Distribution of *Aedes* mosquitoes in the Kilimanjaro Region of northern Tanzania

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Little is known about the presence and distribution of *Aedes* mosquitoes in northern Tanzania despite the occurrence of viruses transmitted by these mosquitoes such as *Chikungunya virus* (CHIKV) and *Dengue virus* (DENV) in the region. Adult and larval mosquitoes were collected from rural and urban settings across a wide range of altitudes in the Kilimanjaro Region using the Mosquito Magnet CO₂ Trap for collection of adults and old tires for breeding of larvae. Polymerase chain reaction assays were performed on captured adult mosquitoes to detect the presence of CHIKV and DENV. A total of 2609 *Aedes aegypti* adult mosquitoes were collected; no other *Aedes* species larvae were found. Mosquito yields were significantly higher in urban settings than rural settings (26.5 vs. 1.9 mosquitoes per day, $p = 0.037$). A total of 6570 *Ae. aegypti* larvae were collected from old tires; no other *Aedes* species larvae were found. Of the 2609 adult mosquitoes collected, none tested positive for CHIKV or DENV. As far as we are aware, this paper reports for the first time the presence of *Ae. aegypti* in the Kilimanjaro Region of northern Tanzania. Although CHIKV and DENV were not isolated from any of the collected mosquitoes in this study, the apparent absence of other *Aedes* species in the area suggests that *Ae. aegypti* is the primary local vector of these infections.

Keywords: *Aedes aegypti*, *Aedes albopictus*, Chikungunya, Dengue, Sub-Saharan Africa

Introduction

The *Aedes* mosquitoes are the principal vectors of many arboviruses of human health importance, such as *Dengue virus* (DENV), *Chikungunya virus* (CHIKV), and *Yellow Fever virus*.^{1,2} In sub-Saharan Africa, *Aedes aegypti* and *Aedes albopictus* have been the vectors responsible for outbreaks of arboviral infections among humans in several settings, including coastal Gabon³ and coastal Kenya.^{4,5} However, in many other parts of sub-Saharan Africa, the presence of these *Aedes* species and the arboviruses that they transmit has not been studied. One such location is the Kilimanjaro Region of northern Tanzania, where the presence of *Aedes* species has not been documented. However, a study of severe febrile illness etiology in northern Tanzania in 2007–2008 demonstrated that 7.9% of febrile inpatients had acute CHIKV infection and 9.5% of febrile inpatients had presumptive acute DENV infection.^{6,7} These findings suggested the presence of at least

one *Aedes* species capable of transmitting these arboviruses to humans. Although *Ae. aegypti* has been reported to be responsible for DENV epidemics in the low-altitude coastal region of Tanzania,⁸ the presence of *Aedes* mosquitoes has not been described in the high altitudes of the Kilimanjaro Region.

In order to identify and characterize the local vector of CHIKV and DENV, we conducted a mosquito trapping study to confirm the presence and identity of *Aedes* mosquitoes in the Kilimanjaro Region of northern Tanzania and their geographic distribution.

Materials and methods

This study was conducted in and around the town of Moshi (human population – 144,000), situated at an altitude of 890 m above sea level in the Kilimanjaro Region (human population >1.4 million) of northern Tanzania. The location was selected to match the catchment area of the study of severe febrile illness etiology.^{6,7}

Mosquito trapping was conducted using the Mosquito Magnet CO₂ Trap™ (Woodstream Corporation, Lilitz, PA, USA). Trapping was conducted from 15 March 2012

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Table 1 Adult mosquito collection sites, northern Tanzania, 2012

Site name	Urban	Altitude (m)	High altitude	Latitude	Longitude
KCMC ^a	Y	976	Y	3°19'04.9"	37°19'46.5"
Majengo	Y	817	N	3°20'12.4"	37°21'07.3"
Njoro	Y	793	N	3°21'32.5"	37°21'08.8"
Pasua	Y	787	N	3°22'54.2"	37°20'13.3"
Rau	Y	830	N	3°19'55.0"	37°20'38.9"
Shanty town	Y	928	Y	3°20'02.8"	37°19'13.6"
Boma Ng'ombe	N	935	Y	3°19'40.0"	37°09'23.5"
Chekereni	N	720	N	3°27'29.4"	37°23'21.7"
Kibosho	N	1369	Y	3°14'26.1"	37°18'59.6"
Machame	N	1263	Y	3°16'30.7"	37°13'44.6"
Mabogini	N	757	N	3°24'46.5"	37°21'55.3"
Njoro forest	N	805	N	3°21'36.0"	37°23'60.0"
Rau forest	N	771	N	3°23'02.5"	37°21'29.4"
Uru	N	1197	Y	3°16'11.1"	37°20'14.9"

^aKCMC: Kilimanjaro Christian Medical Centre; Y = yes, N = no.

through 25 May 2012, a period covering portions of both the annual dry and rainy seasons. Mosquitoes were collected at 14 different sites; 6 of these sites were located within Moshi Urban District, and the remaining 8 sites were in rural settings outside of the city. GPS coordinates of each trapping site were collected using the Garmin (Olathe, KS, USA) Etrex 10™ GPS device. Trapping was conducted at a single site each day; the trap was set from sunrise to sunset in well-vegetated, shaded settings near human dwellings to maximize capture success.^{9–11}

Upon collection, mosquitoes were killed by freezing, sorted by species and sex using appropriate taxonomic keys,^{12,13} and stored at -80°C . At the conclusion of the sampling period, mosquito samples were shipped on dry ice to collaborators at the Duke-National University of Singapore in Singapore for molecular testing.

For each real time polymerase chain reaction (RT-PCR) assay, five mosquitoes of the same species and site were pooled. DENV and CHIKV RNA were extracted from the sample using TRIzol® RNA isolation reagent (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's protocol and reverse transcription was performed using Invitrogen Superscript III First Strand Synthesis System™ (Life Technologies, Carlsbad, CA, USA), according to manufacturer's instructions. RT-PCR assays for DENV and CHIKV were carried out with the LightCycler 480 SYBR Green I Master kit™ (Roche Diagnostics, Penzberg, Germany) using previously described primers and cycling conditions.^{14,15}

Used tires of uniform size served as artificial breeding sites and were placed in five locations in and around Moshi, including two urban locations within the town limits and three rural locations outside of town. Tires were filled with clean water and set in shaded vegetated areas to mimic the natural breeding sites for *Aedes* mosquitoes.¹¹ Tires were first placed in January 2012. For the ensuing 20 weeks, third and fourth stage larvae and pupae were collected from these breeding sites on a weekly basis. These larvae were subsequently reared in an insectary, and identification of species was performed using taxonomic keys^{12,13} once the larvae had fully matured into adult mosquitoes.

In order to reduce the risk of adult emergence from the artificial breeding sites, larvae were collected at least once per week and subsequently stored in sealed containers.

Urban collection sites were defined as those within the limits of the Moshi Urban District. Rural collection sites were defined as those within the outlying villages and forests of Moshi Rural District and Hai District. Because mosquito trapping was performed for a different number of days at different sites, we defined 'mosquito abundance' as the average number of mosquitoes caught per day at each site, to allow for uniform comparison. High altitude sites were defined as those at altitudes ≥ 900 m above sea level; low altitude sites were defined as those at altitudes < 900 m above sea level. Differences in mosquito abundance across groups were compared using Mood's median test.

This study was approved by the Kilimanjaro Christian Medical University College (KCMU College) Research Ethics committee.

Results

Adult mosquito trapping

Characteristics of the 14 collection sites are described in Table 1. Six (43%) of the sites were in urban locations, and the median (range) altitude of these sites was 823.5 (720, 1369). A map of the collection sites is displayed in Fig. 1.

Trapping was conducted for a total of 40 days. A total of 2609 *Aedes* mosquitoes were collected from the 14 sites, and all of the collected mosquitoes were *Ae. aegypti*. No *Ae. albopictus* or forest-dwelling *Aedes* mosquitoes were collected from any trapping site. Of the collected mosquitoes, 1618 (62%) were female.

Table 2 lists the total number of *Aedes* mosquitoes caught from each site as well as the mosquito abundance for each site, measured in mosquitoes per day. The median (range) mosquito abundance was 3.3 (0, 219.3) mosquitoes per day.

The median (range) mosquito abundance among urban sites was 26.5 (0.5, 219.3) mosquitoes per day, compared to 1.9 (0, 14.0) mosquitoes per day among rural sites; this difference was statistically significant ($p = 0.037$). The

Figure 1 Map of adult mosquito collection sites and breeding sites, Kilimanjaro Region, northern Tanzania, 2012. Map source: GinkgoMaps project.

Notes: A map of the Kilimanjaro Region of northern Tanzania with identification of the 14 mosquito collection sites. High altitude sites are represented by squares; low altitude sites are represented by circles. Breeding sites are represented by crosses. Urban sites were those within Moshi Urban District; all other sites were rural sites.

Table 2 Mosquito yields by trapping site, northern Tanzania, 2012

Site name	Urban	Trapping days	n (females)	n (males)	n (total)	Mosquito abundance (mosquitoes/day)
KCMC ^a	Y	10	1434	759	2193	219.3
Majengo	Y	4	97	131	228	57
Njoro	Y	2	0	1	1	0.5
Pasua	Y	2	4	3	7	3.5
Rau	Y	2	4	8	12	6
Shanty Town	Y	2	25	69	94	47
Boma Ng'ombe	N	2	6	9	15	7.5
Chekereni	N	2	1	0	1	0.5
Kibosho	N	2	0	0	0	0
Machame	N	3	36	6	42	14
Mabogini	N	2	4	2	6	3
Njoro forest	N	2	3	0	3	1.5
Rau forest	N	2	0	0	0	0
Uru	N	3	4	3	7	2.3

^aKCMC: Kilimanjaro Christian Medical Centre; Y = yes, N = no.

Table 3 Yields of *Ae. aegypti* larvae from breeding sites, northern Tanzania, 2012

Site name	Urban	Altitude, m	High altitude	Total <i>Aedes</i> larvae collected, n
KCMC ^a	Y	976	Y	4693
Majengo	Y	817	N	130
Mabogini	N	757	N	613
Rau forest	N	771	N	143
Uru	N	1197	Y	991

^aKCMC: Kilimanjaro Christian Medical Centre; Y = yes, N = no.

median (range) mosquito abundance among high altitude sites was 10.8 (0, 219.3) mosquitoes per day, compared to 2.3 (0, 57) mosquitoes per day among low altitude sites; this difference was not statistically significant ($p = 0.298$).

Larval collection

Table 3 lists the five sites used for artificial mosquito breeding. A map of these breeding sites is displayed in

Fig. 1; the breeding sites represented a mix of urban and rural locations in both high altitude and low altitude settings. A total of 6570 *Aedes* larvae were collected from these breeding sites. All of the collected larvae were *Ae. aegypti*; no other *Aedes* larvae were collected from any of the breeding sites. Table 3 lists the total number of larvae collected from each site. The median (range) larval collection per site was 613 (130, 4693). The highest larvae yield

was obtained in a high-altitude urban site, Kilimanjaro Christian Medical Centre (KCMC). Because of the small number of breeding sites, statistical analyses comparing larvae yields among sites of different altitudes or urban vs. rural settings were not pursued.

Viral PCR testing

None (0%) of the 2609 adult *Ae. aegypti* mosquitoes collected in this study tested positive for CHIKV or DENV by RT-PCR.

Discussion

To our knowledge, this study is the first documenting the presence of *Ae. aegypti* in the Kilimanjaro Region of northern Tanzania. Despite collection of both adult and larval *Aedes* mosquitoes from a variety of urban and rural locations in both high and low altitude settings, no other *Aedes* species were collected during this study. Although we were unable to detect CHIKV and DENV in the collected mosquitoes, the apparent absence of *Ae. albopictus* or forest-dwelling *Aedes* species suggests strongly that *Ae. aegypti* is the principal vector of these viruses in the region. This finding stands in contrast to the results of studies conducted in the Congo, Gabon, Kenya, and elsewhere in sub-Saharan Africa^{4,16–18} where *Ae. albopictus* was found to have supplanted *Ae. aegypti* as the primary vector of CHIKV infection. Moreover, the presence of *Ae. aegypti* in the region suggests the possibility of outbreaks of other arboviral infections, such as Yellow fever and West Nile virus disease, in the future.

Adult *Ae. aegypti* mosquitoes were found to be more abundant in urban locations; this result is consistent with the mosquito's well-documented predilection for urban environments around the globe.^{19–22} This study did not demonstrate a correlation between *Ae. aegypti* abundance and altitude, although a more comprehensive study including an analysis of ecologic factors such as vegetation and rainfall is needed to fully elucidate the relationship between altitude and mosquito abundance. Although *Ae. aegypti* invasion to altitudes greater than 1000 m above sea level has traditionally been considered to be very rare,²³ both adult and larval *Ae. aegypti* were collected from sites at altitudes above 1000 m during this study. This finding is in accord with the results of recent studies from the western hemisphere documenting the presence of large numbers of *Ae. aegypti* well above this altitude.²⁴ To our knowledge, this is the first report of the invasion of *Ae. aegypti* to altitudes above 1000 m in the African continent.

CHIKV and DENV were not detected in any of the collected mosquitoes. There are many possible explanations for this finding. First, this study may have been conducted during an inter-epidemic period or period of low CHIKV and DENV transmission, during which time the prevalence of CHIKV and DENV among *Ae. aegypti* is likely to be very low. Because the etiology of febrile illness study was conducted five years prior to this study, the prevalence of

human CHIKV and DENV infection during the mosquito collection period is not known. Second, inadvertent mishandling of the collected mosquitoes during storage and shipping, such as a break in the cold chain, could have compromised our ability to detect CHIKV and DENV when the samples arrived in Singapore.

This study had several limitations. First, mosquito collection was conducted during a relatively brief three-month period. A year-long study would be more useful in detecting temporal trends in mosquito abundance as well as potentially detecting species that were not abundant for whatever reason during the study period. Second, this study did not collect additional data about surrounding vegetation, presence of open containers, and site-specific temperatures which would have allowed for a more robust analysis of the determinants of variation in mosquito abundance across sites. Third, mosquito identification was performed using pictorial taxonomic keys rather than using molecular identification. Although pictorial taxonomic keys are generally sufficient to identify major *Aedes* species such as *Ae. aegypti* and *Ae. albopictus*, molecular identification is the most accurate method and prevents missing species not included in taxonomic keys. Fourth, this study relied on a binary definition of urban and rural settings based on administrative boundaries. Local population density data at each trapping site would have allowed for a more detailed analysis of the interaction between mosquito abundance and population density but unfortunately such comprehensive population density data were not available. Fifth, viral PCR studies were only performed on adult mosquitoes in this study, not on larval mosquitoes. As adult mosquitoes can travel great distances, viral studies on larvae can potentially better characterize local transmission of pathogens.

In conclusion, this study documented for the first time the presence of *Ae. aegypti* in the Kilimanjaro Region of northern Tanzania, even at altitudes above 1000 m. Other *Aedes* species were not found during this study, suggesting that *Ae. aegypti* is likely the principal vector of recently described human CHIKV and DENV infection in the region. Additional study is needed to confirm this finding. Our findings suggest several avenues for further research. First, a more exhaustive year-long trapping study with concomitant temperature, rainfall, and vegetation data would allow for a more detailed analysis of spatial and temporal trends in *Ae. aegypti* populations. Second, comparing temporal trends in *Ae. aegypti* populations to trends in human chikungunya infections in the region could further illuminate pathogen–vector–host dynamics. Such data could ultimately be used to plan and implement vector control strategies to reduce the local burden of arboviral disease.

Conflict of interest

None of the authors have any conflicts of interest to declare.

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