CHIKUNGUNYA KNOWLEDGE, ATTITUDE, AND PRACTICES AND ITS TRANSMISSION INDICES IN TANGA CITY, NORTH-EASTERN TANZANIA

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A DISSERTATION SUBMITTED IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE IN ONE HEALTH MOLECULAR BIOLOGY OF THE SOKOINE UNIVERSITY OF AGRICULTURE. MOROGORO, TANZANIA.

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ABSTRACT

Chikungunya is among the important re-emerging arboviral disease caused by Chikungunya virus (CHIKV), an alphavirus belonging to Togaviridae family. Since the first outbreak in Tanganyika (now Tanzania) in 1952/53, several outbreaks are constantly being reported in different parts of the world. So far, there is no commercially available vaccine or drug effective for Chikungunya management. Vector control is the main option. This study aimed to determine the Chikungunya knowledge, attitudes and practices and its transmission indices in Tanga City, north-eastern Tanzania. This cross-sectional study was conducted in Tanga city, involving Nguvumali, Mzingani and Central wards. In this study, mosquitoes were collected by a Mosquito Magnet Liberty Plus trap in six selected sites (two sites per ward). Larvae surveys were conducted to randomly selected households. A questionnaire on knowledge, attitude and practices regarding Chikungunya was administered to heads/members of households where larval surveys were conducted. Mosquitoes were identified morphologically. Detection of CHIKV in *Aedes aegypti* was done using one step reverse transcription polymerase chain reaction (RT-PCR). Majority of the respondents (88%) were unaware of Chikungunya fever and its associated information. A total of 1469 adult mosquitoes were collected and identified into four species. *Aedes aegypti* was the most abundant (73.52%). Larvae survey involved 101 households and out of them 88 water holding containers were surveyed. The House Index (HI), Container Index (CI) and Breteaux Index (BI) were 40.59%, 60.2% and 52.5% respectively. Female *Ae. aegypti* were pooled into 44 pools (20 mosquito/pool) for detection of CHIKV and 7 pools were positive for CHIKV. In conclusion, *Aedes aegypti* mosquitoes are abundant and local transmission of CHIKV is taking place in Tanga city. The community knowledge and practices as regards to Chikungunya is low. Further research should be carried out to assess the status of Chikungunya transmission in human population in Tanga.

DECLARATION

I, Michael J. Msolla, do hereby declare to the Senate of Sokoine University of Agriculture that this dissertation is my own original work done within the period of registration and that it has neither been submitted nor being concurrently submitted to any other institution.

_____ _____

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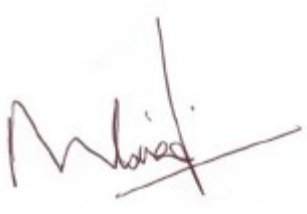
The declaration is hereby confirmed by;

_____ _____

Prof. Gerald Misinzo Date

(Supervisor)



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Dr. Leonard E. G. Mboera Date

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DEDICATION

I dedicate this work to my creator, the Almighty God and to my parents Jackson and Daines Msolla. I also dedicate this work to my siblings Abia Msolla and Herry Msolla for their endless love and support in the course of my education life.

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LIST OF ABBREVIATIONS AND SYMBOLS

µl	microliter
^{o}C	degree Celsius
Ae	Aedes
An	Anopheles
BI	Breteaux index
Bp	base pair
CDC	Centers for Disease Control and Prevention
CHIKV	Chikungunya virus
CI	container index
CO1	cytochrome C oxidase subunit 1
Cx	Culex
DMEM	Dulbecco's Modified Eagle Medium
DNA	deoxyribonucleic acid
GPS	geographical positioning system
HI	house index
Km	Kilometer
Ma	Mansonia
MRCC	Medical Research Coordinating Committee
NC	negative control
NIMR	National Institute for Medical Research
NSP	non-structural protein
ORF	open reading frame
PC	positive control
PCR	polymerase chain reaction
RNA	ribonucleic acid
RT-PCR	reverse transcription polymerase chain reaction
SUA	Sokoine University of Agriculture
TAE	Tris acetate EDTA buffer
URT	United Republic of Tanzania
USA	United States of America
UTR	untranslated region
WHC	water holding container
WHO	World Health Organization

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background

Chikungunya which literary means a “disease that bends up the joint” is a mosquito-borne infection caused by the Chikungunya virus (CHIKV). Chikungunya virus was first isolated in Newala and Masasi districts of southern Tanzania in an outbreak in 1952-53 (Robinson, 1955). Until early 2000s, little was known of its epidemic potential with only a few sporadic outbreaks mainly reported from Africa, Asia, Europe and the Indian and Pacific Ocean islands (Weaver *et al*., 2015). To date, the disease is widespread in the Americas, Oceania/Pacific Islands, Asia, Africa and Europe (CDC, 2019). In Africa, CHIKV infections have been reported in 29 countries (CDC, 2019), with recent outbreaks reported from Angola, Cameroon, Central African Republic, Comoros, The Republic of Congo, The Democratic Republic of Congo, Kenya, Gabon, Guinea, Malawi, Mozambique, Reunion, Nigeria, Seychelles, South Africa, Tanzania and Uganda (Nsoesie *et al*., 2016; Wahid *et al*., 2017; Proesmans *et al*., 2019; Vairo *et al*., 2020).

Chikungunya is caused by an alphavirus belonging to the Togaviridae family (Mascarenhas *et al*., 2018). It is a 12 kilo bases (kb) single-stranded, positive-sense RNA virus and belonging to the Semliki Forest virus complex (Mascarenhas *et al*., 2018). There are four circulating CHIKV genotypes, namely Asia, East/Central/South Africa, West Africa and Indian Ocean Lineage (Mascarenhas *et al*., 2018). Chikungunya virus infection in humans is characterized by febrile illness, skin rashes and severe arthralgia. Severe manifestations including myocarditis, hepatitis, ocular and neurological disorders have also been reported (Galán-Huerta *et al*., 2015).

Chikungunya virus is transmitted mainly by *Aedes aegypti* and *Ae. albopictus* mosquitoes (Thavara *et al*., 2008). Moreover, in Africa, several sylvatic Aedes species have been identified to carry CHIKV. They include *Ae. africanus* (in East Africa); *Ae. furcifer*, *Ae. taylori*, *Ae. dalzieli*, *Ae. luteocephalus*, and *Ae. vittatus* (in West Africa); and *Ae. taylori* and *Ae. cordellieri* in Southern Africa (Weetman *et al*., 2018).

To date there is neither effective antiviral drug nor commercially available vaccine that are used for treatment and prevention of Chikungunya. Vector control remains to be the most reliable method to prevent and control Chikungunya (Mascarenhas *et al*., 2018). It is therefore, critical that surveillance programs in both human and vectors are put in place to provide evidence-based effective control interventions. This study was carried out to determine vectors and the transmission indices of Chikungunya as well as community knowledge, attitudes and practices regarding the disease in Tanga City in north-eastern Tanzania.

1.2 Problem Statement and Justification

In recent years, Sub-Saharan African countries, including Tanzania have experienced periodic outbreaks related to mosquito-borne viral diseases such as Rift Valley fever, Chikungunya, Zika, Yellow fever and Dengue (Braak *et al*., 2018). However, mosquito-borne viral diseases have received little attention in the region most likely due to inadequate knowledge, awareness and availability of diagnostic tools. Thus, Chikungunya is underreported and go unnoticed despite the fact that seroprevalence studies have indicated the presence of the disease in some parts of Tanzania (Chipwaza *et al*., 2014; Kajeguka *et al*., 2016; Kinimi *et al*., 2018). On the other hand, there are only a few studies that have established CHIKV transmission in mosquitoes in Tanzania. Patrick *et al*. (2018) reported an overall CHIKV infection rate of 30% in *Ae. aegypti* in Karagwe,

Kyerwa and Mbeya. Although CHIKV has been reported in Tanga, Tanzania (Kajeguka *et al*., 2016; The Citzen, 2018), there is dearth of information of its vectors and transmission indices as well as community knowledge, attitudes and practices regarding the disease. In order to identify and characterize the local vectors of CHIKV there is need to conduct entomological surveys to confirm the presence and identity of its vectors in the area. Identifying vector species, disease transmission indices and risk factors is critical for devising appropriate prevention and control strategies and as well as future research priorities. Since effective control strategy for mosquito vectors engages the community (Gubler and Clark, 1996), therefore its efficiency depends on information gathered pertaining to community knowledge, attitude and practices regarding the disease.

1.3 Research Questions

i. What are the community knowledge, attitude and practices with regards to Chikungunya in Tanga City?
ii. What are the mosquito species responsible for CHIKV transmission in Tanga City?
iii. What are the infection rates of CHIKV in mosquitoes in Tanga City?

1.4 Objectives

1.4.1 Main objective

The main objective of this study was to determine Chikungunya knowledge, attitudes and practices and its transmission indices in Tanga City in north-eastern Tanzania.

1.4.2 Specific objectives

i. To assess community knowledge, attitudes and practices as regards to Chikungunya in Tanga City.
ii. To determine mosquito abundance and species composition of CHIKV vectors in Tanga City and
iii. To determine CHIKV infection rates in identified mosquito vectors.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Mosquitoes

Mosquitoes are arthropods classified into the order Diptera, sub-order Nematocera and family *Culicidae*. The *Culicidae* family is divided into three subfamilies namely, Anophelinae, Culicinae and Toxorhynchitinae. Adult females of Anophelinae and Culicinae are blood feeders while those of Toxorhynchitinae feed on nectar and other plant juices (Harbach, 2007). The Anophelinae subfamily comprise of three genera while Culicinae has 41 genera (Harbach, 2007). The Culicinae subfamily has been reported to carry and transmit a number of arboviruses and parasites causing diseases in humans and animals (Jeffries *et al*., 2018).

2.1.1 Culicine mosquitoes

There are 30 mosquito genera belonging to culicine subfamily, of which the medically important ones are Culex, Aedes, Mansonia, Sabethes, Haemagogus and Psorophora. In the tropics, Aedes mosquitoes are important vectors of several viral diseases including

Zika, Dengue, Chikungunya, Yellow fever and Rift valley fever (Jeffries *et al*., 2018). Globally, *Ae. aegypti* is the major vector of all of these viruses, but a range of African *Aedes* species are competent and epidemiologically significant vectors (Tedjou *et al*., 2019). Only a few studies in Tanzania have reported on the role of *Aedes* mosquitoes in the transmission of arboviruses (Trpis, 1972; Mboera *et al*., 2016; Patrick *et al*., 2018).

2.1.2 Aedes mosquito ecology and life cycle

The Aedes mosquitoes have a complex life cycle that involves aquatic and terrestrial life (Kweka *et al*., 2018). Before laying eggs, Aedes mosquitoes like most of other mosquito species prefer a blood-meal from the host in order for the eggs to develop. However, in the absence of vertebrate hosts, females can feed on plant nectar (Scott *et al*., 2000). *Aedes aegypti* prefers to breed in both natural and artificial containers where the latter is said to be most important (Trpis, 1972). The artificial breeding sites are mostly water storage containers and discarded containers (Trpis, 1972). Mosquito lays her eggs on the sides of water holding containers, and eggs hatch into larvae after a rain or flooding. A larva changes into a pupa in about a week and into an adult mosquito in two days (Zahouli *et al*., 2016). Adult females of *Ae. aegypti* are mostly diurnal (feeding during the day) and endophagic (indoor feeders) (WHO, 2020).

2.1.3 Mosquito identification

There are various methods or approaches that are used in mosquitoes' identification. They include morphological, molecular, proteomics tools, isoenzyme analysis and others (Jourdan *et al*., 2018). Despite its shortcomings, morphological identification has been frequently used for both research and operational surveillance because it requires little technical facilities and is inexpensive even in situation where large number of mosquitoes

are to be identified (Jourdain *et al*., 2018). Identification based on morphological characteristics is achieved through the use of dichotomous keys (Huang, 2001) which rely on external features such as scales, legs, wings and bristles. This method faces limitations which include the need of trained taxonomists to perform the task and this render them difficult in presence of large number of species, level of preservation of morphological characters, species complexes when they share most of the morphological characteristics and identification of immature stages of mosquitoes (Jourdan *et al*., 2018). Molecular identification of mosquitoes is an accurate method that eliminates complexes that arise in morphological identification. DNA barcoding is among the molecular method that is constantly being used for vector surveillance programs (Batovska *et al*., 2016). It entails the use of short DNA segments which hold much less variation within species than between species (Batovska *et al*., 2016). The 5′-segment of the mitochondrial gene Cytochrome Oxidase I (COI) is frequently being used in barcoding studies. Although other regions of DNA can also be used, the COI segment of mitochondrial is regarded as a standard approved by the Barcode of Life Database (BOLD) (Ratnasingham and Hebert 2007). Isoenyme analysis is commonly used in determination of slight variations from species to species in the mobility and structures of several intracellular enzymes (Nims and Herbstritt, 2005). The steps involved in this technique include extraction of isoenzymes, separation of enzymes in gel electrophoresis and staining (Siddiquee *et al*., 2010). Time consuming is one of the challenges associated with isoenzymes analysis (Haque *et al*., 1998). Molecular identification techniques are however, expensive and need trained personal, hence not readily available in routine mosquito surveillance programmes.

2.2 Chikungunya

2.2.1 Chikungunya virus

Chikungunya virus is an enveloped, spherical, single-stranded positive-sense RNA alphavirus belonging to the family *Togaviridae* with a genome size of approximately 12 kb (11 811 nucleotides). The genome is organised as 5'-UTR-nsP1-nsP2-nsP3-nsP4-J-C-E3-E2-6K-E1-polyA-3'-UTR (Mascarenhas *et al*., 2018). It has two open reading frames (ORFs) placed between the 5'and 3' UTRs. First ORF (7422 nucleotides long) encodes four non-structural proteins, nsP1, nsP2, nsP3, and nsP4. Second ORF encodes five structural proteins, including the capsid and envelope glycoproteins E1, E2, E3 and J (junction) region (used as the promoter for subgenomic RNA synthesis) (Mascarenhas *et al*., 2018) (Fig. 1).

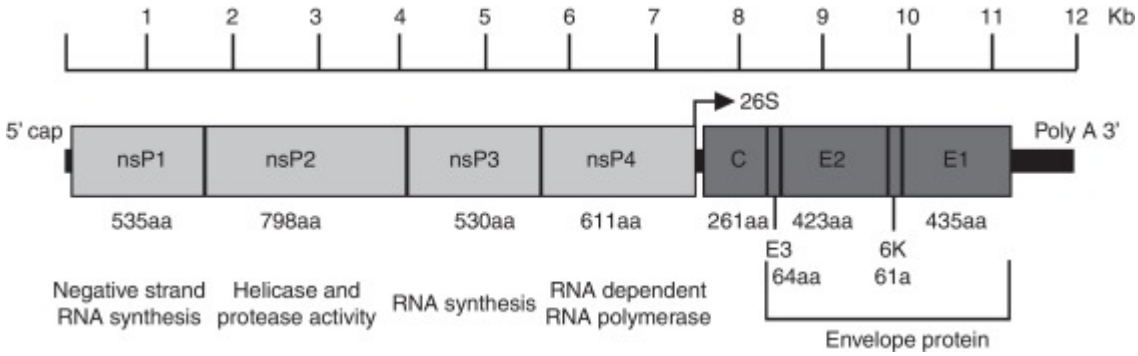


Figure 1: Chikungunya virus genome organization. The figure shows organization of structural proteins, non-structural proteins and non-translatable regions in 5' to 3' direction. For each protein, amino acid size and function are presented. Source: Galán-Huerta *et al*. (2015).

2.2.2 Transmission of Chikungunya virus

Chikungunya virus is mainly transmitted via two cycles: urban and sylvatic. The urban cycle is the one in which a transmission is from human to mosquito to human, whereas the sylvatic cycle involves transmission from animal to mosquito to human (Sigh and Unni, 2011). In more densely populated areas, CHIKV is mainly maintained in an urban cycle, where humans act as key hosts and Aedes mosquitoes as vectors (Sigh and Unni, 2011). *Aedes aegypti* has been documented to be the principal vector of CHIKV (Powers and Logue, 2007). In addition, *Ae. albopictus* has been reported as vector in some parts of the world such as Reunion Island, Gabon and Europe (Sigh and Unni, 2011).

2.2.3 Diagnosis of Chikungunya

Although clinical signs are the most commonly used diagnostic method in most of the low-and- middle income countries, they do not provide definitive diagnosis of the disease since a number of diseases manifest themselves with similar clinical picture (Johnson *et al*., 2016). Laboratory diagnosis of CHIKV infection is accomplished by serologic methods, virus isolation, and viral RNA detection by reverse transcription polymerase chain reaction (RT-PCR) (WHO, 2009; Johnson *et al*., 2017). Molecular tools are rapid, sensitive and useful in both entomological surveillance as well as molecular epidemiology of diseases (Mascarenhas *et al*., 2018). But they are expensive, not available for clinical management and are prone to providing false positive results due to contamination and their high sensitivity (Mascarenhas *et al*., 2018).

2.2.4 Management of Chikungunya

There is no vaccine against Chikungunya that is commercially available but numerous candidates are currently being studied (Gao *et al*., 2019). The management of this disease is typically symptomatic paying more attention on alleviation of fever and joint pain (arthralgia) (Mascarenhas *et al*., 2018). The pains which range from acute, post-acute and chronic are mainly treated by pain killers like ibuprofen, naproxen or other non-steroidal anti-inflammatory agents (NSAID) while antipyretic agents such as acetaminophen or paracetamol can be used to relieve fever (WHO, 2009). Furthermore, patients are advised to take rest, carry out movements and mild exercises as well as plenty of fluids to replenish fluids lost from excessive sweating and vomiting (WHO, 2009). In cases whereby joint pain is prolonged or becomes persistent, long term anti-inflammatory therapy as well as graduated physiotherapy are recommended (WHO, 2009).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Study Design and Study Area

The present cross-sectional study was conducted in Tanga city, north-eastern Tanzania located at -5^0.04’8.15” S and 39^{0}05’55.50 E. Tanga is dominated by warm and wet climate and is located along the Indian Ocean coast. During the hot months (December to March) the average temperature is between 30 ^{0}C and 32 ^{0}C while in cool months (May to October) the average temperature is 23 ^{0}C-28 ^{0}C. On average, Tanga receives 1327 mm of rainfall annually with the peak during April and May whereas the lowest is during January and February. Tanga region economy is mainly based on subsistence agriculture, livestock keeping and fishing. Tanga city is comprised of 24 wards having a total area of 597 km^2 with a population size of 273 332 and the population density of 458.2 per km^2 (URT, 2013).

The study was conducted during December 2019 and involved three wards namely, Central, Mzingani and Nguvumali (Fig. 2). The sampling sites were purposively selected based on demographic and ecological characteristics. Central has a total area of 3.88 square kilometres, a population size of 5739 and a population density of 1478/km^2. Mzingani ward covers a total area of 8.18 km^2, population size of 29 049 and its population density is 3551/km^2 while Nguvumali ward has a total area of 5.79 km^2, population size of 15 133 and 2612/km^2 population densities. All sampling points within the study area were recorded using global positioning system (GPS) and mapped using Geographical Information System (Fig. 2).

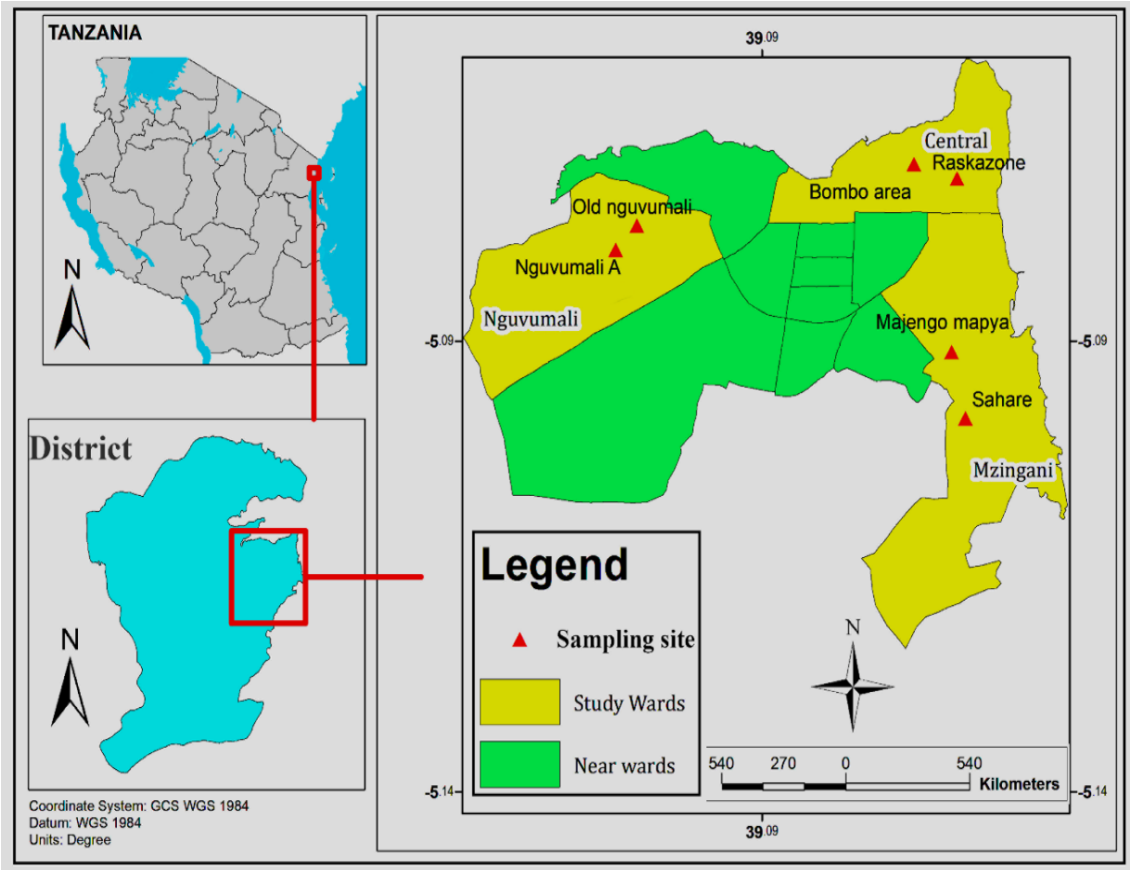


Figure 2: Map of Tanga City showing sampling wards and sites

3.2 Community Knowledge, Attitudes and Practices with Regard to Chikungunya

A questionnaire was administered through face-to-face interview to 99 heads/ members of households (≥18 years old) during the mosquito larvae survey. The questionnaire comprised of four parts: i) demographic and household characteristics; ii) knowledge about Chikungunya, its vectors, transmission, and signs/symptoms; iii) Attitudes toward Chikungunya; and iv) prevention practices against Chikungunya (Appendix 1). The sample size of respondents was determined by the number of houses where the permission to do larvae survey was given.

3.4 Adult Mosquito Collection

Two sampling sites were selected from each of the study wards. Collection of adult mosquitoes was done using carbon dioxide-propane powered Mosquito Magnet Liberty Plus traps (Fig. 3). Two traps were used to collect mosquitoes from two different streets within each ward. The traps in each sampling site were set from 0600 hours then allowed to operate for 24 hours for three consecutive days and mosquitoes were collected the following morning. The species, sex and abdominal status of each mosquito were recorded in a mosquito collection form (Appendix 2).

Figure 3: Mosquito Magnet Liberty Plus trap

3.5 Immature Mosquito Surveys

Water-holding containers (artificial and natural breeding sites) were surveyed for the presence of immature stages (larvae and pupae) of Aedes mosquitoes from the selected houses during the study. Larval dipping technique was done in all sampling sites. Following entrance, the yard and the areas around the house were thoroughly inspected for the presence of water-holding containers. For small discarded containers, sampling was done by first emptying the water from the container into a tray and a plastic Pasteur pipette was used to collect the immature stage of mosquitoes. For medium plastic containers, surveys entailed pouring the water through the sieve into a bowl with a good contrast and the immatures were collected using a small plastic dropper. All containers surveyed were scored for container type, approximate volume of container, water type/state (either clear or turbid otherwise) and approximate water volume within the container.

3.6 Identification of Collected Mosquitoes

On each sampling day, the collected adult mosquitoes were killed by freezing and identified using stereo microscope to genus and species level based on the morphological identification keys (Huang, 2001). Identified mosquitoes were pooled into groups of 20 based on species and site of collection where only females Aedes mosquitoes were packed in cryovials and stored in liquid nitrogen.

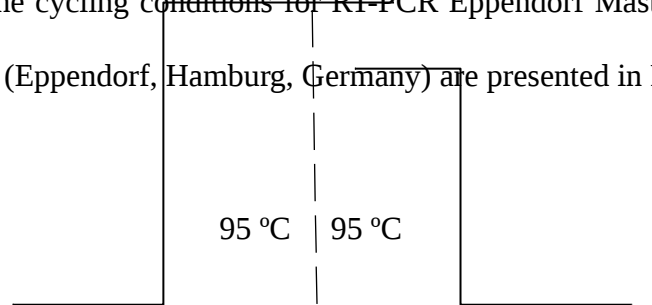
3.7 Screening of Chikungunya Virus in Mosquito Pools

3.7.1 RNA extraction

Mosquito samples in cryovials were mixed with Dulbecco's Modified Eagle Medium (DMEM) and tissues homogenized using a plastic application stick. Total RNA was extracted using QIAamp Viral RNA kit (Qiagen, Hilden, German), according to the manufacturer's instructions with little modification (Appendix 3). Briefly, samples were lysed using lysis buffer (AVL buffer) followed by addition of absolute ethanol to precipitate proteins. The lysate was passed through a QIAamp min column and washed with washing buffers. The RNA was later eluted from the columns with 60 µL of Buffer AVE. The extracted RNA was stored at – 80 ºC for RT-PCR.

3.7.2 Detection of Chikungunya virus

One step reverse transcriptase polymerase chain reaction (RT-PCR) was carried out to detect viral RNA using AgPath-ID™ One-Step RT-PCR Kit (Waltham, MA, USA). Chikungunya virus primer sets (CHIKV E1F: 5'-ACGCAATTGAGCGAAGCA C-3' and CHIKVE1R:5'-CTGAAGACATTGGCCCC AC-3') as designed by Thavara *et al*. (2009) that target E1 gene region with 200 bp were used. A 25µL reaction Master mix for CHIKV RT-PCR was prepared in clean environment using 2× reaction mix, 25× RT-PCR Enzyme Mix, CHIKVE1F primer, CHIKVE1R primer, Magnesium salt and nuclease-free water. The cycling conditions for RT-PCR Eppendorf Mastercycler Nexus Thermocycler machine (Eppendorf, Hamburg, Germany) are presented in Fig. 4.



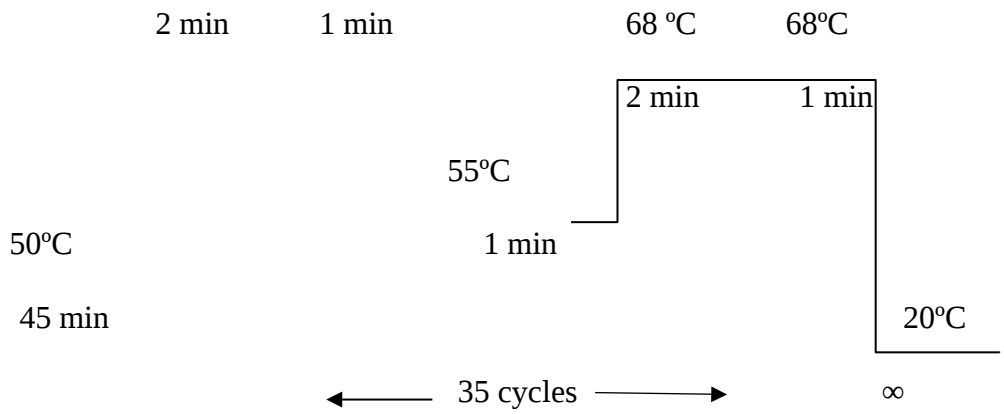


Figure 4: RT-PCR cycling conditions for Chikungunya virus detection

3.7.3 Gel Electrophoresis and visualization of PCR products

RT-PCR products were separated by electrophoresis on a 1.5% agarose gel in 1× Tris acetic acid buffer (SERVA Electrophoresis, Heidelberg, Germany) and then stained with GelRed nucleic acid stain (Phenix Research Products, Candler, USA). Each well was loaded with five µL of the PCR product and three µL of blue/orange 6× DNA loading dye (Promega, Madison, USA). Samples were separated along with a 100bp DNA marker (Promega, Fitchburg, CA) at 100 volts for 35 minutes. Visualization of the Agarose gel was done in ultraviolet fluorescence light using a gel documentation system (EZ Gel Doc, BioRad, USA).

3.8 Data Analysis

Data were entered in MS Excel and later on analysed by R software (University of Auckland, Auckland, New Zealand). Mosquito density was calculated for each sampling site. For immature populations (larvae or pupae), three main methods were used to assess Aedes mosquito infestation indices: (i) house index (HI) as the percentage of houses infested with larvae and / or pupae; (ii) container index (CI) as the percentage of water-

holding containers infested with larvae and/or pupae; and (iii) Breteaux index (BI) as the number of positive containers per 100 houses inspected in a specific location.

3.9 Ethical Consideration

This study received ethical approval from the Medical Research Coordination Committee of the National Institute for Medical Research (NIMR/HQ/R.8a/Vol. IX/3266) (Appendix 4). Prior to data collection, the purpose for the study was explained to the residents through community leaders. Each participant was given an informed consent form to sign after willing to participate (Appendix 5).

CHAPTER FOUR

4.0 RESULTS

4.1 Knowledge, Attitude and Practices with Regard to Chikungunya

4.1.1 Socio-demographic characteristics of studied population

A total of 99 participants were included in this study. One third (33.3%; n=33) were ≥56 years, while the majority (70.7%; n=70) were females. Among the participants, 72.7% were married, majority (45.5%) had attained primary school education and 21.2% mentioned business as their core source of income (Table 1).

Table 1: Socio-demographic characteristics of study participants.

Variables	Response	Number (%)
Age categories	18-25	20 (20.2)
	26-35	19 (19.2)
	36-45	14 (14.1)
	46-55	12 (12.1)

	56 and above	33 (33.3)
Sex	Male	29 (29.3)
	Female	70 (70.7)
Marital status	Single	17 (17.3)
	Married	72 (72.7)
	Cohabiting	1 (1.0)
	Separated/divorced	1 (1.0)
	Widow/widower	8 (8.1)
Education level	None	4 (4.0)
	Primary education	45 (45.5)
	Secondary education	27 (27.3)
	Advanced education	9 (9.1)
	Post-secondary education	14 (14.1)
Occupation	Farmer	4 (4.0)
	Employed	38 (38.4)
	Business	21 (21.2)
	Unemployed	36 (36.4)

4.1.2 Knowledge regarding Chikungunya

Of 99 participants, only few reported to have heard about Chikungunya (Nguvumali, 18.9%; Mzingani, 5.3% and Central 9.7%) (Fig. 5). When participants were asked to mention diseases that are transmitted by mosquitoes, majority (100%) mentioned Malaria. Chikungunya was mentioned by a small proportion across all three wards (Nguvumali, 4.8%; Mzingani, 1.4% and Central 3.7%) (Fig. 6). Across the three wards, only 6.7%, 0.0% and 3.2% of the respondents in Nguvumali, Mzingani and Central respectively responded of being aware of Chikungunya symptoms. Only three participants across all wards mentioned fever and arthralgia as Chikungunya clinical signs.

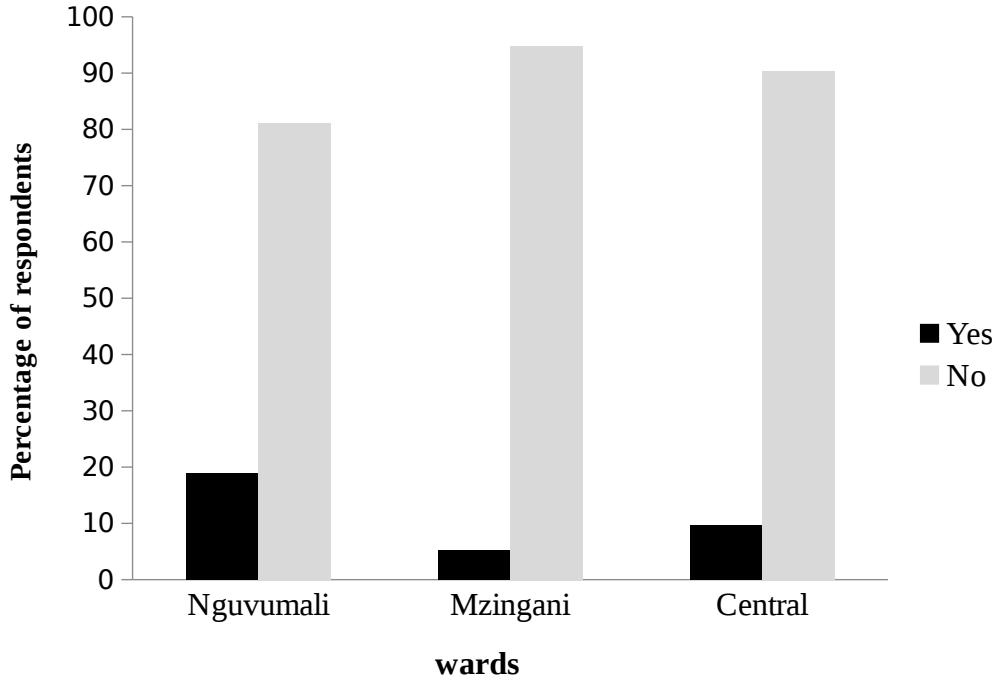


Figure 5: The percentage of respondents who were aware of Chikungunya

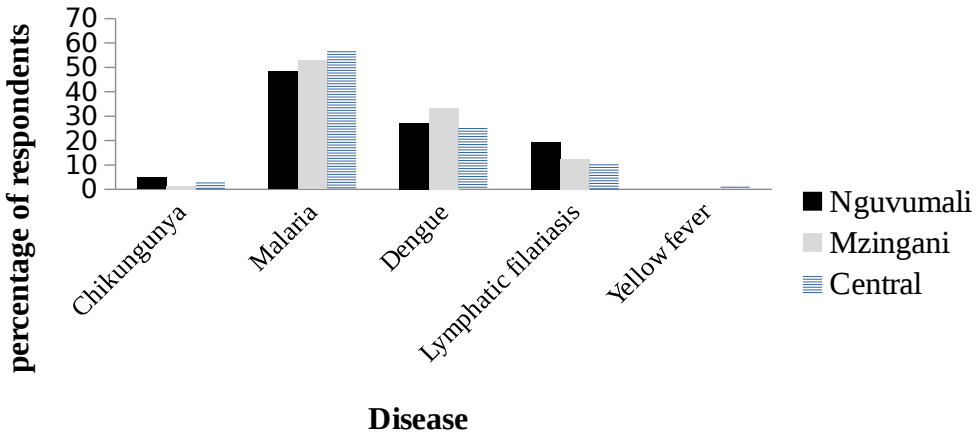


Figure 6: Percentage of respondents with knowledge on mosquito-borne diseases

4.1.3 Attitude towards Chikungunya

Participants were asked about the severity (seriousness) of Chikungunya ranging from high, moderate, low and do not know. High percentage (96.0%) of participants in all wards were not aware of the severity of the disease (Nguvumali, 93.3%; Mzingani, 97.4% and Central, 96.8%) while a small proportion said “high” (Nguvumali, 3.3%; Mzingani, 2.6% and Central, 3.2%) and only in Nguvumali where 3.3% perceived Chikungunya severity as “low” (Fig. 7).

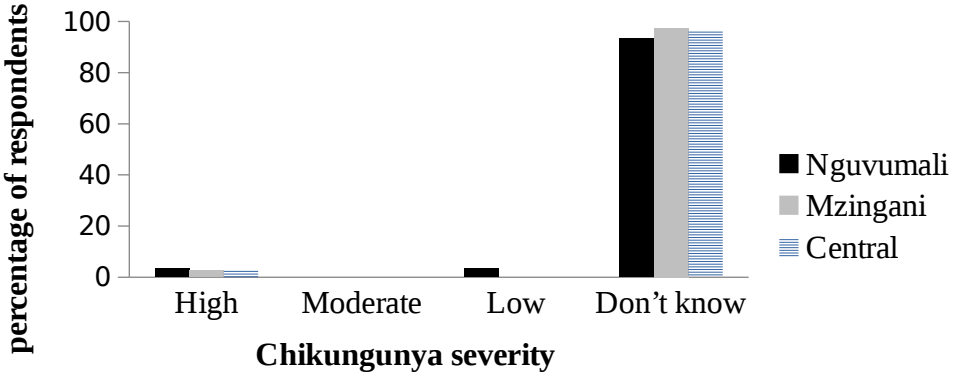


Figure 7: Percentage of respondents as regards to attitude toward Chikungunya severity

4.1.4 Practices toward Chikungunya transmission

Participants were asked about the presence of water holding containers in their premises. A relatively large proportion of participants in Central Ward (87.1%) reported to have water holding containers in their premises (Fig. 8). Majority of the respondents in Nguvumali, 93.3%, Mzingani, 97.4% and Central, 96.8%) reported to have experienced mosquito bite during day times (Fig. 9). However, most of them (Nguvumali, 76.7%; Mzingani, 84.2% and Central, 68.8%) would do nothing to protecting themselves from mosquito bites during the day time (Fig. 10). Few respondents (Nguvumali, 16.7%; Mzingani, 13.2% and Central, 25%) mentioned mosquito repellents as a means to prevent mosquito bites during the day times.

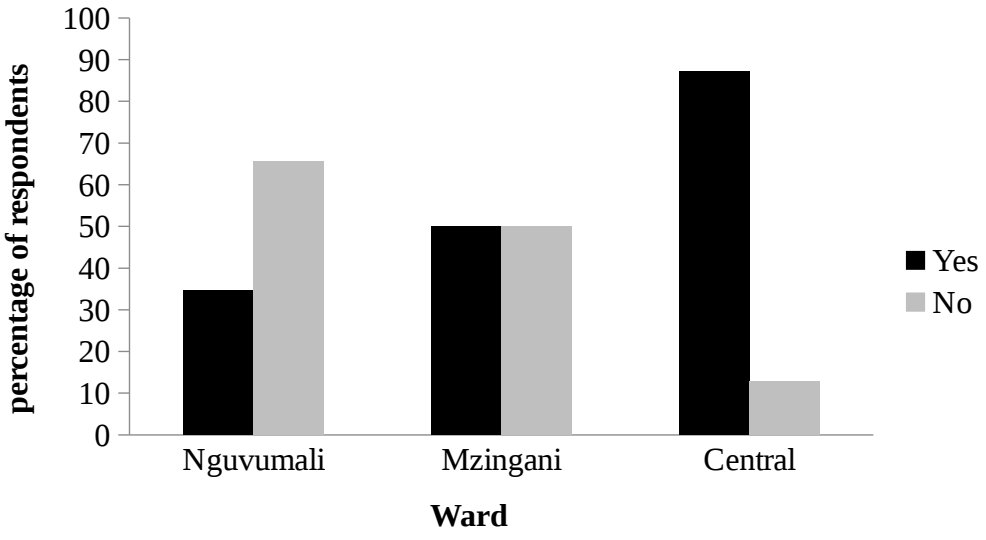


Figure 8: Percentage of respondents with water-holding containers in their house premises

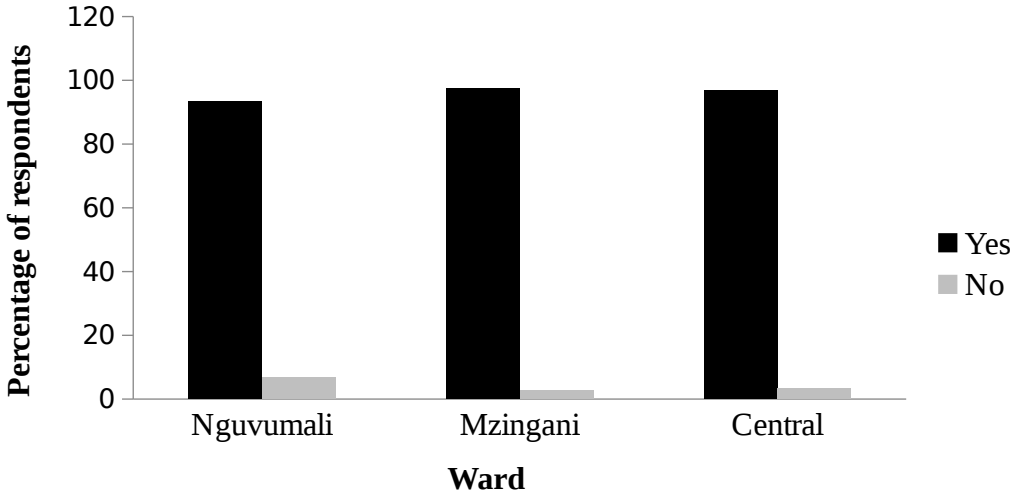


Figure 9: Percentage of respondents as regards to mosquito biting during the day time

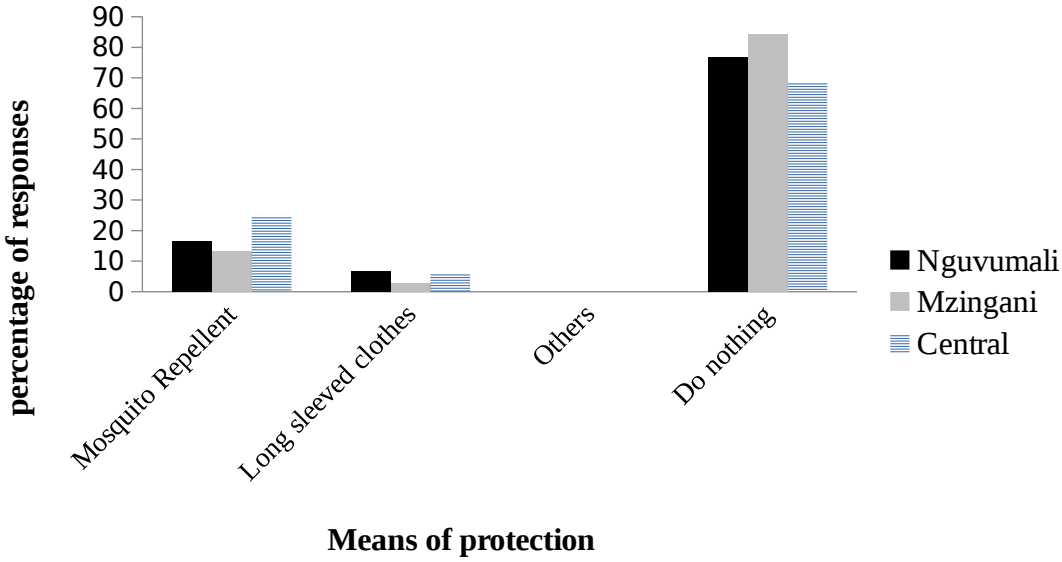


Figure 10: Percentage of respondents as regards to mosquito personal prevention

4.2 Mosquito Abundance

A total of 1469 adult mosquitoes were collected using mosquito magnet traps. Almost a similar proportion of mosquitoes was collected in Nguvumali (36.7 %; n=539) and Mzingani (36.3%; n=534) (Table 2). Three genera namely Aedes (73.66%), Culex (26.21% and Anopheline (0.14%) were identified. *Aedes aegypti* was the most abundant

mosquito species accounting for 73.52% of the total mosquitoes collected. *Culex quinquefasciatus* and *Culex cinereus* accounted for 16.34% and 9.87%, respectively. *Aedes simpsoni* accounted for 0.14% while *Anopheles gambiae* sensu lato accounted for 0.14% (Table 2). The distribution of each species is presented graphically in Fig. 11.

Table 2: Number (%) and mosquito species collected by ward

	Sampling locations (wards)			
Species	**Nguvumali No (%)**	**Central No (%)**	**Mzingani No (%)**	**Total No (%)**
Aedes aegypti	368 (68.27)	279 (70.45)	433 (81.09)	**1 080 (73.52)**
Aedes simpsoni	0 (0.00)	0 (0.00)	2 (0.37)	**2 (0.14)**
Culex quinquefasciatus	124 (23.01)	49 (12.37)	67 (12.55)	**240 (16.34)**
Culex cinereus	46 (8.53)	67 (16.92)	32 (5.99)	**145 (9.87)**
Anopheles gambiae	1 (0.19)	1 (0.25)	0 (0.00)	**2 (0.14)**
Total	**539**	**396**	**534**	**1 469**

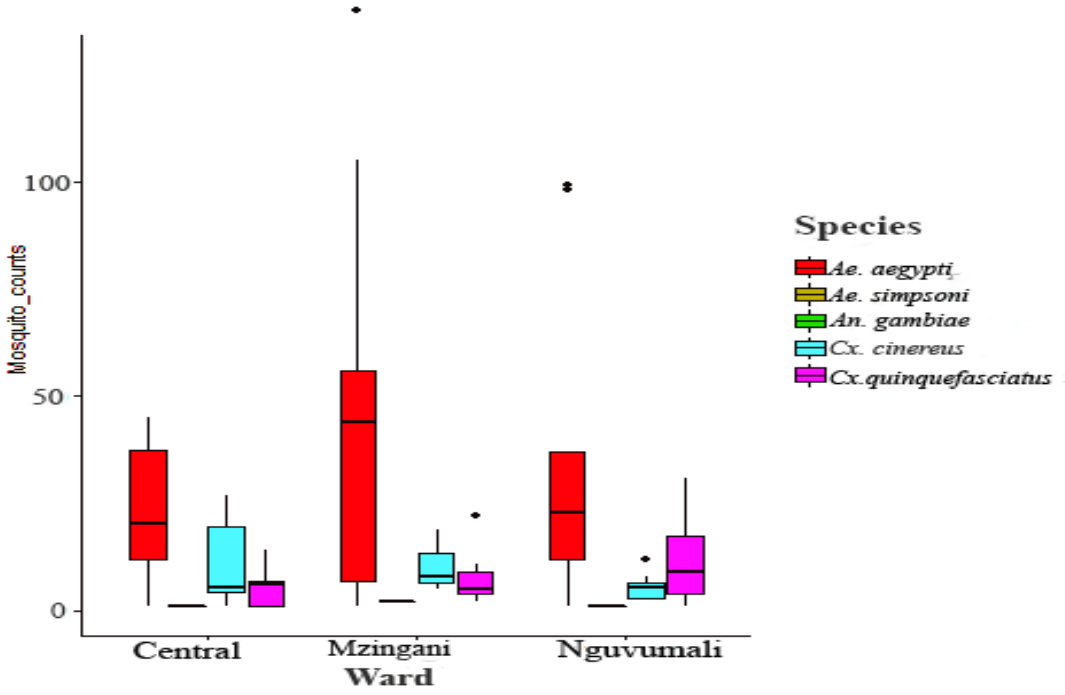


Figure 11: Distribution of adult mosquito species by ward

4.3 Immature Mosquito Survey

A total of 101 houses were inspected for the presence of potential breeding sites for Aedes mosquitoes. Out of these, 60 houses (59.4%) were found having water-holding containers (WHC) in their premises (Fig. 12). Houses in Mzingani were found to have larger proportions of water holding containers (69.44%). This was followed by Nguvumali (56.25%) and Central wards (51.51%) (Fig. 13).

Figure 12: Different water holding containers infested with mosquito larvae/pupae (A) Car tyres, (B), Bicycle tyre (C) Tree log and (D) Flower vase

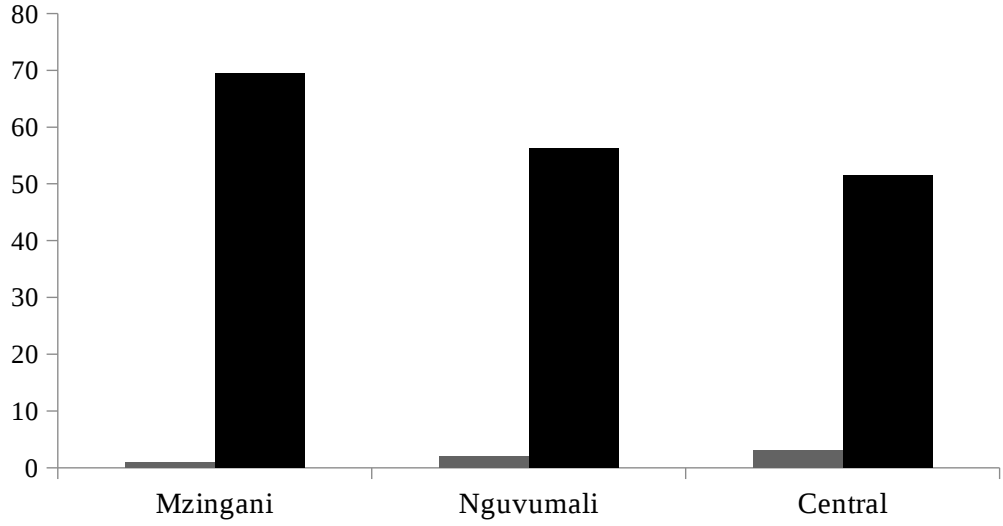


Figure 13: Proportion of houses with water holding containers by ward

The proportion of houses with larvae/pupa was as follows: Mzingani 41.67/25.0%, Central 42.42/30.30% and Nguvumali 34.38/18.75% (Fig. 14).

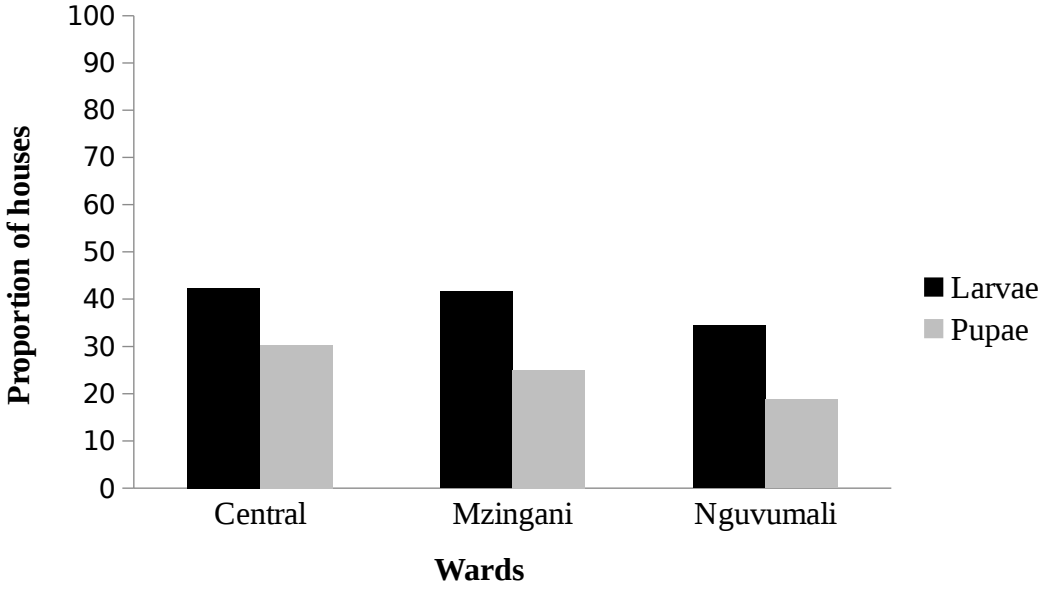


Figure 14: Proportion of houses with larvae and pupae in Tanga city

Among others, discarded tyres (42.59%, n=23) and discarded plastic containers (27.78%, n=15) were the most common breeding sites recorded (Fig.15). The other breeding sites included flower vases, water reservoir (Water drum/ barrel/tanks), domestic buckets, bowls and tree logs (Table 3). Among containers that were infested with immatures stages of mosquitoes, medium sized containers (1-10 liters) were higher (70%) in number when compared to other container groups. Containers that were found to have clear water (57.3%) were more infested with mosquito immature stages than water holding containers with turbid water.

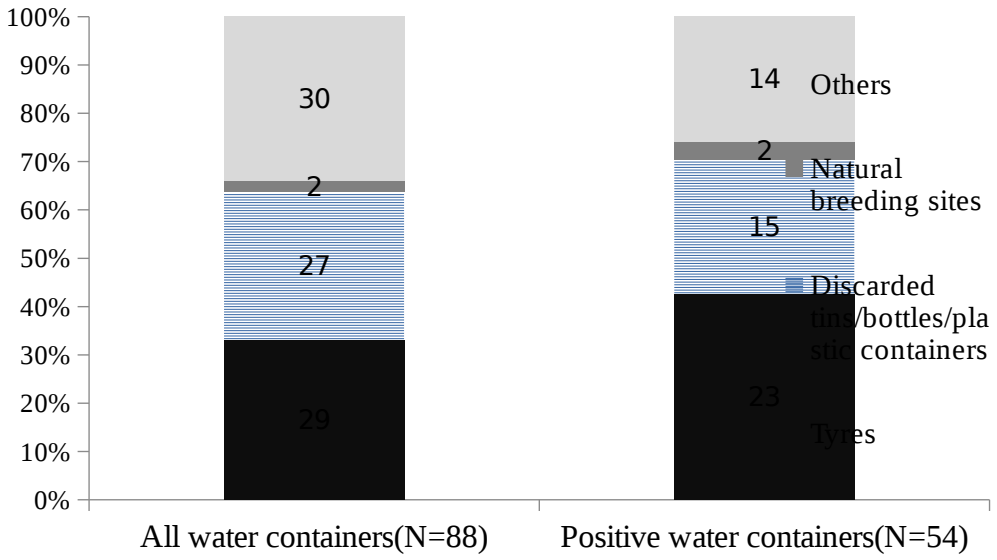


Figure 15: Type and abundance of water holding containers

The overall house index (HI) was 40.59%. Nguvumali, Mzingani and Central had house indices of 34.38%, 41.67% and 42.45%, respectively. The overall container index (CI) was 60.2%; it was 56% for Nguvumali, 53.5% for Mzingani and 80.0% for Central. Similarly, highest pupal index was recorded in Central (30.3%), followed by Mzingani (25.0%) and Nguvumali (18.8%) (Table 4).

Table 3: Types of water-holding containers (with mosquito larvae) by ward

Type of container	Nguvumali	Mzingani	Central	All
Tyres	7 (87.50%)	12 (80%)	4 (67%)	23
Discarded tins/bottles/plastic containers	4 (80.00)	5 (33%)	5 (71%)	15
Coconut shells	1 (25%)	0 (0%)	0 (0%)	1
Flower vase	1 (33%)	2 (50%)	0 (0%)	3
Drum barrel/tank	3 (60%)	0(0%)	2 (100%)	5
Animals drinkers/feeders	0 (0%)	1 (100%)	0 (0%)	1
Bowl/ceramic sink	0 (0%)	2 (100%)	2 (100%)	4
Plant axials, rock pools, tree log	0 (0%)	0 (0%)	2 (100%)	2

Table 4: Number (%) of houses surveyed with water-holding containers and Aedes indices by ward

Ward	**No. houses surveyed**	**No. houses with WHC**	**No. houses with larvae**	**Container Index (%)**	**House index (%)**	**Breteaux index**	**House index (% houses +ve for pupae**
Nguvumali	32	18	11	56	34.38	52.5	18.75
Mzingani	36	25	15	53.49	41.67		25
Central	33	17	14	80	45.45		30.3
Total	101	60	40				

4.4 Chikungunya Virus Detection in Mosquitoes

There was a total of 44 pools of female *Aedes aegypti* mosquitoes, each pool containing 20 mosquitoes. All the pools were screened for Chikungunya virus by RT-PCR assay. Of the 44 mosquito pools screened, seven were positive for CHIKV (Fig. 16) presenting an infection rate of 15.9%. Of the positive mosquito pools, 3 were from Mzingani, 3 from Nguvumali and 1 from Central ward (Fig. 16; Table 5).

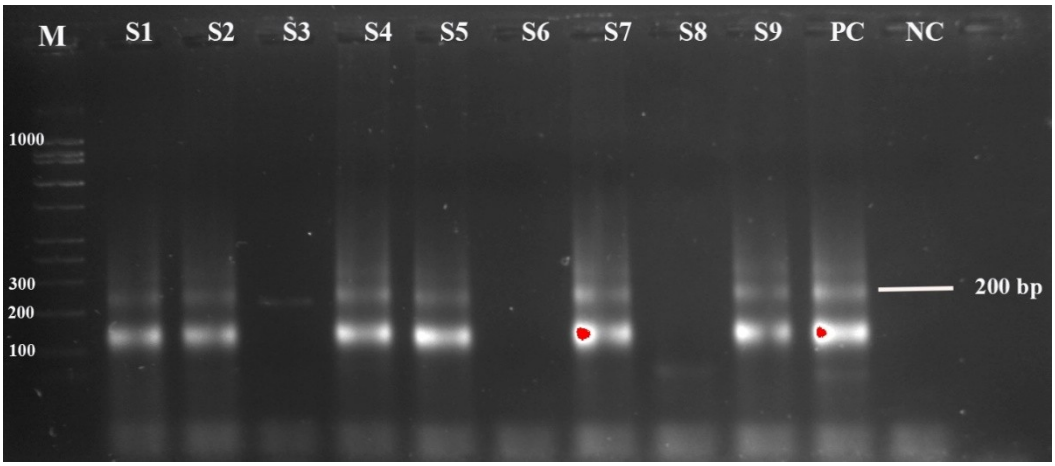


Figure 16: Gel electrophoresis of RT-PCR amplicon of Chikungunya virus.

CHIKV E1F and CHIKV E1R primers were used for detection. The expected band size is 200 bp. M, molecular weight marker; NC, negative control; PC, positive control; S1-S9, mosquito pool samples

Table 5: Chikungunya virus infection rate in Aedes mosquito pools

Ward	**Pool number**	**CHIKV**	**Infection Rate per location (%)**
Mzingani	3	+	
Mzingani	5	+	
Mzingani	15	+	15.8
Nguvumali	24	+	
Nguvumali	28	+	
Nguvumali	29	+	20.0
Central	30	+	10.0
Total		**7**	
Infection Rate		**7/44 (15.9%)**	

CHAPTER FIVE

5.0 DISCUSSION

The world is currently facing the challenge of mosquito-borne viral disease epidemics, with the majority of them occurring in tropical and sub-tropical regions (Gubler, 2002). Due to the recent changes in global climate, human activities, globalization, epidemiological and genetic factors, the need to collect more data regarding the emergence of mosquito-borne viruses has increased. The present study aimed to determine the mosquito abundance and transmission levels of Chikungunya in Tanga city as well as community knowledge, attitudes and practices as regards to the disease.

The findings of this study indicate that community knowledge on Chikungunya is very low. Most of the study participants were not aware of the Chikungunya disease. Only few reported to have heard about the disease. These findings are similar to the study carried out in Kilimanjaro, Tanzania, which found only 3.2% of the study participants were aware of Chikungunya (Kajeguka *et al*., 2017). This could imply lack of considerations for mosquito borne diseases other than malaria due to its observed impacts and control strategies that societies are constantly being involved. With regards to diseases transmitted by mosquitoes, only a small proportion of study participants pointed out that Chikungunya is transmitted by mosquitoes. The majority of the respondents knew that malaria is transmitted by mosquitoes. This shows that malaria is highly known by the community, similar to what has been reported by Chipwaza *et al*. (2014) in Kilosa, Tanzania. A very low proportion of the respondents knew the clinical signs and symptoms of Chikungunya. It was further found that the majority did not know the disease severity (seriousness) indicating the least consideration Chikungunya is given in this part of the

country. Among the surveyed participants, majority knew nothing concerning Chikungunya treatment or management. This conforms with the study conducted in Kilimanjaro where majority of community members and health care workers had little knowledge concerning Chikungunya treatment (Kajeguka *et al*., 2017). This implies that members of the society in all classes are not well informed about this infection. With regards to practices towards Chikungunya, it was found that majority of participants had water holding containers in their premises. A study in Jamaica, reported slightly higher proportions than in our study (Alobuia *et al*., 2015). The use of mosquito repellents as a means to avoid mosquito bites during the day time was recorded in a small proportion of participants with Central ward reporting relatively higher percent as compared to the rest of the wards. This finding is parallel to a systematic review by Corrin *et al*. (2017) where participants' score reported to be using mosquito repellents during day time ranged from 0-92%. This suggests that community is still unaware of the diseases that might be transmitted by day biting mosquitoes.

In terms of mosquito species composition, Aedes mosquitoes accounted for about three-quarters of mosquitoes in Tanga. Similar findings have been reported from a study in Cameroon involving urban and peri-urban areas (Mayi *et al*., 2020). Urban environment plays a significant role in mosquito breeding via presence of water holding devices like discarded tins, car tires, septic tanks and natural breeding sites which together favour breeding of Aedes mosquitoes (Patrick *et al.*, 2018).

The most common Aedes mosquito breeding sites were used car tyres and discarded plastic containers with Central ward having relatively higher proportion of containers positive for larvae/pupae. This may be contributed by presence of residential houses with large backyard area. Visual assessment showed that most of the backyard areas were not properly managed leaving open containers unattended. Similar findings were reported in

Dar es Salaam, Tanzania where discarded plastic containers and used car tyres were most important breeding sites for mosquitoes (Mboera *et al*., 2016). Majority of water holding containers that were infested with immature stages of mosquitoes were man-made. This conforms with the findings from two other studies in Tanzania (Mboera *et al*., 2016; Saleh *et al*., 2018). In this study, discarded tyres had a high positivity rate for larvae of Aedes mosquitoes. This is consistent with the study conducted in Ethiopia (Ferede *et al*., 2018). Clear water was found to habour more immature stages of mosquitoes than turbid water. This is consistent with the findings obtained from a study in Cameroon (Tedjou *et al*., 2020) but contrary to the study in Zanzibar where water turbidity had no association with the presence of immatures (Saleh *et al*., 2018). *Aedes aegypti* prefer to oviposit in clear water and has little adaptation to turbid water when compared to *Aedes albopictus* (Madzlan *et al*., 2016). Physical properties of water like temperature and turbidity have been reported to influence mosquito breeding (Teklu *et al*., 2010). This study reports overall house index and container index for Tanga city to be high. This suggests that Tanga city is at potential risk of Chikungunya outbreaks as the indices are above the recommended levels (<5) (PAHO, 1994). A study in 2014 in Dar es Salaam, Tanzania reported lower house index (27.5%) but higher container index (77.4%) (Mboera *et al*., 2016) as compared to this study in which the house index and container indices were 40.59% and 60.2% respectively. The larvae indices are key indicators toward intervention decisions in the control strategies of mosquito infestation in the environment.

The CHIKV infection rate in mosquitoes was 15.9%. This shows that there is a risk of Chikungunya infections and outbreak incidences in Tanga city. Studies elsewhere in Tanzania reported a relatively higher mosquito infection rate (Patrick *et al*., 2018). Detection of Chikungunya virus in *Ae. aegypti* imply that these mosquito species are the

vectors of the virus. This finding is similar to other studies conducted in Tanzania (Patrick *et al*., 2018) and Thailand (Thavara *et al*., 2009).

CHAPTER SIX

6.0 CONCLUSION AND RECOMMENDATIONS

6.1 Conclusion

This study has reported that *Ae. aegypti* is the most abundant mosquito species in Tanga city. The abundance of *Ae. aegypti* collaborate with a large proportion of potential breeding habitats in the city. The high Aedine indices reported in this study are alarming and pose the risk of Chikungunya transmission. Therefore, it is important that the vector surveillance programs be intensified to generate more data that will assist in intervention activities. The findings that CHIKV transmission is taking place in Tanga city require institutions of appropriate prevention and control interventions. Generally, it has been observed that the community knowledge regarding Chikungunya is very minimal. In order for the community to respond positively in practices toward prevention and control of mosquito borne diseases, the need to raise their awareness in these infections is inevitable.

6.2 Recommendations

i. The presence of significant number of *Aedes aegypti* and infection rate in mosquitoes in Tanga is an alarm that the area is potential for CHIKV outbreaks. Therefore, the community should be informed on the public health importance of these vectors and the diseases they transmit.
ii. The government through Tanga city council should consider instituting appropriate and effective vector control interventions. This would help to keep mosquito population at levels which do not pose infection risk to human population.

iii. Health care personnel should consider Chikungunya as a differential diagnosis of non-malaria febrile illnesses.

iv. For researchers, there is a need to conduct longitudinal epidemiological and entomological studies to consolidate knowledge on the burden and distribution of Chikungunya in Tanga city and other districts of Tanzania. Also, there is a need to establish the genetic characteristics of CHIKV in the region.

REFERENCES

Alobuia, W. M., Missikpode, C., Aung, M. and Jolly, P. E. (2015). Knowledge, Attitude and Practices regarding Vector-borne Diseases in West Jamaica. *Annals of Global Health* 81(5): 654 – 663.

Batovska, J., Blacket, M. J., Brown, K. and Lynch, S. E. (2016). Molecular identification of mosquitoes (Diptera: Culicidae) in southeastern Australia. *Ecology and Evolution* 6(9): 3001–3011.

Braak, L., Gouveia de Almeida, A. P., Cornel, A. J., Swanepoel, R. and de Jager, C. (2018). Mosquito-borne arboviruses of African origin: review of key viruses and vectors. *Parasite and Vectors* 11(29): 1 – 26.

CDC (2019). Chikungunya Virus. Geographical distribution. [https://www.cdc. gov/chikun gunya/geo/index.html] site visited on 10/8/2020.

Chipwaza, B., Mugasa, J. P., Selemani, M., Amuri, M., Mosha, F., Ngatunga, S. D. and Gwakisa, P. S. (2014). Dengue and Chikungunya fever among viral diseases in outpatient febrile children in Kilosa district hospital, Tanzania. *PLoS Neglected Tropical Diseases* 8(5): 1 – 11.

Corrin, T., Waddell, L., Greig, J., Young, I., Hierlihy, C. and Mascarenhas, M. (2017). Risk perceptions, attitudes, and knowledge of Chikungunya among the public and health professionals: a systematic review. *Tropical Medicine and Health* 45(21): 1 – 15.

Ferede, G., Tiruneh, M., Abate, E., Kassa, W. J., Wondimeneh, Y., Damtie, D. and Tessema, B. (2018). Distribution and larval breeding habitats of Aedes mosquito species in residential areas of northwest Ethiopia. *Epidemiology and Health* 40: 1 – 7.

Galán-Huerta, K. A., Rivas-Estilla, A. M., Fernández-Salas, I., Farfan-Ale, J. A. and Ramos-Jiménez, J. (2015). Chikungunya virus: A general overview. *Medicina Universitaria* 17(68): 175 – 183.

Gao, S., Song, S. and Zhang, L. (2019). Recent progress in vaccine development against Chikungunya Virus. *Frontiers in Microbiology* 10: 1 – 10.

Gubler, D. J. (2002). The global emergence/resurgence of arboviral diseases as a public health problem. *Achieves of Medical Research* 33(4): 330 – 342.

Gubler, D. J and Clark, G. G. (1996). Community involvement in the control of *Aedes aegypti*. *Acta Tropica* 61: 169 – 179.

Haque, R., Ali, I. K., Akther, S. and Petri, W. A. (1998). Comparison of PCR, isoenzyme analysis, and antigen detection for diagnosis of *Entamoeba histolytica* infection. *Journal of Clinical Microbiology* 36(2): 449 – 452.

Harbach, R. (2007). The Culicidae (Diptera): A review of taxonomy, Classification and Phylogen. *Zootaxa* 1668: 591 – 638.

Huang, Y. M. (2001). A pictorial key for identification of the subfamilies of *Culicidae*, genera Culicine, and subgenera of Aedes mosquitoes of the Afrotropical region (Diptera: Culicidae). *Entomological Society of Washington* 103(1): 1 – 53.

Jeffries, C. L., Tantely, L. M., Raharimalala, F. N., Hurn, E., Boyer, S. and Walker, T. (2018). Diverse novel resident Wolbachia strains in Culicine mosquitoes from Madagascar. *Scientific Report* 8(17456): 1 – 15.

Johnson, B. W., Russell, B. J. and Goodman, C. H. (2016). Laboratory diagnosis of Chikungunya virus infections and commercial sources for diagnostic assays. *Journal of Infectious Diseases* 214(5): 471–474.

Jourdain, F., Picard, M., Sulesco, T., Haddad, N., Harrat, Z., Sawalha, S.S., Günay, F., Kanani, K., Shaibi, T., Akhramenko, D., Sarih, M., Velo, E., Paronyan, L., Pajovic, I., Faraj, C., Sikharulidze, I., Putkaradze, D., Maric, J., Bosevska, G., Janceska, E., Bouattour, A., Hamidi, A., Sherifi, K., Alten, B., Petrić, D. and Robert, V. (2018). Identification of mosquitoes (Diptera: Culicidae): an external quality assessment of medical entomology laboratories in the MediLabSecure Network. *Parasites and Vectors* 11(553): 1 – 7.

Kajeguka, D. C., Kaaya, R. D., Mwakalinga, S., Ndossi, R., Ndaro, A., Chilongola, J. O., Mosha, F. W., Schioler, K. L., Kavishe, R. A. and Alifrangis, M. (2016). Prevalence of Dengue and Chikungunya virus infections in north-eastern Tanzania: a cross sectional study among participants presenting with malaria-like symptoms. *BMC Infectious Diseases* 16(183): 1 – 9.

Kinimi, E., Shayo, M., Bisimwa, P., Angwenyi, S., Kasanga, C., Weyer, J., van Vuren, P. J., Paweska, J., Mboera, L., Rweyemamu, M. and Misinzo, G. (2018). Evidence of Chikungunya virus infection among febrile patients seeking healthcare in selected districts of Tanzania. *Infection Ecology and Epidemiology* 8(1553460): 1 – 8.

Kweka, E.J., Baraka, V., Mathias, L., Mwang'onde, B., Baraka, G., Lyaruu, L. and Mahande, A. M. (2018). *Ecology of Aedes Mosquitoes, the Major Vectors of Arboviruses in Human Population*. IntechOpen. London, UK. 19pp.

Madzlan, F., Dom, N. C., Tiong, C. S. and Zakaria, N. (2016). Breeding characteristics of *Aedes* mosquitoes in dengue risk area. *Procedia - Social and Behavioral Sciences* 234: 164 – 172.

Mascarenhas, M., Garasia, S., Berthiaume, P., Corrin, T., Greig, J., Ng, V., Young, I. and Waddell, L., (2018). A scoping review of published literature on Chikungunya virus. *PLoS One* 13(11): 1 – 40.

Mayi, M. P. A., Bamou, R., Djiappi-tchamen, B., Fontaine, A., Jeffries, C. L., Walker, T., Antonio-Nkondjio, C., Cornel, A. J. and Tchuinkam, T. (2020). Habitat and Seasonality Affect Mosquito Community Composition in the West Region of Cameroon. *Insect* 11(312): 1 – 17.

Mboera, L. E. G., Mweya, C. N., Rumisha, S. F. and Tungu, P. (2016). The risk of dengue virus transmission in Dar es Salaam, Tanzania during an epidemic period of 2014. *PLoS Neglected Tropical Diseases* 10(1): 1 – 15.

Nims, R. W. and Herbstritt, C. J. (2005). Cell line authentication using isoenzyme analysis. *Biopharm International* 18(6): 76 – 79.

Nsoesie, E. O., Kraemer, M. U., Golding, N., Pigott, D. M., Brady, O. J., Moyes, C. L., Johansson, M. A., Gething, P. W., Velayudhan, R., Khan, K., Hay, S. I. and Brownstein, J. S. (2016). Global distribution and environmental suitability for Chikungunya virus 1952 to 2015. *Euro Surveillance* 21(20): 1 – 22.

PAHO (1994). Dengue and Dengue Haemorrhagic Fever in Americas. Guidelines for Prevention and Control. Pan American Health Organization, Washington DC, USA. 100pp.

Patrick, B. N., Kinimi, E., Shayo, M. J., Angwwenyi, S. O., Weyer, J., van Vuren, P. J., Paweska, J. T., Mboera, L. E. G., Rweyemamu, M. M., Misinzo, G. and Kasanga, C. J. (2018). Distribution and diversity of mosquitoes and the role of Aedes in the transmission of arboviruses in selected districts of Tanzania. *International Journal of Mosquito Research* 5(1): 53 – 60.

Powers, A. and Logue, C. H. (2007). Changing patterns of chikungunya virus: re-emergence of a zoonotic arbovirus. *Journal of General Virology* 88: 2363–2377.

Proesmans, S., Katshongo, F., Milambu, J., Fungula, B., Mavoko, H. M., Mundeke, S. A., da Luz, R. I., Esbroeck, M. V., Arien, K. K., Cnops, L., Smet, B. D., Lutumba, P., Van geertruyden, J. P. and Vanlerberghe, V. (2019). Dengue and Chikungunya among outpatients with acute undifferentiated fever in Kinshasa, Democratic Republic of Congo: A cross-sectional study. *PLoS Neglected Tropical Diseases* 13(9): 1 – 16.

Ratnasingham, S. and Hebert, P. D. N. (2007). The barcode of life data system. *Molecular Ecology Notes* 7: 355–364.

Robinson, M. C. (1955). An epidemic of virus disease in Southern Province, Tanganyika territory, in 1952–1953. *Transaction of the Royal Society of Tropical Medicine*

and Hygiene 49(1): 28 – 32.

Saleh, F., Kitau, J., Konradsen, F., Alifrangis, M., Lin, C., Juma, S., Mchenga, S. S., Saadaty, T and Schioler, K. L. (2018). Habitat characteristics for immature stages of aedes aegypti In Zanzibar City Tanzania. *Journal of the American Mosquito Control Association* 34(3): 190–200.

Scott, T. W., Amerasinghe, P. H., Morrison, A. C., Lorenz, L. H., Gary, G., Strickman, D. and Edman, J. D. (2000). Longitudinal studies of aedes aegypti (Diptera : Culicidae) in Thailand and Puerto Rico : Blood feeding frequency. *Journal of Medical Entomology* 37(1): 89–101.

Siddiquee, S., Soon, G. T. and Umi, K. Y. (2010). Isoenzyme analysis and relationship among three species in Malaysian *Trichoderma* Isolates. *Journal of Microbiology and Biotechnology* 20(9): 1266 – 1278.

Singh, S. K. and Unni, S. K. (2011). Chikungunya virus: Host pathogen interaction. *Review of Medical Virology* 21: 78 – 88.

Tedjou, A. N., Kamgang, B., Yougang, A. P., Njiokou, F. and Wondji, C. S. (2019). Update on the geographical distribution and prevalence of *Aedes aegypti* and *Aedes albopictus* (Diptera: Culicidae), two major arbovirus vectors in Cameroon. *PLoS Neglected Tropical Diseases* 13(3): 1-18.

Tedjou, A. N., Kamgang, B., Yougang, A. P., Wilson-Bahun, T. A., Njiokou, F and Wondji, C. S. (2020). Patterns of ecological adaptation of *Aedes aegypti* and *Aedes albopictus* and *Stegomyia* indices highlight the potential risk of arbovirus transmission in Yaoundé, the Capital City of Cameroon. *Pathogens* 9(491): 1 – 17.

Teklu, B. M., Tekie, H., McCartney, M. and Kibret, S. (2010). The effect of physical water quality and water level changes on the occurrence and density of Anopheles mosquito larvae around the shoreline of the Koka reservoir, central Ethiopia. *Hydrology and Earth System Sciences* 14: 2595 – 2603.

Thavara, U., Tawatsin, A., Pengsakul, T., Bhakdeenuan, P., Chanama, S., Anantapreechal, S., Molito, C., Chompoosri, J., Thammapalo, S., Sawanpanyalert, P. and Siriyasatien, P. (2009). Outbreak of Chikungunya fever in Thailand and virus detection in field population of vector mosquitoes, *Aedes aegypti* (L.) and *Aedes albopictus* skuse (Diptera: Culicidae). *Southeast Asian Journal of Tropical Medicine and Public Health* 40(5): 951 – 962.

The Citizen (2018). Healthy ministry raises alarm over chikungunya fever. [https:// www.thecitizen.co.tz/news/Health-ministry-raises-alarm-over-chikungunya-fever/ 1840340-4630298-view-asAMP-k8mj61/index.html] site visited on 9/8/2019.

Trpis, M. (1972). Seasonal changes in the larval populations of *Aedes aegypti* in two biotypes in Dar es Salaam, Tanzania. *Bulletin of the World Health Organization* 47: 245 – 255.

URT (2013). 2012 *Population and Housing Census: Population Distribution by Administrative Areas*. National Bureau of Statistics, Ministry of Finance, Dar es Salaam and Office of Chief Government Statistician, President's Office, Finance, Economy and Development Planning, Zanzibar. 264pp.

Vairo, F., Mavoungou, M. P. A. C., Ntoumi, F., Castilletti, C., Kitembo, L., Haider, N., Carletti, F., Colavita, F., Gruber, C. E. M., Iannetta, M., Messina, F., Lanini, S., Judicaël, B. U., Giombini, E., Montaldo, C., Portella, C., Diafouka-Diatela, S., Rueca, M., Kock, R., Bartolini, B., Mboera, L., Munster, V., Fischer, R., Seifert, S., Muñoz-Fontela, C., Escudero-Pérez, B., Gomez-Medina, S., Nelson, E. V., Tungu, P. K., Nicastri, E., Puro, V., Di Caro, A., Capobianchi, M. R., Mikolo, J. L., Zumla, A. and Ippolito, G. (2020). Chikungunya Outbreak in the Republic of the Congo, 2019—Epidemiological, Virological and Entomological Findings of a South-North Multidisciplinary Taskforce Investigation. *Viruses* 12(1020): 1 – 15.

Wahid, B., Ali, A., Rafique, S. and Idrees, M. (2017). Global expansion of chikungunya virus: mapping the 64-year history. *International Journal of Infectious Diseases* 58: 69 – 76.

Weaver, S. C. and Forrester, N. L. (2015). Chikungunya: Evolutionary history and recent epidemic spread. *Antiviral Research* 120: 32 – 39.

Weetman, D., Kamgang, B., Badolo, A., Myes, C., Shearer, F. M., Coulibaly, M., Pinto, J., Lambrechts, L. and McCall, P. (2018). Aedes Mosquitoes and Aedes-Borne Arboviruses in Africa: Current and future threats. *Environmental Research and Public Health* 15(220): 1 – 20.

WHO (2009). *Guidelines for Prevention and Control of Chikungunya Fever*. World Health Organization, Geneva. 42pp.

WHO (2020). Dengue control. [https:/ www.who. int/denguecontrol/mosquito/en] site visited on 5/5/2020.

Zahouli, J. B. Z., Utzinger, J., Adja, M. A., Muller, P., Malone, P., Tano, Y and Koudou, B. G. (2016). Oviposition ecology and species composition of Aedes spp. and Aedes aegypti dynamics in variously urbanized settings in arbovirus foci in Southeastern Cote d'Ivoire. *Parasites & Vectors* 9(523): 1 – 14.

APPENDICES

Appendix 1: Questionnaire assessing community knowledge, attitudes and practises

1. Questionnaire No…….
2. Date (dd/mm/yyyy) ……...
3. Area of residence……...

Socio-demographic characteristics of participant

1.	Age (years)	…….
2.	Sex	1.Male 2.Female
3.	Marital status	1. Single 2. Married 3. Cohabiting 4. Separated/Divorced 5.Widow/Widower
4.	Education level	1. None 2. Primary Education 3. Secondary Education 4.Advanced Education 5.Post-Secondary Education (College, University) 6. Others……..
5.	Occupation	1. Farmer 2. Formally employed 3. Self employed 4. Business 5. Student 6. Unemployed 7. Retiree 8. Others, specify

Knowledge about Chikungunya

1. Have you ever heard of the disease called Chikungunya?

Yes ☐ No ☐

(a) If Yes: Where did you get the information? (tick the appropriate answer)

Newspaper	
Television	
Radio	
Health Facility/Health worker	
Social network	
Other; …….	

(b) How is the disease transmitted?

..……

2. Do you know any disease transmitted by mosquitoes?
   Mention: (i)…… (iv)……
   (ii)…….. (v)…….
   (iii)…….. (vi)……

3. Do you know the clinical signs associated with Chikungunya?
   Yes ☐ No ☐
   If Yes, indicate.

Fever	Arthralgia	Headache	Malaise	Skin rashes	Others	Don't know

Attitude toward Chikungunya

1. In your opinion, how severe is the disease? (tick the appropriate answer)

High	Moderate	Low	I don't know

2. Do you know any treatment plan for Chikungunya?
   YES ☐ NO ☐
   If YES: explain…….

Practices toward Chikungunya transmission

1. In your house surroundings, are there flower grown in pots?
   YES ☐ NO ☐

2. Is there any open water holding containers around the house?
   YES ☐ NO ☐

   Tick appropriate answer

Tyre	Discarded bottles	Flower vase	Coconut shells	Bowl/ ceramic sink/	Drinkers/ feeders for animals	Drum barrel/simtank	Natural breeding sites	Others

3. How many members of this household slept under a mosquito net last night?
   a. Total number of members of household _____
   b. Number slept under a mosquito net last night_____

4. Have you ever bitten by mosquito during a day?

YES ☐ NO ☐

5. How do you protect from mosquito bite during a day?

Use mosquito repellent
Wear long sleeved clothes
Other……
Do nothing

THANK YOU FOR YOUR PARTICIPATION

Appendix 2: Adult mosquito collection form

Date: _____ Village/Street: _____

Name of Collector: _____

Latitude: _____ Longitude: _____

Altitude_____

Rain last night: None/light/Heavy/Showers/Storm (Tick)

Presence of cattle/sheep/goats/donkeys/pigs in the house compound (Tick)

Mosquito species	**Female mosquito abdominal status**					**Male mosquitoes**
	M	**UF**	**F**	**HG**	**G**	
TOTAL						

Appendix 3: Procedures for RNA extraction

1. Preparing the samples:
   i. Thaw the homogenised lysate/sample on ice.
   ii. Vortex the sample to mix well
2. Pipette 560 µL prepared Buffer AVL containing carrier RNA into a 1.5 ml microcentrifuge tube.
3. Add 140 µL of sample to the Buffer AVL–carrier RNA in the microcentrifuge tube. Mix by pulse-vortexing for 15 s.
4. Incubate at room temperature (15–25°C) for 10 min (Longer incubation times have no effect on the yield or quality of the purified RNA).
5. Briefly centrifuge the tube to remove drops from the inside of the lid.
6. Add 560 µL ethanol (96–100%) to the sample, and mix by pulse-vortexing for 15 s. After mixing, briefly centrifuge the tube to remove drops from inside the lid.
7. Carefully apply 630 µL of the solution from step 6 to the QIAamp Mini column (in a 2 ml collection tube) without wetting the rim. Close the cap, and centrifuge at 6000 x *g* (8000 rpm) for 1 min. Place the QIAamp Mini column into a clean 2 ml collection tube, and discard the tube containing the filtrate.
8. Carefully open the QIAamp Mini column, and repeat step 7. Repeat this step until all of the lysate has been loaded onto the spin column.
9. Carefully open the QIAamp Mini column, and add 500 µL Buffer AW1. Close the cap, and centrifuge at 6000 x *g* (8000 rpm) for 1 min. Place the QIAamp Mini column in a clean 2 ml collection tube (provided), and discard the tube containing the filtrate.
10. Carefully open the QIAamp Mini column, and add 500 µL Buffer AW2. Close the cap and centrifuge at full speed (20,000 x *g*; 14,000 rpm) for 3 min.

Continue directly with step 11, or to eliminate possible Buffer AW2 carryover, perform step 10 and then continue with step 11.

11. Place the QIAamp Mini column in a clean 1.5 ml microcentrifuge tube (not provided). Discard the old collection tube containing the filtrate. Carefully open the QIAamp Mini column and add 60 µL Buffer AVE equilibrated to room temperature. Close the cap, and incubate at room temperature for 1 min.
12. Centrifuge at 6000 x *g* (8000 rpm) for 1 min. Perform a double elution using 2 x 40 µL Buffer AVE
13. Aliquot eluted RNA into 4 aliquots of 20 µL each while maintaining cold temperature using Ice Park.
14. Store at –80°C for future use

Appendix 4: Ethical clearance certificate

THE UNITED REPUBLIC OF TANZANIA

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20th November 2019

Michael J. Msolla
Sokoine University of Agriculture
College of Veterinary Medicine and Biomedical Sciences
Department of Veterinary Microbiology, Parasitology and Biotechnology
P.O. Box 3015,
Chuo Kikuu, Morogoro,
Tanzania

RE: ETHICAL CLEARANCE CERTIFICATE FOR CONDUCTING MEDICAL RESEARCH IN TANZANIA

This is to certify that the research entitled: "Chikungunya virus vectors and transmission indices in Tanga city, north-east Tanzania." (Msolla MJ. et al), has been granted ethical clearance to be conducted in Tanzania.

The Principal Investigator of the study must ensure that the following conditions are fulfilled:

1. Progress report is submitted to the Ministry of Health, Community Development, Gender, Elderly & Children and the National Institute for Medical Research, Regional and District Medical Officers after every six months.
2. Permission to publish the results is obtained from National Institute for Medical Research.
3. Copies of final publications are made available to the Ministry of Health, Community Development, Gender, Elderly & Children and the National Institute for Medical Research.
4. Any researcher, who contravenes or fails to comply with these conditions, shall be guilty of an offence and shall be liable on conviction to a fine as per NIMR Act No. 23 of 1979, PART III Section 10(2).
5. Sites: Tanga region.

Approval is valid for one year: 20th November 2019 to 19th November 2020.

Name: Prof. Yunus Daud Mgaya

Signature
CHAIRPERSON
MEDICAL RESEARCH
COORDINATING COMMITTEE

Name: Prof. Muhammad Bakari Kambi

Signature
CHIEF MEDICAL OFFICER
MINISTRY OF HEALTH, COMMUNITY DEVELOPMENT, GENDER, ELDERLY & CHILDREN

CC: Director, Health Services-TAMISEMI, Dodoma
RMO of Tanga region
DMO/DED of respective districts.

Appendix 5: Informed consent statement

SOKOINE UNIVERSITY OF AGRICULTURE (SUA)
SACIDS Foundation for One Health
P.O Box 3297, Chuo Kikuu, Morogoro, Tanzania
Tel: +255 23 264 0037; +255 787 011 677
e-mail: secretariat@sacids.org
url: www.sacids.org

CHIKUNGUNYA VIRUS VECTORS AND TRANSMISSION INDICES IN TANGA CITY, NORTH-EASTERN TANZANIA

The following information will be read to every participant (age ≥18 years) in this survey.

My name is ……., from the Sokoine University of Agriculture, Morogoro. We are carrying out a research study on Chikungunya transmission in Tanga. We would like to know the level of understanding, attitudes and practices of community in this City. You are one of those few selected to represent views of residents in this area. The views we get from you and several others will help us to get evidence to be used by the Government to develop appropriate interventions and policies. This interview will take approximately half an hour to complete. Please feel free to participate in this survey.

I would like to assure you that the responses and comments that you are going to share will be a secret between you and us researchers. If you choose not to participate in this study, that is fine too. If you participate, you are free to skip any questions you do not wish to answer or to stop at any time. You may ask the researchers any questions you have at any time.

Do you wish to participate?	YES	☐
	NO	☐

(If the answer is "YES", continue with the interview. if the answer is "no", then thank the participant and move to the next interviewee)

Participant signature……. Date …….

NB: If you have any questions regarding this research, you may ask the research staff or contact Mr. Michael Msolla, Sokoine University of Agriculture, P.O. Box 3297, Telephone: +255 752185594; E-mail: michaelmsolla@gmail.com. For further information regarding this project, you may wish to contact the Secretariat of National Health Research Ethics Review Committee, National Institute for Medical Research at 3 Barack Obama Drive, P.O Box 9653 Dar-es-Salaam, Email: ethics@nimr.or.tz; Telephone: +255222121400.