 1150 and 20 mosquitoes were respectively identified as *Aedes aegypti* and *Culex quinquefasciatus*. The abundance of *Ae. aegypti* (in percentage) collected from each region is presented in Figure 3; and the percentage abundance and distribution of *Ae. aegypti* collected from various sampling locations is presented in Figure 4.

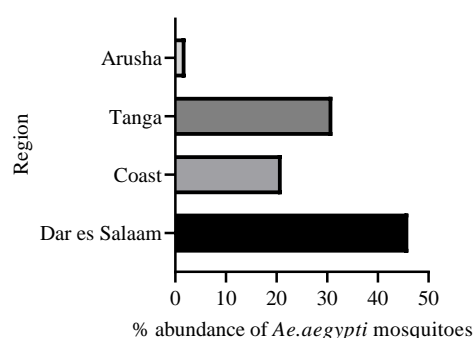


Figure 3: Percentage distribution of *Aedes aegypti* mosquitoes in different regions.

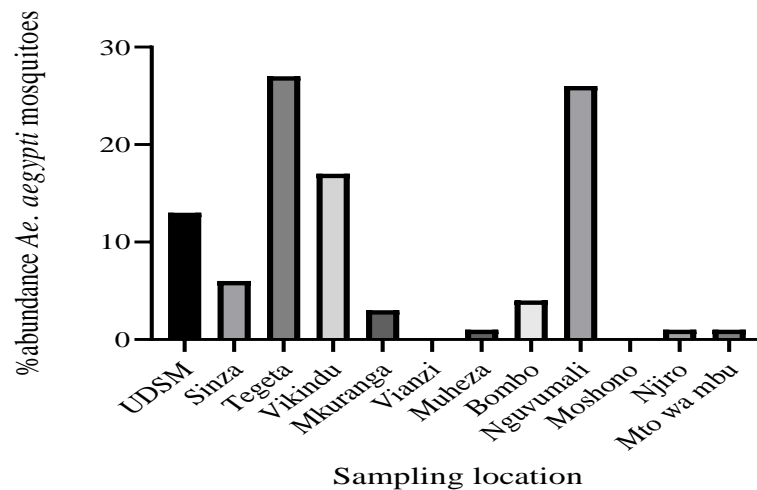


Figure 4: The percentage abundance and distribution of *Aedes aegypti* mosquitoes from all the sampling locations.

In Dar es Salaam region, significant variations in mosquito abundance was observed ($\chi^2 = 41.7013$, DF = 2, $p = 0.0245$) with further analysis showing significant numbers to exist in Tegeta, and no significant variations between UDSM and Sinza ($\chi^2 = 1.2759$, DF = 1, $p = 0.2587$). In Coast region, the abundance of *Ae. aegypti* mosquitoes was relatively higher at Vikindu ward as compared to Mkuranga ward sampling locations and the difference was statistically significant ($\chi^2 = 4.4545$, DF = 2, $p = 0.03481$), and no specimen was recorded in Vianzi ward. For Tanga region, very few mosquitoes were trapped in Muheza sampling location, in most cases there were no mosquitoes in the traps or there were *Culex* mosquitoes. Nevertheless, there was a significant difference in mosquitoes abundance collected in Tanga region ($\chi^2 = 18.0621$, DF = 2, $p = 0.0001$) with many mosquitoes being collected from Nguvumali, and no significant difference between Bombo hospital and Muheza sampling locations ($\chi^2 = 1.1644$, DF = 1, $p = 0.2805$).

The abundance of *Ae. aegypti* in Arusha region was very low, a total of 20 *Ae. aegypti*

mosquitoes were collected from the 3 sampling sites. All the sites in Arusha showed no significant difference in mosquito abundance ($\chi^2 = 0.2280$, DF = 2, $p = 0.8922$). Similarly, there was no significant difference in abundance between the sampled genera ($\chi^2 = 1.3592$, DF = 3, $p = 0.7151$). However, the difference in abundance between the sampling locations was significant ($\chi^2 = 41.7013$, DF = 3, $P = 4.6425E-09$). The information on abundance and statistical analyses of *Ae. aegypti* from different location is presented in Table 1.

Detection of Dengue virus by RT-PCR

Dengue viruses (DENV) were detected from 2/99 pools screened, a total of 160 mosquitoes were moribund and were not screened for virus detection, the positive pools contained specimen that were collected at Tegeta during the 2019 dengue epidemic, therefore the viral infection rate was 2%. The results showing number of mosquitoes and their corresponding pools screened for detection of the DENV by RT-PCR are summarized in Table 2.

Table 1: Percentage abundance and distribution of *Ae. aegypti* mosquitoes between the sampling locations collected between December, 2018-February and June, 2019

Region	Location	Abundance (N)	Abundance (%)	p Value
Dar es Salaam	UDSM	150	13	0.0245*
	Sinza	70	6	
	Tegeta	310	27	
Tanga	Nguvumali	300	26	0.0001*
	Muheza	10	1	
	Bombo hospital	50	4	
Coast	Vikindu	200	17	0.03481*
	Mkuranga	40	3	
	Vianzi	0	0	
Arusha	Moshono	2	0	0.8922
	Njiro	8	1	
	Mto wa Mbu	10	1	
Overall analysis of <i>Ae.aegypti</i> abundance	All	N = 1150	100	4.6425E-09*

* = significant at 95% CI

Table 2: Number of individual mosquitoes and the pools tested for DENV infection in vector species by study sites

Sampling regions	Sampling sites	Pool number	Number of individuals tested	Species	DENV	
Dar es Salaam	UDSM	1-15	150	<i>Ae.aegypti</i>	-	
	Sinza	16-22	70	<i>Ae.aegypti</i>	-	
	Tegeta	23-53	300	<i>Ae.aegypti</i>	+	
Tanga	Nguvumali	54-73	280	<i>Ae.aegypti</i>	+	
		Muheza	74	10	<i>Ae.aegypti</i>	-
		Bombo	75-79	40	<i>Culex</i>	-
Coast	Vikindu	80-95	160	<i>Ae.aegypti</i>	-	
		Mkuranga	96-98	30	<i>Ae.aegypti</i>	-
					<i>Culex</i>	-
Arusha	Moshono	99	10	<i>Ae.aegypti</i>	-	
		Njiro			<i>Ae.aegypti</i>	-
		Mto wa Mbu			<i>Ae.aegypti</i>	-
Total	All	99	990		2	

DENV = Dengue Virus, + = positive, - = negative

Discussion

The composition of mosquitoes collected from all the four regions (Dar es Salaam, Coast, Tanga and Arusha) was dominated by *Aedes* genera (75%); others were *Culex*

(13%) *Anopheles* (10%) and 2% were mosquitoes from other genera. The preponderance of *Aedes* mosquitoes in this study is attributed to sampling techniques employed including the traps used (mosquito

magnet traps) and the time of sampling (day time) and sampling outdoor. The findings are contrary to the findings of the previous work by Mboera et al. (2016) following the 2014 dengue outbreak in Dar es Salaam, and a Brownsville, Texas study that characterized arboviral vector species abundance in attempt to mitigate chikungunya, dengue and zika viruses (Srinivasan et al. 2017). Both studies reported *Culex quinquefasciatus* as a dominant species and *Ae. aegypti* was a secondary vector. *Aedes aegypti* initially regarded to be an urban species is a common outdoor breeding mosquito even in rural areas and it is rapidly expanding its geographical range around the globe. Assessments of arbovirus vectors distribution and their roles in virus transmission are fundamental aspects for the determination of high risk areas for target control should an outbreak occur. Considering that the current control interventions for adult vectors such as Indoor Residual Sprays (IRS) and Insecticide Treated Nets (ITNs) target vectors that feed and rest indoors, *Ae. aegypti* species is known to bite outdoor and increasingly adapting outdoor environment (Saifur et al. 2012), therefore is not likely to be controlled by the existing interventions. It is important that interventions specific for targeting this particular species be designed to complement the existing ones.

The distribution of *Ae. aegypti* by places of collection shows that high vector abundance was recorded in Dar es Salaam and Tanga constituting 46% and 31% of the total collections, respectively, others were Coast region (21%) and Arusha (2%). Previous studies in Tanzania reported high abundance of *Ae. aegypti* in urban Dar es Salaam (Mathias et al. 2017, Mboera et al. 2016, Philbert and Ijumba 2013), but the vectors are also available in rural areas (Bisimwa et al. 2018, Trpis 1972), and this indicates that residents are at high risk of contracting dengue and other arboviral infections should outbreaks occur. Dar es Salaam has been the

epicenter for all the previous dengue epidemics (Mboera et al. 2016), and this is attributed to the availability of many mosquito breeding habitats in the area (Mathias et al. 2017, Mboera et al. 2016, Philbert and Ijumba 2013) and optimum temperature that favours vector occurrence and survival (Kraemer et al. 2015). The findings from this study corroborate the previous studies and suggest that, Dar es Salaam is consistently at higher risk for dengue and other arboviral infections transmission.

Dengue viruses were detected in *Ae. aegypti* mosquitoes, and the infection rate was 2%. Although dengue viruses were detected in *Aedes* mosquito pools collected at Tegeta, Dar es Salaam, the occurrence of the same vectors in many other Tanzanian regions and their roles in transmission and maintenance of viruses cannot be disregarded. It was interesting also to note that viruses were only detected from the samples collected during the 2019 dengue outbreak in Dar es Salaam. All the samples collected during the period when there were no outbreaks tested negative for the virus. This implies that there are hosts other than mosquitoes that maintain viruses in circulation for the periods when there are no outbreaks. Such reservoir hosts need to be identified for the purpose of control of dengue and other arboviral infections vectored by *Ae. aegypti* mosquitoes. A similar study that was conducted in Dar es Salaam by Mboera et al. (2016) reported 8% infection rate of dengue viruses in vector mosquitoes collected. Elsewhere, *Ae. aegypti* has been implicated for transmission of arboviruses during epidemics (Dupont et al. 2012, Rasheed et al. 2013). Nonetheless, the low virus infection rate in this study could also be attributed to the time lag between sample collection and laboratory analysis.

Dengue viruses spread has increased dramatically over the last 50 years, the

disease is spreading to new geographic locations and is increasing in incidence within their range (Weaver 2014), their expansion poses significant health threats to humans. The global expansion of dengue and other arboviruses is of public health importance due to lack of proper diagnosis especially in developing countries, lack of treatment (antivirals) and or vaccines. Studies show that the global expansion of arboviruses was preceded by the global expansion of their vectors, namely *Aedes aegypti* and *Aedes albopictus*. The global expansion of dengue vectors *Aedes aegypti* is traced back during the slave trade and believed to have originated in Africa and spread to the tropical and subtropical regions of the world (Brown et al. 2014). Dengue vectors and the virus expansion are also associated with trade and travel via shipping, their spread being driven by human movements and transport routes. The spread is also made possible due to the anthropophilic behaviour of *Aedes aegypti*. Indeed, the global spread of dengue and other arboviral infections have undoubtedly been consequences of increasing global interconnectedness. In that regard, as these processes continue and the world becomes increasingly connected and urbanized, the risks of importation and subsequent introduction of arboviruses to new areas will definitely continue to increase (Hofhuis et al. 2009, Khan et al. 2014, Kraemer et al. 2015). This calls for continuous surveillance of all potential vectors. The detection of dengue viruses for samples collected from Dar es Salaam is an evidence of active circulation of the virus in the region. The findings further suggest that residents of Dar es Salaam, especially in certain geographic locations such as Tegeta, are highly likely to be exposed to dengue infections.

Conclusion

The current study shows that dengue vectors (*Aedes aegypti* mosquitoes) are abundant in the surveyed regions. These vectors are also responsible for transmission

of other arboviruses apart from dengue; this implies that residents are at risk of the dengue and other arboviral infections should outbreaks occur. The detection of dengue viruses, albeit at low infection rate and only during the epidemic, indicates that viruses are constantly circulating in unknown reservoirs. Considering that currently there are no vaccines and or proper treatments for dengue fever, it is vital to prioritize mosquito control programs and support entomological surveillance capacity especially in high risk areas such as Dar es Salaam. It is also important that reservoir hosts that maintain viruses during when there are no epidemics are identified for target control. In addition, public education on the causes and transmission of dengue is also paramount because unknowingly people create mosquito-genic environments thus maintaining the vector population. Combinations of these can provide better understanding of the local dengue infection epidemics and transmission potentials for management, control and prevention of future outbreaks.

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Declaration of interest

Authors declare that they have no competing interests.

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