

International Journal of Mosquito Research

ISSN: 2348-5906
CODEN: IJMRRK2
IJMR 2018; 5(1): 53-60
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Received: 18-11-2017
Accepted: 25-12-2017

Bisimwa N Patrick
Department of Animal Science, Faculty of
Agricultural and Environmental Studies, Université
Évangélique en Afrique, P.O. Box 3323, Bukavu,
Democratic Republic of Congo

Edson Kinimi
Department of Microbiology, Parasitology and
Biotechnology, College of Veterinary Medicine and
Biomedical Sciences, Sokoine University of
Agriculture, P.O. Box 3019, Morogoro, Tanzania

Mariana J Shayo
Department of Microbiology, Parasitology and
Biotechnology, College of Veterinary Medicine and
Biomedical Sciences, Sokoine University of
Agriculture, P.O. Box 3019, Morogoro, Tanzania

Samuel O Ang wenyi
Department of Microbiology, Parasitology and
Biotechnology, College of Veterinary Medicine and
Biomedical Sciences, Sokoine University of
Agriculture, P.O. Box 3019, Morogoro, Tanzania

Jacqueline Weyer
Center for Emerging, Zoonotic and Parasitic
Diseases, National Institute for Communicable
Diseases of the National Health Laboratory Services,
Private Bag X4, Sandringham 2131, Republic of
South Africa

Petrus Jansen van Vuren
Center for Emerging, Zoonotic and Parasitic
Diseases, National Institute for Communicable
Diseases of the National Health Laboratory Services,
Private Bag X4, Sandringham 2131, Republic of
South Africa

Janusz T Paweska
a) SACIDS African Centre of Excellence for
Infectious Diseases of Humans and Animals in
Southern and Eastern Africa, Sokoine University of
Agriculture, P. O. Box 3297, Morogoro, Tanzania
b) Center for Emerging, Zoonotic and Parasitic
Diseases, National Institute for Communicable
Diseases of the National Health Laboratory Services,
Private Bag X4, Sandringham 2131, Republic of
South Africa

Leonard EG Mboera
National Institute for Medical Research, 3 Barack
Obama Drive, P. O. Box, Dar es Salaam, Tanzania

Mark M Rweyemamu
a) Department of Microbiology, Parasitology and
Biotechnology, College of Veterinary Medicine and
Biomedical Sciences, Sokoine University of
Agriculture, P.O. Box 3019, Morogoro, Tanzania
b) SACIDS African Centre of Excellence for
Infectious Diseases of Humans and Animals in
Southern and Eastern Africa, Sokoine University of
Agriculture, P. O. Box 3297, Morogoro, Tanzania

Gerald Misinzo
a) Department of Microbiology, Parasitology and
Biotechnology, College of Veterinary Medicine and
Biomedical Sciences, Sokoine University of
Agriculture, P.O. Box 3019, Morogoro, Tanzania
b) SACIDS African Centre of Excellence for
Infectious Diseases of Humans and Animals in
Southern and Eastern Africa, Sokoine University of
Agriculture, P. O. Box 3297, Morogoro, Tanzania

Christopher J Kasanga
a) Department of Microbiology, Parasitology and
Biotechnology, College of Veterinary Medicine and
Biomedical Sciences, Sokoine University of
Agriculture,
P.O. Box 3019, Morogoro, Tanzania
b) SACIDS African Centre of Excellence for
Infectious Diseases of Humans and Animals in
Southern and Eastern Africa, Sokoine University of
Agriculture, P. O. Box 3297, Morogoro, Tanzania

Correspondence
Bisimwa N Patrick
Department of Animal Science, Faculty of
Agricultural and Environmental Studies, Université
Évangélique en Afrique, P.O. Box 3323, Bukavu,
Democratic Republic of Congo

Distribution and diversity of mosquitoes and the role of *Aedes* in the transmission of arboviruses in selected districts of Tanzania

Bisimwa N Patrick, Edson Kinimi, Mariana J Shayo, Samuel O Ang wenyi, Jacqueline Weyer, Petrus Jansen van Vuren, Janusz T Paweska, Leonard EG Mboera, Mark M Rweyemamu, Gerald Misinzo and Christopher J Kasanga

Abstract

Arboviruses belong to various families of viruses that are transmitted by arthropods, mainly mosquitoes and often cause diseases in humans. The objective of this study was to determine mosquito diversity and transmission of arboviruses by *Aedes* in selected ecosystems in Tanzania. Adult mosquitoes were collected from rural and urban settings using carbon dioxide-baited CDC light traps, Biogent sentinel traps, and the Mosquito Magnet traps. Reverse Transcription-Polymerase Chain Reaction assay was performed on pooled adult *Aedes* mosquitoes to detect the presence of Chikungunya, Dengue, Rift Valley fever (RVF) and Yellow fever (YF) viruses. A total of 42, 77 mosquitoes belonging to five genera (*Aedes*, *Anopheles*, *Culex*, *Mansonia* and *Mimomyia*) and 18 species were collected. *Culex* accounted for the largest (62.7%; n=2,682) proportion of the mosquitoes while *Anopheles* for the lowest proportion (5.7%; n=245). Of the total mosquitoes collected, *Culex quinquefasciatus* accounted for more than a half (53.4%; n=2692), followed by *Aedes aegypti* 12.1% (n=520). Of the 34 adult *Ae. aegypti* pools tested, arboviruses were detected in 33(97%) pools. Dengue virus was detected in 47.6% (10/ 21) pools which tested positive for Flaviviruses. Chikungunya virus was detected in 30% (3/ 10) pools which were positive for Alphavirus genera. Of 2 pools tested positive for Bunyavirus genus, Rift Valley fever virus was detected in 1 pool (50%). The presence of various mosquito vectors and detection of arboviruses in aedes mosquitoes leave the population of Tanzania at great risk of transmission of different pathogens and highlight a need for vector control measures in the country.

Keywords: mosquito vectors, diversity, arboviruses, aedes, Tanzania

Introduction

Mosquitoes are nuisance species distributed throughout the world occupying many biotopes and are potential transmitters of human and animal infections [62]. The spatial distribution and abundance of arbovirus vectors are related to climatic and topographic factor as well as the effects of anthropogenic changes in the environment [4]. Rainfall, temperature, and relative humidity play a major role in influencing mosquito population density [18].

Several mosquito species are the vectors of many viruses affecting human and animals. These include Dengue virus (DENV), Chikungunya virus (CHIKV), Rift Valley fever virus, Zika virus and Yellow fever virus [17]. Human acquire infection during blood feeding by an infected arthropod vector, also laboratory-acquired infections can occur after handling tissues and body fluids from an infected patient [1].

Two families of Anophelines (all *Anopheles* mosquitoes) as well as Culicines (*Aedes*, *Mansonia* and *Culex*) have been identified as potential mosquito vectors across eastern Africa countries for arboviruses such as malaria, filariasis, dengue, chikungunya, Rift valley fever and of recent Zika viruses [34]. In the coastal part of Tanzania, they transmit filariasis because of high humidity and presence of microfilaria in human population [40].

Among the Culicines mosquito family, *Aedes aegypti* has been described as the most important vector of arboviruses listed above that are considered to be present in Tanzania as well as ZIKA virus circulating outside Africa [32]. Most of These arboviruses have a variety of types of RNA genomes and replication strategies, suggesting that the arthropod-borne transmission strategy has arisen several times during the evolution of RNA viruses [10].

Humans and animals are considered to be dead-end hosts by the fact that they do not produce significant viremia and do not contribute to the transmission cycle of arboviruses; their infection depends mostly on factors ranging from epidemiology to viral genetics [64].

Several mosquito-borne virus disease outbreaks have been reported in Tanzania. Rift Valley fever has been reported in Tanzania on average 8-10 years since 1930 [53]. During the last RVF outbreak (2006/2007), human cases were reported in Arusha, Dar es Salaam, Dodoma, Iringa, Manyara, Mwanza, Morogoro, Pwani, Singida and Tanga Regions [13]. Chikungunya was first reported in Tanzania in 1953 where patients were described to have acute onset of fever associated with rigor headache, joint pain and rash [44]. During recent years, Chikungunya has been reported in Hai, Moshi, Tanga, Kilosa, Karagwe, Kilombero, Kyela, and Sengerema [10, 25]. Cases of dengue have been reported in Iringa, Zanzibar and Dar es Salaam [17, 60, 59]. Zika virus disease, first identified in human blood samples in Tanzania and Uganda in 1952, has been reported in high prevalence in a recent study in Tanzania [34].

Currently, no vaccine or specific treatment are available for most of arboviral infections, prevention are focused mainly on vector control, public education in order to avoid high-risk locations, wearing protective clothing, house modification by avoiding house with caves and use of DEET-containing insect repellents [7].

However these control measures have led to several challenges in Tanzania where for example the intensive use of insecticides for both public health and Agricultural pests control has created mosquito insecticide resistance [23]; similarly, the quality of some houses in unplanned urban areas is quite poor and cannot confer effective protection for habitants against disease vector which have house entry behavior such as mosquitoes [27]. Furthermore, the increased population in urban has led to demand for more agriculture produce, which has created potential breeding sites that are difficult to be attended at a point of time, hence leading to adult vector productivity in urban [23].

The recent increase in the density and distribution of *Aedes aegypti* as well as the rise in air travel has increased the risk of introduction and spread of these viruses in non-endemic regions [2]. Developing strategic measures to control the spread and transmission of arboviruses needs the understanding of the abundance and distribution of mosquito vectors with transmission patterns of the disease. In most of African countries including Tanzania, little is known about arboviruses, potential arbovirus vectors diversity as well as their competence in disease transmission [32, 58]. Therefore, this study was conducted to determine vector abundance and transmission of arboviruses by *Aedes* in selected ecological zones of Tanzania. Information from this study will add to understanding distributions of major arbovirus vectors and their competence in virus transmission in Tanzania and guiding vector sampling during inter-epidemic periods.

Materials and Methods

Study sites

This study was conducted in Kagera, Mwanza, Morogoro and Mbeya regions. These regions were chosen based on variation in their ecological characteristics (altitudes, climate) and prevalence of fever and malaria [47]. A recent survey has indicated that the respective prevalence of fever and malaria in the regions was 17.5% and 41% in Kagera, 22.8% and

15.1% in Mwanza, 18.3% and 22.5% in Morogoro and 15.1% and 0.7% in Mbeya [56]. Districts namely Karagwe, Sengerema, Kilombero, Kyerwa, and Ulanga were selected (Fig. 1). Most of these areas are characterized by mountain ranges which are reported to have swampy valley bottoms and wetland lying on the flood plains of Lake Victoria, Lake Nyasa and Kilombero River. The annual average temperature is about 26 °C in Kilombero, Sengerema and Karagwe characterized by tropical climate. The majority of inhabitants are involved in subsistence farming, fishing and livestock keeping. Ulanga district experiences a bi-modal rainfall pattern with long rains between March and May and short rains between November and January. The average annual rainfall varies between 800 mm and 1600mm every year. The daytime temperature ranges from 18 °C minimum (July) to 26 °C maximum (November).

In addition, Kyerwa district is characterized by mountain ranges, which are separated by swampy valley bottoms and wet lands. The District has a tropical highland climate and annual average temperature is 26 °C. Rainfall distribution is bi-modal with peak rains from September to December and from March to May. The District receives rainfall between 800mm/yr - 1000mm/yr annually. The high ridges get over 1000mm/yr and generally rainfall decreases from East to West.

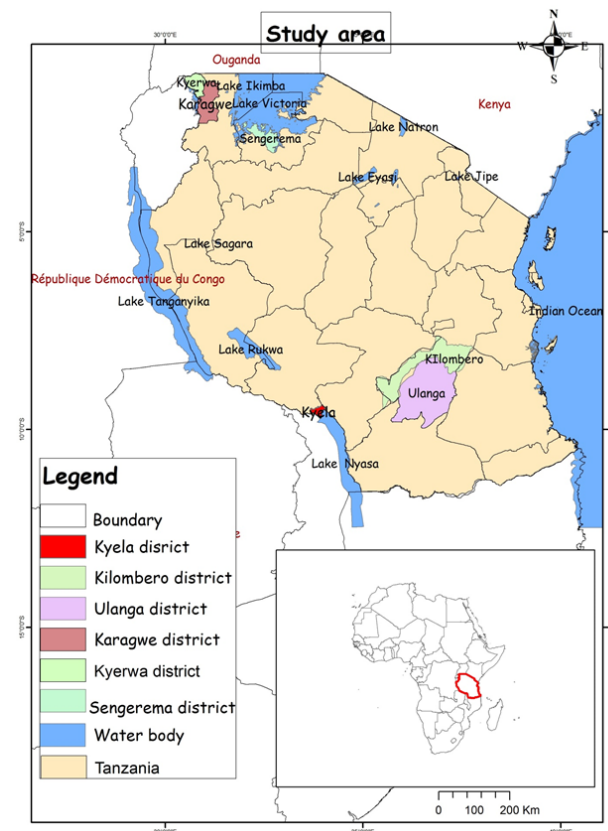


Fig 1: Map of Tanzania showing study locations which included five districts located within four regions

Study design and mosquito trapping

This study has utilized a cross-sectional panel design and was carried out during and after rainy season from March 2015 to June 2015 which is suitable period for mosquitoes breeding. Different mosquito traps were used to collect adult mosquitoes in different urban locations including Carbon

dioxide -baited CDC light traps which were run from 6:00 hours to 1:00 hours inside the houses for indoor mosquito collection. Biogent sentinel traps and the Mosquito Magnet traps were used to collect outdoors mosquitoes and where set outside the houses besides garages, around temporary ponds, in proximity to animal shelters, around rice and banana plantations between 14:00hr and 19:00hr.

Mosquito preservation and identification

The collected adult mosquitoes were immobilized by using absolute ethanol (100%), packed in labeled 1.5 ml eppendorf tubes and transported in dry ice to molecular biology laboratory at Sokoine University of Agriculture in Morogoro where they were kept in freezer at -20°C before identification. Mosquitoes were then identified using morphological identification keys [22] under a stereo light microscope. After identification, *Aedes aegypti* pools were selected, preserved in RNA later and were used for mobovirus screening.

Screening of Mosquito-borne viruses in Aedes Mosquitoes Pooling and Lysis of Aedes Mosquitoes

The *Aedes* mosquitoes collected from the selected districts were used for the detection of Alphavirus, Bunyavirus and Flavivirus. Mosquitoes were pooled in groups of fifty nine in which an average of 20 mosquitoes were pooled together in Eppendorf tubes. Thereafter, 300 µl of phosphate buffered saline (PBS) (Sigma Aldrich, California, USA) was added into each of Eppendorf. Mosquitoes were afterwards crushed using a micropestal, followed by centrifugation at 10,000 rpm within three minutes to obtain cells-free supernatant.

RNA extraction

Ribonucleic acid was extracted from the homogenized lysates using QIAamp Viral RNA Kit (Qiagen, Hilden, Germany), according to the manufacturer's instructions. Briefly, 140 µl of mosquito lysate supernatant was put into an RNase-free Eppendorf tube followed with 560 µl of lysis buffer (AVL). The mixture was pulse vortexed for 15 seconds and incubated for ten minutes at room temperature. Protein precipitation was conducted by adding 560 ml of absolute ethanol followed by pulse-vortexing for 15 seconds. The supernatant was carefully withdrawn and passed through a silica-gel column, followed by washing of the column twice with 500 µl of each of the washing buffers AW1 and AW2, respectively and the tubes containing filtrate were discarded. Finally, 60 µl of elution buffer AVE equilibrated to room temperature was added into QIAamp Mini Column, incubated for one minute and centrifuged at 8,000 rpm. The viral RNA in the eluate was stored at -80 °C until cDNA synthesis.

cDNA synthesis

Virological detection of mosquito-borne viruses in *Aedes*

mosquito pools was carried out using reverse transcription PCR (RT-PCR). Aliquots of extracted RNA were used for cDNA synthesis. RNA was converted into cDNA by using the Superscript III first strand synthesis system kit (Invitrogen, Carlsbad, and California, United States of America), containing random hexamers. Aliquot eight microlitre of RNA from each pool were transferred into PCR micro plates and RNA was heat denatured at 65 °C for five minutes. Plates were immediately incubated on melting ice to prevent renaturation of RNA. Ten microliters of the mastermix were dispensed into each of the 96 wells of the PCR microplate. The plates were incubated at 25 °C for five minutes in order to maximize the annealing of the hexanucleotides to RNA targets. The plates were then incubated at 50 °C for 50 minutes; 85 °C for five minutes and 4 °C hold temperature and finally the cDNAs were stored at -20 °C until PCR.

Arbovirus detection by Reverse Transcriptase-Polymerase Chain Reaction (RT-PCR)

The cDNA amplicons were used for RT-PCR amplification (AgPath-ID™ One-Step RT-PCR Kit, Applied Biosystems, USA) using primers targeting virus genera or specific arboviruses. A total of 20µL of master mix was prepared containing 10µL of 2xDream Taq green PCR Master Mix (Thermo Scientific, Calsband, USA), 1µL of both forward and reverse primer, 1µL of cDNA and 7 µL of nuclease-free water up to 20µL. Primers targeting Bunyavirus, Alphavirus and Flavivirus (Table 1) were used to detect the presence of arbovirus specific genera. For a sample which tested positive with genus primers, were tested further with primers that target conserved genes in the specific viruses belonging to the genus in question such as CHIKV, RVFV, YFV and DENV (Table 2). The PCR cycling performed was the following: an initial denaturation step at 94 °C for 15 min, following by 35 cycles of denaturation at 94 °C for 30 sec, annealing temperatures at 57 °C for 60 sec each, extension at 72 °C for 30sec. The reaction mixture in each PCR tube was then subjected to a final extension step at 72 °C for 10 min. The PCR amplification of targeted gene for specific virus in the cDNA was performed in a 25 µL reaction containing: 12.5 µl of 2xDream Taq Green PCR master mix (Thermo Scientific), 0.5 µL each of forward and reverse primer, 2 µl of the cDNA and 9.5 µl of water to top up to 25 µl. Thermal profiles were performed on a GeneAmp PCR system 9700 (Applied Biosystems, USA). PCR products were separated by electrophoresis, 1.5% of agarose gel, 0.5% of Tris acetic acid and 2µl of GelRed (Phenix, Candler, USA) were used for staining. Five µl of RNA sample, one µl of 6x loading dye and six µl of one kb DNA maker (Promega, Fitchburg, CA) were loaded and run at 100 volts for 40 min. The agarose gel was then visualized through 19 ultraviolet fluorescence light by using gel documentation system (EZ Gel Doc, BioRad, USA).

Table 1: Primers used for detection of selected moboviruses

Virus	Primers	Sequence (5'-3')	Position	Region	Size (bp)
CHIKV	CHIK3F	CAC ACG TAG CCT ACC AGT TTC	14-112	5'NTR	98
	CHIK3R	GCT GTC AGC GTC TAT GTC CAC	14-112		
DENV	D1 38-65	TCA ATA TGC TGA AAC GCG CGA GAA ACC G	10520-10541	3'UTR	345
	D2 455-483	TTG CAC CAA CAG TCA ATG TCT TCA GGT TC	10674-10694		
RVFV	RVF3	CAG ATG ACA GGT GCT AGC	876	GlyM,	550
	RVF4	CTA CCA TGT CCT CAA T	2817-2840		
YFV	CAG	CGA GTT GCT AGC AAT AAA CAC ATT TGG A	43 - 71	Polypro.	1269
	YF7	AAT GCT CCC TTT CCC AAA TA	1293-1312		
<i>Alphavirus</i>	VIR2052F	TGG CGC TAT GAT GAA ATC TGG AAT GTT	6971-6997	NSP4	138

	VIR2052R	TAC GAT GTT GTC GTC GCC GAT GAA	7086-7109		138
<i>Bunyavirus</i>	BCS82C	ATC ACT GAG TTG GAG TTT CAT GAT GTC	86-114	N Protein	243
	BCS332V	GCTGT TCC TGT TGC CAG GAA AAT	309-329		243
<i>Flavivirus</i>	FU1	TAC AAC ATG ATG GGA AAG AGA GAG AA	9007-9032	NS5	276
	CFD2	GTG TCC CAG CCG GCG GTG TCA TCA GC	9308-9283		276

Source: [41]

Statistical analysis

The comparison of counts of mosquito species captured in the selected districts and in different periods was determined by calculating the relative density of every mosquito species [52] in which RD (relative density) = NA (number of all specimens of each species collected during each period)/N (the number of specimens of all species collected during each period) × 100. The infectious status of mobovirus in mosquitoes were determined by RT-PCR and analyzed with Epi Info 7 software (Centers for Disease Control and Prevention). Chi-square test was used to evaluate heterogeneity of rates among the different districts.

Ethical Consideration

Ethical clearance to conduct this study was obtained from the Medical Research Coordinating Committee (MRCC) of the Tanzania National Institute for Medical Research (Certificate number NIMR/HQ/R.8a/Vol.1x/194). Districts, village leaders and house residents were sensitized and asked for their permission before installation of mosquito traps in their houses or premises.

Results

Mosquito genera composition and distribution

A total of 4277 mosquitoes were collected from March to June 2015 in 5 selected districts such as Karagwe, Kyerwa, Ulanga, Sengerema and Kilombero. After morphological identification, a total of 18 mosquito species belonging to 5

genera namely Culex, Aedes, Anopheles, Mansonia and Mimomyia were identified. Among them, 8 species of Culex, 5 species of Aedes, 2speciesofAnopheles, 2 species of Mansonia, and 1 species of Mimomyia genus were identified (Table 3). Culex represented the most abundant genera 62.7% (n= 2682), followed by Aedes 13.6% (n=584), Mimomyia 9.1% (390), Mansonia8.7 % (n=376), and Anopheles species being the minority 5.7% (n=245) (Table 2).

Overall, the abundance of mosquito genera varied significantly (p<0.05) (Table 2). In addition, there was significant differences in mosquito abundance between the study districts (P<0.05).The most abundant mosquito collection was recorded in Karagwe 31.9% (n=1,367) followed by Sengerema 24.2% (n= 1,039) (Table 2).

Species composition and density

Among the 18 species collected, the most prevalent species was *Culex quinquefasciatus* 53.4% (n=2,285), followed by *Aedes aegypti* 12.1% (n=520); *Mimomyiasplendes* 9.1% (n=390); *Culex pipiens* 7.6% (n= 328); *Mansonia uniformis* 7.08% (n= 303); *Anopheles gambiae* complex 5.5% (n=238)), *Mansoniaafricanus* 1.7%(n=73),*Culexcinereus* 0.95% (n=41), *Ae.albopictus* 0.8% (n=35); *Cx. poicipiles*0.53% (n= 23) and *Ae.africanus* 0.4%(n=20), respectively. Few numbers of mosquitoes (n< 20) were also collected from the following 9 species, such as: *Cx. eretmapodites*, *Ae. simpsoni*, *An. funestus*, *Ae. pembaensis*, *Cx. duttoni*, *Cx. uranotaeniahenrardi* and *Cx. tigripes* (Table 3).

Table 2: Mosquito distribution by genus in the selected district, Tanzania

Site	Aedes	Culex	Mansonia	Anopheles	Mimomyia	Total
	n(%)	n(%)	n(%)	n(%)	n(%)	n(%)
Karagwe	70 (11.9)	809(30.1)	0(0)	178(72.6)	310(79.4)	1367(31.9)
Kilombero	131(22.4)	699(26.06)	10 (2.6)	0(0)	0(0)	840 (19.6)
Kyerwa	150 (25.6)	230(8.5)	0 (0)	60 (24.4)	80(20.5)	520 (12.1)
Sengerema	227(38.8)	441(16.4)	364(96.8)	7(2.8)	0(0)	1039 (24.2)
Ulanga	6(1.02)	503(18.7)	2(0.53)	0(0)	0(0)	511 (11.9)
Total	584 (13.6)	2682(62.7)	376 (8.7)	245(5.7)	390(9.1)	4277 (100)

Table 3: Mosquito species relative density by district

Species	Karagwe	Kyerwa	Kilombero	Ulanga	Sengerema	Total	RD
<i>Ae. aegypti</i>	65	120	123	5	207	520	12.1
<i>Ae. africanus</i>	-	-	-	-	20	20	0.4
<i>Ae. albopictus</i>	5	30	-	-	-	35	0.8
<i>Ae. simpsoni</i>	-	-	5	1	-	6	0.14
<i>Ae. pembaensis</i>	-	-	3	-	-	3	0.07
<i>An.gambiae</i>	178	60	-	-	-	238	5.5
<i>An. funestus</i>	-	-	-	-	7	7	0.16
<i>Cx. quinquefasciatus</i>	610	189	678	475	333	2285	53.4
<i>Cx. eretmapodites</i>	-	-	-	1	-	1	0.02
<i>Cx.cinereus</i>	-	-	16	25	-	41	0.95
<i>Cx.tigripes</i>	-	-	1	-	-	1	0.02
<i>Cx.duttoni</i>	-	-	2	-	-	2	0.04
<i>Cx. uranotaeniahenrardi</i>	-	-	1	-	-	1	0.02
<i>Cx.pipiens</i>	199	41	1	2	85	328	7.6
<i>Cx. poicipiles</i>	-	-	-	-	23	23	0.53
<i>Mimomyiasplendes</i>	310	80	-	-	-	390	9.1
<i>Ma. uniformis</i>	-	-	10	1	292	303	7.08
<i>Ma. africanus</i>	-	-	-	1	72	73	1.7
Total	1367	520	840	511	1039	4277	

Molecular detection of mosquito-borne viruses in *Aedes* mosquitoes by RT-PCR

For the screening of arboviruses, only *Aedes* mosquitoes were used and pooled into 59 (20 mosquitos in each pool) included 53 of *Ae. aegypti*, 2 *Ae. africanus*, 1 of *Ae. simpsoni* and 3 pool of *Ae. albopictus*. We considered only the genera of *Aedes* for virus infections screening as is well known that they are the major vector of most of arboviruses worldwide. Specific arbovirus genus (Bunyavirus, Alphavirus and Flavivirus) were screened from them. Of 34 *Aedes* pools tested, arboviruses were detected in 33 (97.05%). They

included Flavivirus (21 pools), Alphavirus (10 pools) and Bunyavirus (2 pool) (Table 4). From a total of 21 *Aedes* mosquito pools tested positive for Flavivirus genus, virus was identified into 10 pools by RT-PCR, giving an infection rate of 47.6%. Moreover, Chikungunya virus was detected in 3 pools out of 10 (30%) Alphavirus genus positive pools while Rift Valley fever virus was detected in 1 pool out of 2 (50%) Bunyavirus positive pools (Table 4). No pool was positive for yellow fever virus. Positive pools were detected in all study districts.

Table 4: Mosquito-borne viruses detected in *Aedes* mosquitoes

Sites	Mosquito species	No. pools	Flav.	Bunya.	Alpha.	DFV	RVFV	CHIKV	YFV
Kilombero	<i>Ae.aegypti</i>	6	-	1	5	-	1	-	-
	<i>Ae. simpsoni</i>	1	-	-	-	-	-	-	-
Ulanga	<i>Ae.aegypti</i>	1	1	-	-	-	-	-	-
	<i>Ae.aegypti</i>	13	12	-	-	9	-	-	-
Sengerema	<i>Ae.aegypti</i>	1	1	-	-	1	-	-	-
	<i>Ae. africanus</i>	3	2	1	2	-	-	1	-
Karagwe	<i>Ae.aegypti</i>	1	-	-	-	-	-	-	-
	<i>Ae. albopictus</i>	6	5	-	3	-	-	2	-
Kyerwa	<i>Ae.aegypti</i>	2	-	-	-	-	-	-	-
	<i>Ae. albopictus</i>	2	-	-	-	-	-	-	-
Total		34	21	2	10	10	1	3	0

Discussion

A geographical assessment of mosquito vectors and their role in virus transmission is a fundamental aspect for the determination of high risk transmission areas. This could provide necessary information for a rapid response plan against emerging arbovirus infections. In several African countries, mosquito-borne virus diseases have been reported and caused outbreaks afflicting human as well as livestock with devastating public health and economic consequences in recent times [17, 18]. Our results show that *Aedes aegypti* to be major vector of arboviruses with the genera and the abundance and infection rate varies from one district to another in Tanzania.

This variability of mosquito abundance depends on ecological and climatic factors [3]. Findings of a similar study in Algeria conform that the climatic factors (temperatures and rainfall), and chemical composition of the water, are among the most important elements for the development and the distribution of mosquito species in certain areas [33]. Variation in arbovirus vectors abundance observed in this study across ecological zones indicates potential risk areas for mosquito-borne transmission and circulation.

The largest number of mosquitoes collected from Karagwe district could actually be explained by its geographical location, wetlands, and swampy valley bottoms. The wet lands in Karagwe provide suitable conditions for mosquito productivity. This finding was in accordance with a research carried out in Nigeria [51] which proved that mosquito distribution and abundance are related to population, land use and human activities. Adult *Cx. quinquefasciatus* and *Ae. aegypti* mosquitoes were found to be more abundant in all study regions; the reason could be because most of mosquito were collected in urban locations; this result is consistent with works showing that the mosquito's well-documented predilection for urban environments around the globe [43, 57]. Similarly, our findings show that *Cx. quinquefasciatus* was the specie with high relative density and more abundant in all the study sites followed by *Ae. aegypti* because these mosquito species have long been known to use dirty and

polluted urban habitats. *Culex quinquefasciatus* breeds in polluted water while *Aedes aegypti* breed in clear water [21]. In addition, the presence of favorable breeding sites such as wet pit latrines, septic tanks, cesspits, drains and canals containing stagnant water polluted with organic compound waste find in most urban regions constitute favorable breeding sites for *Cx. Quinquefasciatus* and *Ae. aegypti* to be developed [21].

Following the results on mosquito infection rates observed in this study, there is a very high risk of arbovirus infection (Rift valley fever, Dengue and Chikungunya) and outbreaks occurrence in the study areas. The presence of *Aedes* mosquitoes in this area exposes the people to mosquito-borne arboviruses [5, 15, 50].

Distinct arboviruses were detected in *Aedes* mosquito pools collected the distinct districts. A recent seroprevalence study in Mbeya region reported a high prevalence of Alphavirus in Kyela district, which indicate the viruses are circulating in the area [6, 63]. Our results correlate with the recent studies carried out in the different parts of Tanzania, Kenya and in Democratic Republic of Congo reporting high prevalence of Alphaviruses, Bunyavirus and Flaviviruses [63, 41, 6, 28, 31]. This suggests that *Aedes* mosquitoes are likely to play an important role in the transmission of arboviruses in eastern and central Africa.

Amplification with specific primers of distinct viruses from suspected positive arbovirus genera showed the presence of Chikungunya virus, Dengue fever virus and Rift valley fever virus. The high level of dengue and Chikungunya virus tested in our study could be explained by the fact that most of our study regions are characterized by wetlands and are inhabited by communities whose economy mainly depend on banana, paddy farming systems. These conditions provide an ideal habitat for different *Aedes* mosquitoes³. Similar results were obtained in studies carried out in Tanzania [32, 25], New California [12] and in Argentina [11] confirming the implication of *Aedes aegypti* in Chikungunya and Dengue transmission during epidemics. *Aedess sp* has been reported to be responsible for the 2004-2006 Chikungunya outbreak in the Indian Ocean Islands [45, 49]. However, our results are in

contrast with a study in northern Tanzania where *Aedes* mosquitoes were screened for arboviruses and no pool was positive for either dengue virus or chikungunya virus [20].

Conclusion

Our findings highlight an active circulation of arboviruses of public health significance in various parts of Tanzania with possible undetermined human impact. Vector distribution generated from this study will guide designing of appropriate mosquito-borne virus infection prevention and control strategies.

Acknowledgments

We wish to thank you the Southern African Centre for Infectious Diseases Surveillance (SACIDS) for funding this study. We are indebted to District Commissioners, District Executive Directors and District Medical Officers in all study districts who permitted this study to be carried out in their jurisdiction areas. We thank the Center for Emerging, Zoonotic and Parasitic Diseases, National Institute for Communicable Diseases of the National Health Laboratory Services, Republic of South Africa for laboratory training of EK, MJS, PNB and SOA.

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