EVIDENCE OF ANTI-CHIKUNGUNYA VIRUS IGG AND IGM ANTIBODIES AMONG PATIENTS SEEKING TREATMENT IN DIFFERENT HEALTH FACILITIES IN KYELA DISTRICT, TANZANIA

Bisimwa N. Patrick[1*], Samuel Angwenyi[2], Kinimi Edson[2], Shayo Meriana[2], Mushi Hugo, Christopher J. Kasanga

[1]Université Evangélique en Afrique; Faculty of Agricultural and Environmental studies, Department of Animal Science and Production, Po Box 3323, Bukavu/ DRCongo
[2]Sokoine University of Agriculture, Faculty of Veterinary Medicine, Department of Microbiology and Parasitology, PO Box 3019, Morogoro, Tanzania
[3]Kyela District Hospital, PO Box 330 Mbeya region, Tanzania

ABSTRACT

Chikungunya is an arboviral disease transmitted by *aedes* mosquitoes, caused by Chikungungunya virus. It consists of an acute illness characterized by fever, rash, and incapacitating arthralgia. This study aimed to estimate the prevalence of Chikungunya fever in patients presenting fever at different health facilities located in Kyela district. Out of 132 recruited patients, 94(71.2 %) were female and 38 (28.8 %) were male. The majority of them 80 (60.6%) were adults (≥25 years). Anti-Chikungunya virus anti-immunoglobulin G (IgG) and anti-immunoglobulin M (IgM) antibodies were detected in serum samples using indirect enzyme-linked immunosorbent assay. Chikungunya virus IgG or IgM antibodies were detected in 19 among 132 serum specimens tested indicating a seroprevalence of 14.3%. Out of 132 sera tested, 14 (11%) had IgG antibodies and 5(3.8%) had IgM antibodies. The higher anti-CHIKV IgG seroprevalence was found in female patients (OR= 3.22; 95% CI: 1.03-10.06) than in male. Similarly patients who took some medication before going to the health centre were found with high CHIKV IgG antibodies (OR= 13.912; 95% CI: 1.76-109.78) as well as in patients who never been vaccinated (OR=4.6; 95%CI: 0.02 – 1.71). Additionally, the uni-variate analysis results revealed, feeling nausea as the symptom of significant association with Chikungunya IgG seropositivity (OR = 4.5; 95% CI: 1.3– 14.4). These findings confirm that CHIKV infection seems to be among the common causes of febrile illness in Kyela district and appears to be actively circulating in the population but is routinely misdiagnosed. This suggests a need to raise awareness among health facilities and policy makers on the use of specific diagnosis for better control of arbovirus diseases in the study region.

Key words: Chikungunya virus, Diagnosis, Health centre, Kyela district

No: of Figures: 1 **No: of Tables: 5** **No: of References: 27**

INTRODUCTION

Chikungunya fever is an acute febrile illness caused by Chikungunya virus (CHIKV) which is an RNA arbovirus that belongs to the Alphavirus genus of the family Togaviridae. The virus is transmitted primarily by the urban vector *Aedes aegypti* and has been responsible for epidemics in cities of Africa and Asia Kervin *et al.,* (2004). It was first recognized as a human pathogen during the 1950s in Africa, and since then, cases have been identified in many countries in Africa and Asia (Robinson, 1955). The diseases are manifested as mild febrile illness to severe polyarthritis and encephalitis Kumari *et al.,* (2010).

The illness caused by CHIK is associated with distinct clinical manifestations that include a rather abrupt onset of fever, rash, crippling arthralgia, and occasional frank arthritis; the incubation period is usually two to ten days, with the constitutional symptoms lasting one to seven days. The articular symptoms usually resolve within days to a few weeks, but in severe cases, these symptoms may last for months Kumar *et al.,* (2011).

In Tanzania, while CHIKV was first isolated from a human serum in 1953 in Newala, where patients were described to have acute onset of fever associated with rigor headache, joint pain and rash Robinson, (1955). The name Chikungunya originates from Makonde, a language spoken in Tanzania where it was first isolated, and means "that which bends up" or "stooped over" or "walking bent over" or "bent walker", referring to the posture patients assume with the resulting arthralgia Kondekar *et al.,* (2006).Currently only few data on the distribution and medical importance of CHIKV and other Alphaviruses in Africa are available. Since the 1960s, especially CHIKV was repeatedly isolated throughout African and Asian countries Power and Logue (2008), and small outbreaks were frequently reported. Outbreak of CHIKV occurred in Lamu Island and Mombasa in Kenya in 2004 Sergon, *et al.,* (2008); Kariuki *et al.,* (2008) and the disease subsequently spread eastward, causing millions of disease cases throughout countries in and around the Indian Ocean in 2005/2006, alone Kariuki *et al.,* (2008). With nearly 280.000 people infected on the island of La Reunion Bonn (2006). By 2007, CHIKV was imported into Europe, causing an outbreak of chikungunya fever in Italy Rezza *et al.,* (2007). This outbreak suggested for the first time the significant potential of the virus to move to novel ecological niches, including Europe, Australia, and the Western Hemisphere through returning travelers Pialoux *et al.,* (2007); Epstein, (2007); Enserink (2006).

Southwestern Tanzania is a malaria endemic area and mosquito vectors for both malaria and others arbovirus, especially CHIKV transmissions are prevalent Bisimwa *et al.,* (2016); Weller *et al.,* (2014). Recent studies have demonstrated that in Tanzania, a high proportion of fevers are due to non-malaria febrile illnesses Crump *et al.,* (2013; D'Acremont *et al.,* (2014); patients with non-malaria febrile illnesses such Dengue, Chikungunya, Yellow fever and Rift valley fever may be presented with

similar manifestations but they are not routinely tested at the health facilities and therefore go undiagnosed, as such their prevalence is likely underestimated; thus laboratory confirmation is essential. This region was not affected by the 2004–2007 outbreak, and diagnosis or laboratory verification of acute Chikungunya fever infection is not commonly used locally. This study was therefore designed to estimate the prevalence of Chikungunya fever in patients presenting fever at health facilities in Kyela district. The survey gave us the opportunity to elucidate the importance role of this pathogen in febrile illness occurrence in the study region and its dependence on certain social factors in an endemic transmission cycle in a typical local setting.

Materials and Methods

Study area

This study was conducted in Kyela district of Mbeya region, located in the South Western corner of the Southern Highlands of Tanzania. The district lies between longitudes 33°41' and 33°30' East of Greenwich and between latitudes 9°25' and 9°40' South of Equator (Fig. 1). The district lies in the flood plains of Lake Nyasa and thus receives heavy rains of about 3000 mm per year. Kyela has a hot and humid climate with a mean daily temperature of 23°C. The study site was chosen to include the most health facilities located in the region, such: Kyela District Hospital, Matema hospital, Ipinda health centre, Kajunjumele health centre and Njisi dispensary.

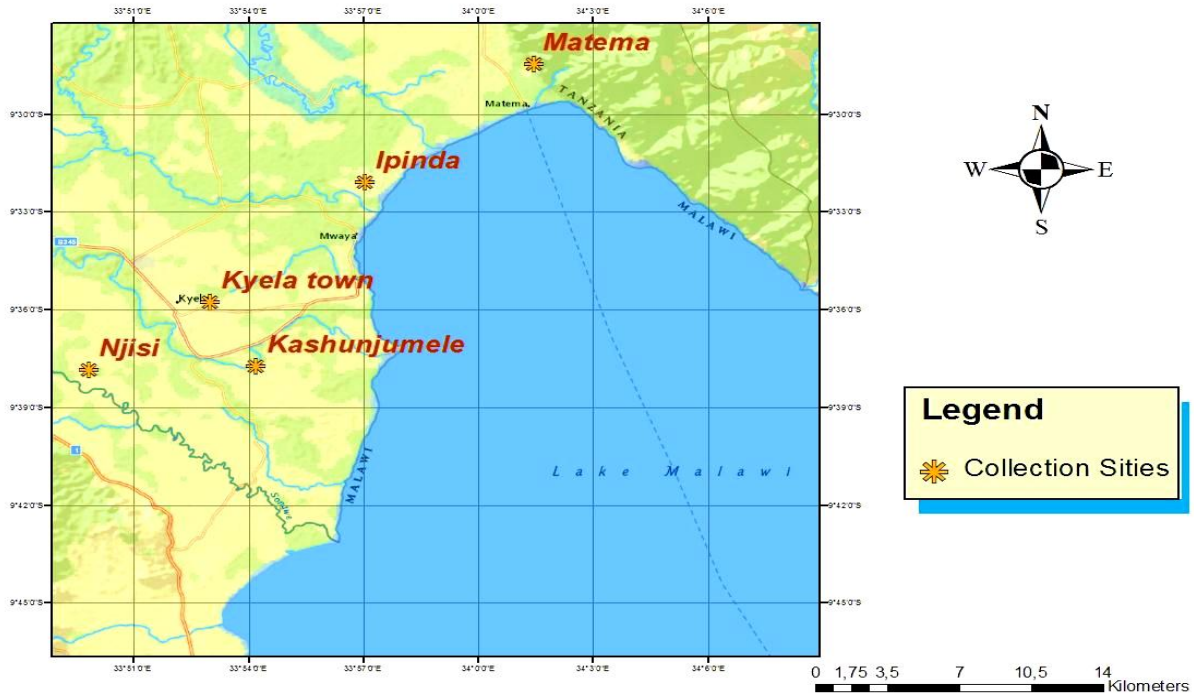

Figure 1: Kyela district showing where blood samples were collected (Source of map: GPS coordinates)

Study design and Participant recruitment

This study has utilized a cross-sectional panel design and was conducted from May to June 2015. Patients aged more than 2 years with specific symptoms (fever, headache, vomiting, joint pain with or without swelling, and the presence or absence of rash on the body) coming for treatment at the health facilities at Kyela district hospital, Matema health centre, Ipinda health centre, Kajunjumele health centre and Ipinda dispensary were considered for blood sample collection. A short questionnaire was used to collect demographic and clinical data such as age, gender and history of illness for each of the participants.

Sample size and sample collection

To estimate the seroprevalence of CHIKV, blood samples were taken from sick persons coming for treatment on different health facilities in Kyela district. To avoid maternal antibodies, blood samples of 1-3 mL was taken into plein vacutainer tubes without EDTA by the aid of qualified laboratory technician from all consenting from febrile patients aged from 1 year and above presenting symptoms or clinical features suggestive of arbovirus infections. A subject number was assigned to each participant and used to label blood samples. Approximately, 258 patients was registered for treatment at the different health facilities from which only 168 patients agreed to participate and provided blood samples, for serum separation. However, 36 blood samples were removed from the samples because of hemolysis and transport problems, only 132 blood samples were been successfully collected for testing for the presence of CHIKV antibodies; blood samples were centrifuged at 5000 r.p.m for 5 minutes for serum extraction and transported to Kyela district hospital where they were stored at -80˚C before being transported at Sokoine University of Agriculture molecular biology laboratory for analysis.

Serological analysis

After the blood was being centrifuged, serum sample was tested for anti-CHIKV anti-immunoglobulin G (IgG) and anti-immunoglobulin M (IgM) antibodies using indirect enzyme-linked immunosorbent assay (ELISA) (EUROIMMUN Medizinische Labordiagnostika AG) according to the manufacturer's instructions. For both tests, we used a positive control serum specimen obtained from a previous Chikungunya virus outbreak in East Africa; a negative control serum specimen was from a person from a non-endemic region who tested negative for arbovirus infection.

Briefly, 100 µl of the calibrator, positive and negative controls and diluted patient samples was transferred into the individual microplate wells according to the pipetting protocol; the micro plate was then incubated for 60 minutes at 37°C. The wells were empted manually and washed 3 times using 300 µl of working strength wash buffer for each wash.

After washing (manually), the whole liquid was thoroughly disposed from the microplate by tapping it on absorbent paper with the openings facing

downwards to remove all residual wash buffer; after that, 100 µl of enzyme conjugate (peroxidase-labelled anti-human IgG or IgM) has been added into each of the microplate wells and plate was incubated for 30 minutes at room temperature; wells were re-washed as described previously. A volume of 100 µl of chromogen/substrate solution was added into each of the microplate wells, and incubated for 15 minutes at room temperature. The reaction was stopped by adding 100 µl of stop solution into each of the microplate wells. However, the photometric measurement of the color intensity has been made at a wavelength of 450 nm within 30 minutes after adding the stop solution. A semi quantitative analysis was performed using incubate calibrator 2 with samples diluted 1:100 in sample buffer. The results were evaluated semi-quantitatively by calculating a ratio of the extinction value of the patient samples over the extinction value of the calibrator. Samples with a ratio < 0.8 was considered to be negative; samples with a ratio ≥0.8 to <1.1 were borderlines and supposed to be re-tested, and samples with ratio ≥1.1 were considered as positive.

Statistical analysis

The seroprevalence of CHIKV infection determined by ELISA was analyzed by Epi Info 2002 software (Centers for Disease Control and Prevention) and then, age, sex, symptom as well as distribution of the seropositive and seronegative participants were compared using the chi-square test ($\chi 2$) while Fisher's exact test was used in cases when expected counts were less than five.

Ethical consideration

The approval to carry out this study was sought from the ethics review subcommittee of the National Research Coordinating Committee (MRCC) and ethical clearance from National Institute for Medical Research of Tanzania (NIMR). Confidentiality of the study participants was strictly observed by coding the participant's name and its provenance region.

Results

Demography and distribution of recruited patients in Kyela district

Among 258 patients who were seeking treatment from May to June 2015 at Kyela district hospital, Matema health centre, Ipinda health centre, Kajunjumele health centre and Njisi dispensary, 168 (65.1%) agreed to participate and provided blood samples for testing. Thirty six serum specimens had inadequate volume or were hemolyzed and were not tested; thus, 132 serum specimens were successfully analyzed for IgG and IgM antibodies to CHIKV of which 94 (71.2 %) were female and 38 (28.8 %) were male. Furthermore, 80 (60.6%) of participants were adults (≥25 years) and a big number of participants include in this study 49(37.1%) were found at Kyela hospital district (Table 1).

Table 1: Distribution of participants tested for CHIKV IgG and IgM antibodies

Variables	Categories	No of patients	Proportion (%)
Sex	Male	38	28.8
	female	94	71.2
Age(years)	0 to 5	12	9.1
	5 to 18	23	17.4
	18 to 25	17	12.9
	>25	80	60.6
Health centres	Ipinda	30	22.8
	Kajunjumele	18	13.6
	Kyela Hospital	49	37.1
	Matema	22	16.7
	Njisi	13	9.9
Total		**132**	**100**

Seroprevalence of IgG or IgM anti Chikungunya antibodies from patients in different health centres of Kyela district

Chikungunya virus IgG or IgM antibodies were detected in 19 among 132 serum specimens tested using indirect ELISA indicating a total seroprevalence of 14.3%; 14 (11%) serum specimens tested positive for IgG antibodies and 5(3.8%) had IgM antibodies (Table 2).

The highest seroprevalance of CHIKV IgG antibodies were found in patients coming for treatment at the Njisi dispensary followed by Kajunjumele health centre and Kyela district hospital with 3(23%), 3(16.66%) and 5(10.2%) respectively. The lowest CHIKV IgG prevalence was found in patient coming for treatment at Ipinda health centre 1(3.33%) while the highest IgM antibodies seroprevalence were found in participants from Kyela district hospital 4(8.1%) and the lowest IgM seroprevalence was found in Kajunjumele health centre 1(5.5%) (Table2). Moreover, there was significant statistical difference ($p< 0.05$) for patients with detectable IgG and IgM antibodies; the proportion of patients who were IgG seropotive was higher (OR = 5.04, 95% CI: 1.129 - 22.496) compared with participants who were IgM seropositive.

Table 2: CHIKV disease serostatus among patients at different health facilities in Kyela district, May to June 2015

Sites	Ipinda n (%)	Kajunj n (%)	KDH n (%)	Matema n (%)	Njisi n (%)	Total n (%)	OR(95% CI)	p-value
Sample size	**30**	**18**	**49**	**22**	**13**	**132**	**-**	
CHIKV IgM	0 (0)	1(5.5)	4 (8.1)	0 (0)	0(0)	5 (3.8)	1	
CHIKV IgG	1(3.33)	3(16.66)	5(10.2)	2(9)	3(23)	14(11)	5.04(1.1-22.5)	0.012

IgM: Immunoglobulin M, IgG: Immunoglobulin G; KDH: Kyela Distict Hospital ; OR: odds ratio; Kajunj.HC:Kajunjumele Health centre; CI: Confident Interval

Different clinical signs and symptoms presented by recruited patients and their association with IgG and IgM seropositivity

Among the symptoms and signs presented by the recruited patients, 108(81.9%) had fever, and 95(72%) had headache while 36(27.2%) had joint pain and 23(17.4%) had nausea. The uni-variate analysis results revealed, feeling nausea as significant predictor of Chikungunya IgG seropositivity with OR = 4.5 (95% CI 1.3 – 14.4), p=0.012 (Table 3). However, no clinical signs were found to be statistically associated with Chikungunya IgGM seropositivity (Table 4).

Table 3: Association of symptoms presented by recruitment patients and Chikungunya IgG seropositivity

Symptoms	n(%)	Positive n (%)	Negative n (%)	OR (95 % CI)	p-value
Fever					
Yes	108(81.9)	10(71.4)	98(83.0)	0.5(0.14 - 1.7)	0.15
No	24(18.2)	4(28.6)	20(17.0)	Reference	
Nausea					
Yes	23(17.4)	6(42.9)	17(14.4)	4.5(1.3 – 14.4	0.012
No	109(82.6)	8(57.1	101(85.6)	Reference	
Headache					
Yes	95(72)	9(64.2)	86(72.9)	0.7(0.2 – 2.1)	
No	37(28)	5(35.8)	32(27.1)	Reference	
Joint pain					
Yes	36(27.2)	3(21.4)	33(28.0)	0.7(0.2 – 2.6)	0.25
No	96(72.8)	11(78.6)	85(72.0)	Reference	
Rash					
Yes	21(16)	1(7.1)	20(17.0)	0.3(0.04 – 3.04)	0.19
No	111(86)	13(92.9)	98(83.0)	Reference	
Back pain					
Yes	34(27.8)	2(14.2)	32(27.1)	0.4(0.09 – 2.1)	0.16
No	98(74.2)	12(85.8)	86(72.9)		

Table 4: Association of symptoms and Chikungunya IgM seropositivity

Symptoms	n	Positive n (%)	Negative n (%)	OR (95 % CI)	p-value
Fever					
Yes	108	4	104	0.9(0.09 – 8.28)	0.43
No	24	1	23	Reference	
Nausea					
Yes	23	2	21	3.36(0.5 – 21.3)	0.12
No	109	3	106	Reference	
Headache					
Yes	95	4	91	1.5(0.17 – 14.6)	0.37
No	37	1	36	Reference	
Joint pain					
Yes	36	1	35	0.65(0.07– 6.08)	0.39
No	96	4	92	Reference	
Rash					
Yes	21	1	20	1.3(0.14 – 12.59)	0.38
No	111	4	107	Reference	
Back pain					
Yes	34	1	33	0.7(0.07 – 6.6)	0.41
No	98	4	94	Reference	

Univariate analysis of factors associated with anti-Chikungunya IgG seropositivity

The results found from the regression logistic revealed a significant association (P < 0.05) of anti-CHIKV IgG status with sex and patients who took some treatment before going to the health facilities; the higher anti-CHIKV IgG seroprevalence was found in female patients (OR= 3.22, 95% CI 1.037-10.06) than in male, while patients who took some medication before going to the health centre were found with high CHIKV IgG antibodies than the one who did not take medication (OR= 13.912; 95% CI 1.762-109.789) (Table 2). In addition, high CHIKV IgG has been detected from patients who never been vaccinated (OR= 4.63; 95% CI 0.02-1.71) than the ones who have been vaccinated. However, no significance association was found with CHIKV seropositivity in term of age of patients, body temperature, and the use or not of the bed net (p<0.5). (Table 5).

Table 5: Factors associated with anti-CHIKV IgG status in Kyela district

Variables	Categories	No patients	No IgG +(%)	OR	95%CI	P value
Sex						
	Male	38	7	1.000		0.0224
	female	94	7	3.22	1.03-10.06	
Age						
	0 to 5 years	12	1	1.000		
	5 to 18 years	23	3	1.650	0.12-3.98	0.588
	18 to 25 years	17	3	2.357	0.51-9.7	
	>25 years	80	7	1.055	0.37-6.6	
Body temperature	High fever	109	12	1.000		0.485
	Normal fever	23	2	0.770	0.27-6.24	
Use of bed net						
	Yes	123	13	1.000	0.12-9.08	0.611
	Not	9	1	1.058		
Vaccination						
	Yes	32	1	1.000	0.02-1.71	0.047
	Not	100	13	4.63		
Treatment						
	Not	62	1	1.000	1.76-109.7	0.0041
	Yes	70	13	13.91		

Discussion

This study was carried out in order to determine the seroprevalence of CHIKV among patients seeking treatment in different heath facilities located in Kyela district, Tanzania using ELISA technique. The findings of this serosurvey suggested CHIKV is present in Kyela district based on the number of cases detected in health facilities. These results reveal that the magnitude of the CHIKV status was substantially greater than what was predicted based on the number of cases detected in health facilities.

During our investigation, a seroprevalence rate of 14.3% for CHIKV infections were found, these results show that CHIKV is common and is among possible causes of febrile illness in Southwestern part of Tanzania. This can be justified by the presence in the study region of vectors principally responsible for transmission of the virus specifically *Aedes* mosquitoes Bisimwa *et al.,* (2016). Similar study carried out in the Mbeya region where our study area is located reveled potential endemic circulation CHIKV with higher seroprevalence of Alphavirus IgG seroprevalence found in Kyela district Weller *et al.,* (2014) but also detection of CHIKV in Aedes mosquitoes collected in the same areas using molecular techniques Bisimwa *et al.,* (2016).

However, the prevalence rate of CHIKV infections (14.3%) found in the present study was lower than the one found in previous studies carried out in Kenya, which reported a prevalence rate of 75% Sergon, *et al.,* (2008), and 63% in the Union of Comoros (Sang, *et al.,* 2008). The difference in prevalence rate could be attributed to the few study sites, fewer samples tested and limited study period. The current study was conducted in 5 different health centres for a period of two months testing 132 human serum samples, while the study at Lamu Island was carried out for five days (5-9) in October 2004 although it was a response to an outbreak of unknown febrile illness. In addition, the higher CHIKV seropositivity found in Njisi Dispensary, followed by Kyela District Hospital and Kajunjumele health centre could be explained by the geographical and ecological factors of the location of the mentioned health facilities. They are located in regions classified as semi-arid, surrounding flood plains of Lake Malawi and are 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 Bisimwa *et al.,* (2016).

Immunoglobiline G (IgG) seropositivity was higher compare to the IgM seropositivity in most of the participants with detectable antibodies. This may be justified by the raison that our survey was carried out after the outbreak of a febrile illness; therefore, it may be possible that IgM antibodies might be already waned in many participants exposed during the febrile illness outbreak peak. The high IgG seropositivity in participant between 18 to 25 years compare to others (but not statistically significant different) could be because of concurrent mosquito net campaigns in both pregnant mothers and infants which may have reduced exposure to mosquito bites in young populations. The positive association

between CHIKV seropositivity and patient who have been taking some treatment related to malaria before going to the health facilities justify that CHIKV is mistreated. This is correlated to the recent finding in Tanzania which revealed that patients with acute dengue and chikungunya infection are often misdiagnosed and treated with anti-malarials or antibiotics Crump *et al.,* (2013); Hertz *et al.,* (2012). CHIKV-infected patients were more likely to receive antibacterial or antimalarial therapy than other febrile patients; such therapy constitutes wasted health resources and potentially promotes antimicrobial resistance.

The consequences of misdiagnosis and underreporting of other diseases than malaria include economic loss Hum *et al.,* (2008), development of drug resistant malaria strains (due to over-prescribing of anti-malarials) Wongsrichanalai *et al.,* (2007) and risks of increased morbidity and mortality Oladosu *et al.,* (2013).
The clinical manifestations of CHIK infection presented by the participants (nausea, headache, fever, joint pain, rash…) in Kyela district were similar to those reported from other epidemics Rezza *et al.,* (2007); Borgherini. et al., (2007); Lakshmi *et al.,* (2008). The common clinical sign observed was fever and headache while rashes were only reported in 21% of patients. This may be explained because much of the clinical information obtained relied on volunteer recall, the appearance of a rash may have been overlooked in some instances Kevin *et al.,* (2004). The observed seroprevalence explains that conditions for CHIKV occurrence are endemic in the study area and goes undetected likely misdiagnosed as malaria Mwongula *et al.,* (2013). Symptoms associated with CHIKV infection such as fever, joint pains, and myalgias are non-specific and could be mistakenly identified with a variety of other diseases including dengue, malaria, Rift Valley fever, and influenza. However, pronounced persistent severe joint pains that affect wrists, elbows, fingers, and knees in some patients should raise the suspicion of alphavirus infection, especially chikungunya disease or O'nyong nyong fever, which also occurred in epidemic form in East Africa in the late 1990s Jeandel *et al.,* (2004); Sander *et al.,* (1999).

Conclusion

CHIKV infections is among the common causes of febrile illness in Southwestern part of Tanzania and appears to be actively circulating in the population but is routinely misdiagnosed, most commonly as malaria. As symptoms of chikungunya are most often clinically indistinguishable from those observed in dengue fever and O'nyong ' nyong virus. Therefore, laboratory confirmation of chikungunya virus infection using molecular approach will be critical.

Acknowledgements

We gratefully acknowledge financial support from Southern African Centre for Infectious Diseases Surveillance (SACIDS) to undertake this study. Our Special thanks are also extended to the Kyela District Hospital for providing storage and centrifuge equipments for our samples during the field work. We thank Prof.

Mushagalusa Nachigera Gustave and Prof. Katcho Karume (Chancelor and Dean of the Faculty of Agricultural and Environment Studies of the Université Evangélique en Afrique respectively) for their hospitality and support.

REFERENCES

Bisimwa NP, Angwenyi S, Kinimi E, Shayo M, Bwihangane BA, Kasanga CJ. (2016). Molecular detection of arboviruses in *Aedes* mosquitoes collected from Kyela district, Tanzania. Rev. Méd. Vét. 167, 5-6, 138-144.

Bonn D (2006). How did chikungunya reach the Indian Ocean? Lancet Infect Dis 6: 543. doi: 10.1016/s1473-3099(06)70559-x

Borgherini G, Poubeau P, Staikowsky F, Lory M, Le Moullec N, Becquart JP, Wengling C, Michault A, Paganin F. (2007). Outbreak of chikungunya on Reunion Island: early clinical and laboratory features in 157 adult patients. Clin Infect Dis. Jun 1;44(11):1401-7.

Crump JA, Morrissey AB, Nicholson WL, Massung RF, Stoddard RA, Galloway RL, Galloway RL, Ooi EE, Maro PV, Saganda W, Kinabo DG, Muiruri C, Bartlett AJ (2013). Etiologyof severe non-malaria febrile illness in Northern Tanzania: a prospective cohort study. PLoS Negl Trop Dis.;7:e2324.

D'Acremont V, Kilowoko M, Kyungu E, Philipina S, Sangu W (2014). Beyond malaria-causes of fever in outpatient Tanzanian children. N Engl J Med. 370: 809.

Enserink M. (2006). Infectious diseases. Massive outbreak draws fresh attention to little-known virus. Science, 311, 1085.

Epstein PR. (2007). Chikungunya Fever resurgence and global warming. American Journal of Tropical Medecine and Hygiene. 76(3):403-4.

Hertz JT, Munishi OM, Ooi EE, Howe S, Lim WY, Chow A, Morrissey AB, Bartlett JA, Onyango JJ, Maro VP, Kinabo GD, Saganda W, Gubler DJ, Crump JA (2012). Chikungunya and dengue fever among hospitalized febrile patients in northern Tanzania.Am J Trop Med Hyg. 86:171–7.

Hume JCC, Barnish G, Mangal T, Armázio L, Streat E, Bates I (2008). Household cost of malaria overdiagnosis in rural Mozambique. Malaria Journal;7:33.

Jeandel P, Josse R, Bagambisa G, Durand JP (2004). Exotic viral arthritis: role of alphaviruses. Med Trop (Mars) 64: 81–88.

Lakshmi V, Neeraja M, Subbalaxmi MV, Parida MM, Dash PK, Santhosh SR, Rao PV (2008). Clinical features and molecular diagnosis of Chikungunya fever from South India. Clin Infect Dis. 46(9):1436-42.

Kariuki MN, Nderitu L, Ledermann JP, Ndirangu A, Logue CH, Kelly CHL, Sang R, Sergon R, Breiman R, Powers AM (2008). Tracking epidemic Chikungunya virus into the Indian Ocean from East Africa. J Gen Virol.; 89(Pt 11): 2754–2760.

Kevin RP, Ratna T, Yunry I, Wuryadi SS, Susana W, Chairin M, Erlin L, Herman K, Linda H, James M, Mohammad J (2004).

A serological study of Chikungunya virus transmission in Yogyakarta, Indonesia: Evidence for the first outbreak since 1982 Southeast Asian Journal of trop Med and Public Health, 35 No. 2.

Kondekar S, Gogtay NJ (2006). Why Chikungunya is called Chikungunya. *J Postgrad Med*. ;52:307.

Kumar NP Suresh A, Vanamail P, Sabesan S, Krishnamoorthy KG.; Mathew J, Jose VT, Jambulingam P (2011). Chikungunya virus outbreak in Kerala, India, 2007: a seroprevalence study. Mem Inst Oswaldo Cruz, Rio de Janeiro, Vol. 106(8): 912-916.

Kumari R, Nand P, Mittal V, Lal S, Saxena VK (2010). The first Chikungunya case from Sonipat district near the national capital city of Delhi, India. J of Inf Devel Countries 4: 262-263.

Mwongula AW, Mwamburi LA, Matilu M, Siamba DN, Wanyama FW (2013). Seroprevalence of Chikungunya Infection in Pyretic Children Seeking Treatment in Alupe District Hospital, Busia County Kenya. Int J Curr Microbiol App Sci 2(5): 130-139.

Oladosu OO, Oyibo WA (2013). Overdiagnosis and Overtreatment of Malaria in Children That Presented with Fever in Lagos, Nigeria. ISRN Infect Dis: 1–6.

Pialoux G, Gaüzère BA, Jauréguiberry S, Strobel M (2007). Chikungunya, an epidemic arbovirosis ; http://infection.thelancet.com Vol 7.

Powers AM and Logue CH (2007). Changing patterns of chikungunya virus: re-emergence of a zoonotic arbovirus. J Gen Virol. 88:2363-2377.

Rezza G, Nicoletti L, Angelini R, Romi R, Finarelli AC, Panning M, Cordioli P, Fortuna C, Boros S, Magurano F, Silvi G, Angelini P, Dottori M, Ciufolini MG, Majori GC, Cassone A (2007). CHIKV study group.Infection with chikungunya virus in Italy: an outbreak in a temperate region. Lancet. 370(9602):1840-1846.

Robinson MC (1955). An epidemic of virus disease in Southern Province, Tanganyika Territory, in 1952-53. Clinical features. Soc Trop Med Hyg 49:28-32.

Sanders EJ, Rwaguma EB, Kawamata J, Kiwanuka N, Lutwama JJ, Ssengooba FP, Lamunu M, Najjemba R, Were WA, Bagambisa G, Campbell GL (1999). Nov. O'nyong-nyong fever in south-central Uganda, 199-1997: description of the epidemic and results of a household-based seroprevalence survey. J Infect Dis 180: 1436–1443.

Sang RC, Ahmed O, Faye O, Kelly CL, Yahaya AA, Mmadi I, Toilibou A, Sergon K, Brown J, Agata N, Yakouide A, Ball MD, Breiman RF, Miller BR, Powers AM (2008). Entomologic investigations of a chikungunya virus epidemic in the Union of the Comoros. Am J Trop Med Hyg.78(1):77-82.

Sergon K, Njuguna R, Kalani V, Ofula C, Onyango S, Limbaso S, Bedon H, Burk AM, Dumila KM, Njenga R, Sang RE, Breiman F (2008). Seroprevalence of chikungunya virus (CHIKV) infection on Lamu Island,

Kenya. Amer J of Trop Med and Hygiene 78: 333–337.

Weller N, Clowers P, Dobler G, Saathof E, Kroidl I (2014). Seroprevalence of Alphavirus Antibodies in a Cross-Sectional Study in Southwestern Tanzania Suggests Endemic Circulation of Chikungunya. Negl. Trop. Dis. 8, 1371-2979.

Wongsrichanalai C, Barcus MJ, Muth S, Sutamihardja A, Wernsdorfer WH (2007). A review of malaria diagnostic tools: Microscopy and rapid diagnostic test (RDT). Am J Trop Med Hyg; 77:119–27.