Research Application Summary

Molecular detection of arboviruses in mosquitoes collected from Kyela district, Tanzania

Bisimwa, N.P.[1], Angwenyi, S.[2], Kinimi, E.[2], Shayo, M.[2], Bwihangane, B.A.[2] & Kasanga, C.J.[3]

Sokoine University of Agricuture, P. O. Box 3000, Tanzania

Corresponding author: patronntagereka@yahoo.fr

Abstract

Arboviruses belong to a group of viruses that are transmitted by arthropods, mainly mosquitoes and ticks causing clinical disease symptoms in humans and animals ranging from febrile illnesses to hemorrhagic fevers. The present study aimed at examining the circulation of Chikungunya, Dengue, Yellow fever and Rift valley fever viral genomes in *Aedes* mosquitoes from Kyela district in Tanzania. A systematic vector surveillance spanning two months and covering five sites in Kyela district was carried out in order to evaluate the potential role of *Aedes* spp. in arbovirus transmission in the study area. Mosquitoes were collected, identified to species level by using morphological keys, pooled in respect species and collection sites and screened for arboviruses by RT-PCR. Adult mosquitoes were collected from April to May, 2015 using CO_2-baited CDC light traps, magnet traps as well as human landing collection (HLC). The study sites included Kyela town, Kajunjumele, Ipida, Matema and Njisi villages. A total of 480 blood-feed *Aedes* ssp. were collected, identified and grouped into 24 pools (1-20 mosquitoes per pool) according to species level and location. Out of the 480 *Aedes* spp. collected, *Aedes aegypti*r epresented the most abundant species totaling 338 (70.4%), followed by *Aedes africanus* 102 (21.2%) and *Aedes natalensis* being the minority, 40 (8.3%). Arboviruses were detected in nine pools (37.5%) including *Alpha viruses* (8 pools) and *Flavi viruses* (1 pool). No sample was positive for *Bunya viruses*.Chikungunya virus (CHIKV) was detected in 6 (75%) *alpha virus* positive pools that were collected mostly in the areas where rice cultivation was common. The findings of this study suggest that inhabitants in this region are highly likely to be exposed to arbovirus infections which may represent significant public health concerns.

Keys words: Abundance, *Aedes*, arboviruses, Kyela district, molecular diagnosis

Résumé

Les arboviruses appartiennent à un groupe des viruses qui sont transmis par des arthropodes, principalement des moustiques et des tiques, responsables de maladies chez l'homme et animaux. Cette étude visait à examiner la circulation des génomes viraux de CHIKV, de DENV, de YFV et de RVFV chez des moustiques du genre *Aedes* collectés dans la zone de Kyela en Tanzanie. Une surveillance systématique de deux mois et couvrant 5 différents sites dans la zone de Kyela a été effectuée afin d'évaluer le rôle potentiel des espèces d'*Aedes* dans la transmission d'arboviruses dans la zone d'étude. Les moustiques ont été

collectés, identifiés en utilisant des clefs morphologiques et rassemblés selon les espèces et les sites de collection. La détection des arbovirus a été effectuée par la technique de RT-PCR. Les moustiques adultes ont été capturés d'Avril à Mai 2015 dans la ville de Kyela, les villages de Kajunjumele, d'Ipida, de Matema et de Njisi. Quatre cents quatre-vingts moustiques du genre *Aedes* ont été capturés, identifiés et groupés dans 24 lots (1-20 moustiques par lot) selon les espèces et l'endroit de collection. Sur les 480 moustiques attrapés, *Aedes aegypti* a représenté l'espèce la plus abondante (338 spécimen, 70.4%), suivi de *Aedes africanus* (102 spécimen, 21.2%) et *Aedes natalensis* (40 specimen, 8.3%). Des arbovirus ont été détectés dans 9 lots (37.5%) comprenant alphavirus (8 lots) et flavivirus (1 lot). Aucun échantillon n'était positif pour bunyavirus. Le virus de Chikungunya (CHIKV) a été détecté dans 6 lots qui étaient positifs pour l'alphavirus (soit 75%) qui ont été collectés la plupart dans les sites où la culture de riz était commune. Les résultats de cette étude suggèrent que les animaux et habitants de cette région seraient fortement exposés aux infections d'arbovirus qui peuvent représenter des problèmes significatifs de santé publique.

Mots cles: Abondance, *Aedes*, arboviruses, Kyela, diagnostic moléculaire

Background

Arboviruses (Arthropod-borne viruses) is composed of a large group of viruses that are commonly transmitted to humans and animals mainly by the bite of mosquitoes and less frequently by ticks, which results in diseases (Figueiredo, 2007). They are classified into *Togaviridae, Flaviviridae, Bunyaviridae* and *Reoviridae* families having mostly single-strand ribonucleic acid (RNA) genome with spherical morphology and a diameter that ranges from 45-120 nm (Gubler, 2002). The Chikungunya virus (CHIKV), Dengue virus (DENV), Yellow fever virus (YFV) and Rift Valley fever virus (RVFV) are included in the group of these viruses. They are considered to be the most common emerging pathogens transmitted to humans by *Aedes* mosquitoes and cause major disease burdens in tropical and subtropical countries worldwide (Carpenter *et al.*, 2015).   They constitute a growing international public health problem for which a licensed vaccine, therapeutic drugs, and effective vector control programs are lacking (Harrington *et al*., 2001). Tanzania has had multiple arbovirus outbreaks resulting in economic and public health distress including RVFV which was reported for the first time in 1930 followed by periodic epidemics of 10-20 years in 1947, 1957, 1977, 1997 and 2007. By the end of 2007, the disease had claimed thousands of cases in ruminants and several hundred human cases (Figueiredo, 2007). CHIKV was reported in 1953 where patients were described to have acute onset of fever associated with rigor headache, joint pain and rash (Figueiredo, 2007).

The lack of efficient prophylactic and therapeutic measure make infection with these pathogens a serious public health concern not only in endemic developing countries, but also in many non-endemic industrial countries. There are no studies reported from Kyela district to investigate the presence of the selected arboviruses in their potential vectors. The diagnosis of arboviruses is still a challenge due to clinical manifestation similarity to other diseases; in most cases the diseases are underreported. This study examined the presence of CHIKV, DENV, YFV and RVFV viral genome in *Aedes* mosquito from Kyela district in Tanzania.

Study description

This study was conducted in Kyela district of Mbeya region, located in the South Western corner of the Southern Highlands of Tanzania.The study utilized a cross-sectional panel design and was conducted from April to May 2015, which are the rainfall months suitable for mosquitoes breeding. Approximately 1830 mosquitoes were collected from which 480 were the *Aedes* spp. using Mosquito Magnet (MM) for the outdoor mosquito; the Center for Disease Control (CDC) light traps and human landing collection (HLC) aspirators were also used for collection of indoors mosquitoes. *Aedes* spp. were selected and analysed for virus detection after morphological identification.The RNA was extracted from pooled mosquito samples using the QIAamp Viral RNA Mini kit (QIAGEN, Hilden, Germany) according to manufacturer's recommendations.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.Primers targeting *Bunyavirus*, *Alphavirus* and *Flavivirus* (Table 1) were used to detect the presence of arbovirus specific genera. Samples 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. PCR products were separated by electrophoresis and visualized under UV light. A statistical tool, Epi Info7 software (CDC) was used to calculate the proportion of infected mosquito pools and the difference of infection according to each study village at P Â 0.05 using Chi-squared test.

Results

Three species of *Aedes* genera were identified including *Aedes natalensis, Aedes aegypti* and *Aedes africanus.* Out of 480 mosquito *Aedes* collected, *Aedes aegypti* represented the most abundant species, 338 (70.4%), followed by *Aedes africanus,* 102 (21.2%) and *Aedes natalensis* being the minority, 40 (8.3%). In general, there were significantly differences in mosquito abundance between sampling sites (P < 0.05; IC 9.54 – 30.57). Specific arbovirus (*Bunyavirus*, *Alphavirus* and *Flavivirus*) were screened from the 24 *Aedes* pools; arboviruses were detected in nine pools (37.5%) including *Alphavirus* (8 pools) and *Flavivirus* (1 pool). No sample was positive for *Bunyavirus.* From a total of 8 *Aedes* mosquito pools that tested positive for *Alphavirus* genus, Chikungunya virus was identified in six pools by RT-PCR , giving an infection rate of 75% (n=6) (Table 1).The highest percentage (66.6%) of Chikungunya infection was detected in mosquitoes sampled in Kyela town.

Discussion

A geographical assessment of arbovirus vectors and their role in virus transmission is a fundamental aspect for the determination of high risk areas where emergence and circulation of arthropod viral diseases might occur. This research showed that mosquito vectors of arboviruses endemic or epidemic in East Africa such *Aedes* spp. are distributed throughout the Kyela district, although in varying densities.

Table 1. List of arboviruses detected in *Aedes* mosquitoes using RT-PCR

Location	Mosq. genus	Pool #	AlphaV	BunyV	FlaviV	CHIKV	DENV	RVFV	YFV
Kyela	*Aedesaegypti*	1	-	-	-	-	-	-	-
	Aedesaegypti	2	+	-	+	+	-	-	-
	Aedesaegypti	3	+	-	-	-	-	-	-
	Aedesaegypti	4	+	-	-	+	-	-	-
	Aedesaegypti	5	-	-	-	-	-	-	-
	Aedesaegypti	6	+	-	-	-	-	-	-
	Aedesafricanus	7	+	-	-	-	-	-	-
	Aedesafricanus	8	+	-	-	+	-	-	-
	Aedesafricanus	9	-	-	-	-	-	-	-
	Aedesafricanus	10	-	-	-	-	-	-	-
	Aedesnatalensis	11	-	-	-	-	-	-	-
Kajunjumele	*Aedesaegypti*	12	-	-	-	-	-	-	-
	Aedesaegypti	13	+	-	-	+	-	-	-
	Aedesaegypti	14	-	-	-	-	-	-	-
	Aedesaegypti	15	-	-	-	-	-	-	-
	Aedesaegypti	16	-	-	-	-	-	-	-
	Aedesafricanus	17	-	-	-	-	-	-	-
Ipinda	*Aedesaegypti*	18	-	-	-	-	-	-	-
	Aedesaegypti	19	-	-	-	-	-	-	-
	Aedesafricanus	20	-	-	-	-	-	-	-
Matema	*Aedesaegypti*	21	-	-	-	-	-	-	-
	Aedesaegypti	22	-	-	-	-	-	-	-
Njisi	*Aedesaegypti*	23	-	-	-	-	-	-	-
	Aedesaegypti	24	+	-	-	+	-	-	-

The abundance of these floodwater *Aedes* in this district may be attributed to the nature of the terrain, soil types and vegetation cover, and rainfall which may influence availability of favorable vector breeding and resting grounds (Ang *et al.*, 2007; Lutomiah *et al.*, 2013).

From 24 pools of *Aedes* species, arboviruses were detected in 9 including *Alphavirus* (8) and *Flavivirus* (1) genus; no sample tested positive for *Bunyavirus*. These results correlate with the recent studies carried out in the same district reporting high seroprevalence of *Alphaviruses* and *Flaviviruses* in human serum collected particularly in Kyela and Kajunjumele (Heinrich *et al.*, 2012; Weller *et al.*, 2014). This suggests that *Aedes* mosquitoes may have played an important role in the transmission of arbovirus in Kyela district. In addition, this region is classified as semi-arid, surrounding flood plains of Lake Malawi and is inhabited by communities whose economy mainly depend on rice or paddy cultivation. These conditions provide an ideal habitat for different *Aedes* species considered as major vectors for most arboviruses. These finding are similar to previous studies suggesting that

most of arboviruses primarily affect inhabitants of the dry lands of the Rift Valley regions and the outlying semi-arid and arid grazing lands (Anyamba *et al.*, 2001).

In conclusion, this study has shown the presence of different arboviruses vectors in Kyela district in Tanzania. Molecular analysis confirmed an evidence of active circulation of Chikungunya virus in the region. This study suggests that people and livestock from Kyela, especially in certain geographic locations, are highly likely to be exposed to arboviruses over the course of their lifetime.

Acknowledgement

References

Carpenter, S., Veronesi, E., Mullens, B. and Venter, G. 2015. Vector competence of Culicoides for arboviruses: Three major periods of research, their influence on current studies and future directions. *Rev. Sci. Tech. Off. Int. Epiz.* 34: 97-112.

Domingo J.L, Cabanne, G., Campos, R. and Tonon, S.A. 2005. Molecular detection of dengue viruses in field caught *Aedesaegypti*mosquitoes from northeastern Argentina. *Rev. Latin. Microb.* 47(3-4):82-87.

Figueiredo, L.T. 2007. Emergent arboviruses in Brazil. *Rev daSocie. Bras.deMedi. Trop.* 40:224-229.

Gubler, D.J. 2002. The global emergence/resurgence of arboviral diseases as public health problems. *Archive Medicine Research* 33:330-342.

Harrington, L.C., Edman, J.D. and Scott, T.W. 2001. Why do female Aedesaegypti (Diptera: Culicidae) feed preferentially and frequently on human blood? *J. of Med Ent.* 38:411-422.