MOSQUITO SPECIES COMPOSITION, ABUNDANCE AND TRANSMISSION RISK OF DENGUE IN KINONDONI DISTRICT, DAR ES SALAAM, TANZANIA

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ABSTRACT

Dar es Salaam city of Tanzania has experienced continued Dengue outbreaks with increased incidence since 2010. However, there is inadequate evidence of vector dynamics and transmission risk in the region. This study aimed to determine mosquito species composition, abundance and transmission risk of Dengue in Kinondoni district, Dar es Salaam, Tanzania. Specifically, this study aimed to: (i) determine mosquito species composition and abundance in Kinondoni district; (ii) examine Aedes mosquito breeding sites and establish container productivity rates; (iii) determine Dengue virus (DENV) infection rate and genetically characterize DENV in mosquito vectors. This cross-sectional study was conducted in Kinondoni district, Dar es salaam, Tanzania. Three wards: Mikocheni, Mwananyamala and Mzimuni were purposively selected. In each ward, three streets were randomly selected as sampling sites. The study involved sampling adult and immature mosquitoes, morphological identification and screening for DENV in collected female Aedes mosquitoes using a one-step reverse transcription polymerase chain reaction (RT-PCR). Three mosquito species were identified in 2001 collected mosquitoes namely *Culex quinquefasciatus* (53.1%), *Aedes aegypti* (23.2%) and *Mansonia mosquitoes* (23.6%) of which *Culex quinquefasciatus* was observed to be the most abundant species in Kinondoni district. The common water-holding containers observed to be breeding sites of Aedes mosquitoes included used car tires, flowerpots and plastic water buckets. The overall House Index (HI), Container Index (CI) and Breteaux Index (BI) of Kinondoni district were 55.1%, 60.4% and 114.2, respectively. DENV was not detected in all collected female Aedes Mosquitoes. Generally, Kinondoni district continues to be at risk of transmission of Dengue as *Aedes aegypti*, a DENV vector was observed to be present. Although DENV was not detected in mosquito vectors, the presence of potential breeding

sites around Kinondoni district and higher Aedes HI, CI and BI put Kinondoni at risk of DENV transmission. Vector control interventions specifically integrated mosquito control approaches are recommended to be directed towards the elimination of breeding sites and adult mosquitoes.

DECLARATION

I, Baraka Laurian Ngingo, 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 in any other institution.

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DEDICATION

My work is dedicated to my wife Paskalina Bilas and our daughter Sarah Baraka for courage and patience during my entire study period.

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LIST OF ABREVIATIONS AND SYMBOLS

Ae	Aedes
BI	Breteaux Index
Bs	*Bacillus sphaerieus*
Bti	*Bacillus thuringiensis israelensis*
C	capsid
CA	California
CDC	Centers for Disease Control and Prevention
CI	Container Index
CO_2	carbon dioxide
COI	cytochrome oxidase
Cx	Culex
DENV	Dengue virus
DNA	deoxyribonucleic acid
E	envelope
ELISA	enzyme-linked immunosorbent assays
HI	House Index
HI	hemagglutination inhibition
IgG	immunoglobulin G
IgM	immunoglobulin M
ITNs	insecticide treated mosquito nets
ITS	internal transcribed spacer
LMICs	low-and to middle-income countries
LTD	limited
MOHSW	Ministry of Health and Social Welfare

MUHAS	Muhimbili University of Health and Allied Sciences
NatHREC	National Health Research Ethics Committee
NIMR	National Institute for Medical Research
NS	non structural
ORF	open reading frame
PCR	polymerase chain reaction
PrM	membrane precursor
PRNT	plaque reduction neutralization test
qRT-PCR	quantitative reverse transcription polymerase chain reaction
RNA	ribonucleic acid
RT-LAMP	reverse transcription loop mediated isothermal amplification
RT-PCR	reverse transcription polymerase chain reaction
SACID-ACE	Southern African Center for Infectious Disease Surveillance-Africa Centre of Excellence for Infectious Diseases of Humans and Animals in Eastern and Southern Africa
SACIDS	Southern African Center for Infectious Disease Surveillance
SL	stem loop
SLA	stem loop a
SUA	Sokoine University of Agriculture
TAE	Tris acetate EDTA
Taq	*Thermos aquaticus*
TBPL	Tanzania Biotech Product Limited
USA	United State of America
URT	United Republic of Tanzania
WHO	World Health Organization

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background Information

Dengue is a mosquito-borne viral disease of global importance affecting over half of the world's population (Messina *et al.*, 2019). Dengue is endemic in 128 countries, mostly low and middle-income countries (LMICs), putting more than 2.05-3.74 billion people at risk (Brady *et al.*, 2012). The disease is caused by an RNA Dengue virus (DENV) with four serotypes, DENV1-4 (WHO, 2016). It is driven by poor urban environmental conditions, globalization and climate change (Zambrano *et al.*, 2012). There is no specific treatment for Dengue, but appropriate medical care frequently saves the lives of patients (WHO, 2016). The most effective way to prevent Dengue virus transmission is to combat its mosquito vectors through mosquito vector management (WHO, 2016).

Dengue virus is transmitted to humans through a bite of an infected *Aedes* mosquito (WHO, 2016). The main vectors that are geographically widespread include *Ae. aegypti* and *Ae. albopictus* (Kraemer *et al.,* 2015). *Aedes aegypti* is a highly domestic and a day-biting species that prefers to feed on humans. It breeds in stagnant water collecting both natural and man-made containers such as flower vases, uncovered barrels, buckets, discarded cans, roof gutters and discarded car tires (Mathias *et al.,* 2017).

In the United Republic of Tanzania, Dengue cases were first reported during the 15th century (Gubler, 1997; Halstead, 2008). Dengue outbreaks were also reported between 1823 and 1870 in the Zanzibar archipelago (Amarasinghe *et al.,* 2011). In Mainland Tanzania, Dengue outbreaks have been reported in 2010, 2012, 2013-2014, 2018 and 2019 (Mboera *et al.,* 2016; Vairo *et al.,* 2016; Okada *et al.,* 2019).   Moreover,

some studies have reported seroprevalence of Dengue in several regions in Tanzania including Arusha, Dar es Salaam, Iringa and Kilimanjaro (Hertz *et al.,* 2012; Chipwaza *et al.,* 2014; Vairo *et al.,* 2014; Mboera *et al.,* 2015; Vairo *et al.,* 2016). Dengue serotypes reported to be circulating in Tanzania include DENV-1 (Okada *et al.,* 2019), DENV-2 and DENV-3 (Mboera *et al.,* 2015). The most recent Dengue outbreak in Tanzania was reported between January and November 2019. The outbreak affected mainly Dar es Salaam City. A total of 6917 confirmed cases and 13 deaths (case fatality rate of 0.19%) were reported (WHO, 2019).

1.2 Problem Statement and Justification of Study

While many African countries are reported to be at risk of Dengue, entomological studies to identify mosquito vectors and transmission indices are limited. So far, only five entomological surveys have been carried out in Tanzania; of which two have established transmission levels of DENV (Mboera *et al.,* 2016; Hertz *et al.,* 2016; Mathias *et al.,* 2017; Patrick *et al.,* 2018; Mselemu *et al.,* 2020).

Dar es Salaam, the commercial city of Tanzania is facing continued Dengue outbreaks with increased frequency and occurrence since 2010 (Mboera *et al.,* 2016; Vairo *et al.,* 2016; WHO, 2019). However, there is insufficient information on mosquito bionomics and transmission risk of Dengue in Dar es Salaam and other parts of the country.

1.3 Research Questions

 i. What is mosquito species composition and abundance in Kinondoni District?

 ii. What are the mosquito breeding habitats and container productivity rates?

 iii. What is the DENV infection rate in mosquito vectors and what are the DENV currently circulating in infected mosquito vectors?

1.4 Research Objectives

1.4.1 Overall objective

To determine mosquito species composition, abundance and transmission risk of Dengue in Kinondoni Municipal, Dar es Salaam, Tanzania.

1.4.2 Specific objectives

i.   To determine mosquito species composition and abundance in Kinondoni Municipal.

ii. To examine Aedes mosquito breeding habitats and establish container productivity rates, and

iii. To determine the DENV infection rate and genetically characterize DENV in mosquito vectors.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Mosquito Taxonomy

Mosquitoes are slender, long-legged insects with long proboscis and have scales on most parts of their body. They belong to the family *Culicidae* order Diptera, class Insecta (Hexapoda) and phylum Arthropoda (Rueda, 2008; Foster and Walker, 2019). There are two recognized subfamilies, the Anophelinae and Culicinae. Subfamily Culicinae has 11 tribes: Aedeomyiini (*Aedeomyia*), Aedini (*Aedes, Armigeres, Ayurakitia, Eretmapodites, Haemagogus, Heizmannia, Opifex, Psorophora, Tanakius, Udaya, Verrallina, Zeugnomyia*), Culicini (*Culex, Deinoce- rites, Galindomyia, Lutzia*), Culisetini (*Culiseta*), Ficalbiini (*Ficalbia, Mimomyia*), Hodgesiini (*Hodge- sia*), Mansoniini (*Coquillettidia, Mansonia*) *or*thopodomyiini (*Orthopodomyia*), Sabethini (*Isos- tomyia, Johnbelkinia, Limatus, Malaya, Maorigoeldia, Onirion, Runchomyia, Sabethes, Shan- noniana, Topomyia, Trichoprosopon, Tripteroides, Wyeomyia*), Toxorhynchitini (*Toxorhynchites*) and Uranotaeniini (*Uranotaenia*) (Rueda, 2008; Foster and Walker, 2019). There are about 3500 species and subspecies, under 140 subgenera in 42 genera of mosquitoes worldwide of which Anophelinae and Culicinae has three and 39 genera, respectively (Harbach, 2007; Rueda, 2008).

2.2 Mosquito Distribution

Mosquitoes have an almost worldwide distribution, being found throughout the tropics and temperate regions (Rueda, 2008). They can thrive in a variety of habitats with freshwater, brackish water or any water (clear, turbid or polluted) except in marine habitats with high-salt concentration (Rueda, 2008). The biodiversity of mosquitoes is very evident, with many genera having worldwide distribution and some genera with the limited or endemic

distribution. For example, the genera Anopheles, Aedes, Coquillettidia, Culex, Culiseta, Lutzia orthopodomyia, Toxorhynchites and Uranotaenia, have at least one species found in all five regions of the world (Rueda, 2008).

2.3 Mosquito Habitats

Environmental conditions, such as temperature and humidity affect mosquito survival (Reinert *et al.*, 2004; Rueda, 2008). Since mosquitoes are delicate insects, they are always found where the air is relatively cool and the humidity is high (Reinert *et al.*, 2004). The immature stages of mosquitoes can be found in a variety of aquatic habitats, such as ponds, streams, ditches, swamps, marshy areas, temporary and permanent pools, rock holes, tree holes, plant containers (leaves, fruits, husks), artificial containers (tires, tin cans, flower vases) and other habitats (Reinert *et al.*, 2004; Rueda, 2008). The source of food for mosquitoes is dependent on species and stage of development. The majority of larvae feed on suspended particulate matter and microorganisms that they extract from the water with filamentous mouth brushes (Reinert *et al.*, 2004; Harbach, 2007). Other species are obligatory or facultative predators that capture and feed largely on the immature stages of other mosquitoes (Reinert *et al.*, 2004; Harbach, 2007). Adult mosquitoes of many species feed on plant liquids, including nectar, honeydew, fruit juices and exudates. Most female mosquitoes feed on the blood of vertebrate animals to produce viable eggs (Reinert *et al.*, 2004; Harbach, 2007). Warm-blooded vertebrates including humans are a common source of blood for most species (Reinert *et al.*, 2004).

2.4 Mosquito Flight and Resource Seeking Behavior

Mosquito dispersal and foraging may occur only a few dozen meters from their larval habitats and most species fly less than 2 km in a lifetime (Foster and Walker, 2019). Mosquitoes tend to have repeated foraging flights as a way of searching for mates, sugar

sources, hosts, resting sites and oviposition sites (Foster and Walker, 2019). Other species enter a specific dispersal mode that is wind-assisted or light-directed and carries them dozens or even hundreds of kilometers from their origins. For example, some salt-marsh species and African anophelines tend to make extended, round-trip flights to complete their gonotrophic cycles when development sites and blood-feeding sites are many kilometers apart (Gillies and De Meillon, 1968).

Each mosquito species has a characteristic pattern of adult diel activity, under the control of an endogenous circadian rhythm that is entrained by the daily light-dark cycle. Generally, one or two flight periods occur every 24 hours, characterized as being diurnal, nocturnal or crepuscular (dawn and dusk) (Gillies and De Meillon, 1968). During these periods, both sexes will take flight without external cues other than ambient light. Mosquitoes likely engage in a generalized search pattern during foraging flights, then respond to specific stimuli associated with either mating sites, sugar sources, hosts or oviposition sites as they encounter them, depending on their needs (Gillies, 1980; Takken, 1991; Takken *et al.,* 1997; Mboera *et al.,* 1998; Mboera *et al.,* 2000; Foster and Walker, 2019). Human biting mosquito either bite during the day or during the night. *Aedes aegypti* has two peaks, early morning for 2 to 3 hours after daybreak and in the afternoon for several hours before dark (Gubler, 1998). Mosquito species vary in the habitats where they forage for mates and food. Some fly over varied terrain; others tend to be active in either wooded or open areas; still, others perform all activities close to larval and resting sites. There is some evidence that adult females become familiar with local habitats that have provided either food or oviposition sites in the past and tend to return there, based on experience (Foster and Walker, 2019).

2.5 Mosquito Life Cycle

The life cycle of mosquitoes is largely influenced by biotic and abiotic factors. The biotic factors include species, blood meal types and their natural predation enemies, whereas the abiotic factors include physicochemical properties of their habitat such as water type and its contents, vegetation and the prevailing environmental conditions such as temperature and rainfall (Takken, 1991; Takken *et al.*, 1997; Juliano, 2009). The females usually mate only once but produce eggs at intervals throughout their life (Gilles, 1988). Depending on the species, the female adult lays either single eggs as in Aedes and Anopheles species or in clusters of up to several hundred at a time as in Culex (Rueda, 2008). Once hatched, it undergoes four distinct stages in their life cycle: egg, larva, pupa and adult (Figure 1).

The development of mosquito larvae and pupae requires an environment with standing water (Rueda, 2008). In the process of development, the fully-grown larva changes into a comma-shaped pupa. The pupal skin later splits and a fully developed adult mosquito emerges. In warm climates, the larval period lasts about four to seven days or longer (Rueda, 2008). The pupal period can last between one to three days before becoming adults. Only adult female mosquitoes bite humans and animals (including mammals and birds) to obtain a blood meal in order to produce viable eggs. Male mosquitoes feed primarily on flower nectars. The entire period from egg to adult takes about 7-13 days under favorable conditions (Rueda, 2008; Juliano, 2009).
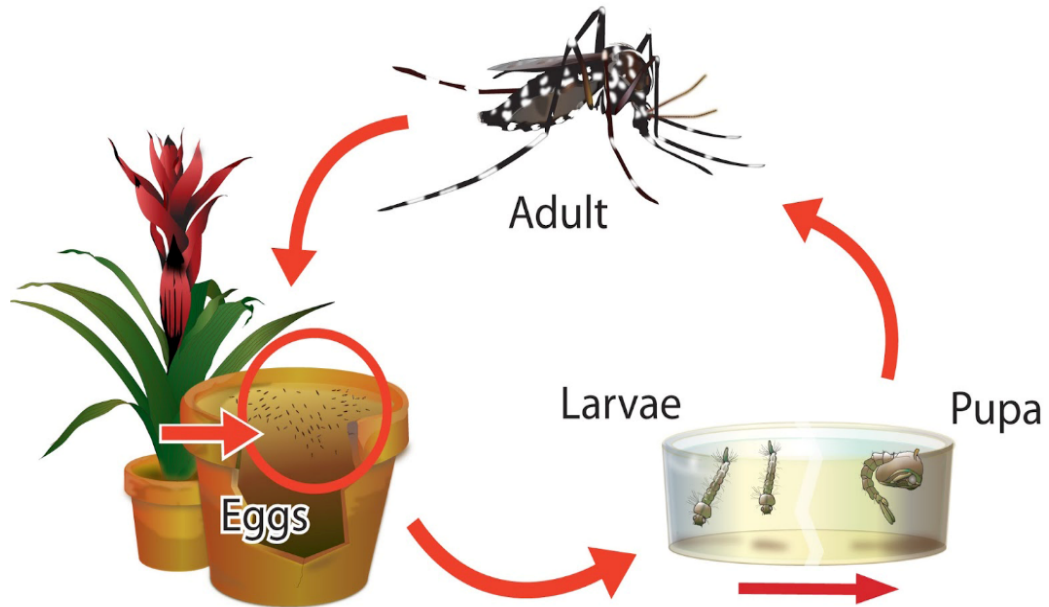
**Figure 1: Mosquito life cycle showing four different stages; egg, larvae, pupae and
adult.** Source: http://denguepatrolskptm. blogspot.com/2015/10/ lifecycle-of-
aedes-mosquito.html accessed on 23 September 2020.

2.6 Mosquito Identification

Mosquitoes can be identified by morphological features (Gillies and Coetzee, 1987;
Coetzee, 2020) and molecular-based techniques such as PCR and DNA barcoding
(Cook *et al*., 2006; Rueda, 2008; Helmersson, 2013). Morphological methods use
distinguishing critical characters of larvae and adults by observing the dorsal, ventral,
lateral, frontal and caudal of the mosquitoes. Mosquito species vary geographically in
their morphological characteristics and biological traits. Their physiological, behaviour
and population biology however are also considered in the identification and
classification of new species (Rueda, 2004).

The use of morphological identification keys is mainly specific to only a few
developmental stages and cannot be possible if a specimen or sample collected is
damaged (when bristles and scales are lost) (Batovska *et al*., 2016). Due to the

limitations of morphological identification, DNA-based technologies for identification can be used. For example, nuclear ribosomal internal transcribed spacer (ITS) markers and mitochondrial cytochrome oxidase (*COI*) genes which are the most conserved have a distinct advantage in distinguishing the variation that exists among mosquito species (Helmersson, 2013; Batovska *et al*., 2016).

2.7 Medical and Veterinary Importance of Mosquito

Mosquitoes have been implicated and found to transmit the most important diseases that affect human and other animals. They are responsible for the transmission of pathogens and parasites such as protozoans (malaria), nematodes (lymphatic filariasis and heart worm in dogs) and viruses (Dengue virus, Chikungunya virus, Rift Valley fever virus, Zika virus, etc.) which cause serious diseases in animals and human (Braack *et al.,* 2018).

Mosquito species of medical importance include *Anopheles gambiae* and *Culex pipiens* complexes and the Aedes subgenus Stegomyia (Foster and Walker, 2019). The *Anopheles gambiae* complex of Africa consists of eight recognized species. Of these, *An. gambiae* and *An. arabiensis*, are widespread and important vectors of malaria and lymphatic filariasis (Foster and Walker, 2019). *Anopheles arabiensis* tends to occur in somewhat drier regions than does *An. gambiae*. Both prefer to bite humans, but *An. gambiae* is more anthropophilic, endophilic and endophagic and therefore it is the more important vector. The *Culex pipiens* species assemblage is an abundant group of two closely related domestic and peridomestic species distributed worldwide which are *Cx. pipiens* (the temperate species) and *Cx. quinquefasciatus* (the tropical and subtropical species) (Foster and Walker, 2019). They are reported to be vectors of several human pathogens such as St. Louis encephalitis virus, West Nile virus and lymphatic filariasis, as well as bird malaria. Two possible members of the species assemblage, *Cx. australicus* and *Cx. globocoxitus,* inhabit

Australia. A form occurring in temperate China and Japan, *Cx. pallens* has no formal status as a species (Foster and Walker, 2019).

Aedes aegypti has a worldwide distribution in the tropics and subtropics (Reinert *et al.,* 2004; Wilkerson *et al*., 2015). *Ae. aegypti* is the primary vector of dengue, yellow fever, Chikungunya, Rift valley fever and Zika viruses (Wilkerson *et al.,* 2015; Braak *et al.,* 2018). *Ae. aegypti* exists in at least two forms, *Ae. aegypti aegypti and Ae. aegpyti formosus*, considered to be either subspecies or separate species. *Ae. aegypti formosus* is the original feral form and is found in large parts of Africa (Braak *et al.,* 2018). It has a black body, develops in small water containers, feeds on a wide variety of animals and rarely enters houses. It has adapted to some domestic situations in Africa, where it develops in rain-filled containers (Powell, 2016). *Aedes aegypti aegypti* is a paler, brownish-black domestic form. It occurs mainly in coastal regions of Africa and is distributed throughout much of southern Asia and most of the warmer parts of the southern United States (Powell, 2016). In Africa, it has become independent of rain, developing in hand-filled water jars without regard to season. On other continents, where it does not compete with *Ae. aegypti. formosus*, it uses both rain-filled and hand-filled containers (Powell, 2016). *Aedes albopictus*, the Asian tiger mosquito, similarly occupies water-filled containers and also transmits Dengue, Chikungunya and Zika viruses (Medlock *et al*., 2015). It was largely confined to Asia, where it occurs in tropical and subtropical rural settings. It readily oviposits in tree holes. In most of its range in the southern United States, *Ae. albopictus* has replaced *Ae. aegypti* as the predominant mosquito in artificial containers in suburban and rural environments (Medlock *et al*., 2015). Other important members of the subgenus Stegomyia include *Ae. africanus, Ae. bromeliae and Ae. luteocephalus*, which transmits yellow fever and dengue viruses in parts of Africa; and *Ae. polynesiensis* and *Ae.*

pseudoscutellaris, which transmit lymphatic filariasis in the South Pacific islands (Foster and Walker, 2019).

2.8 Mosquito Control Methods

Control measures are directed at different stages of the insect in its life cycle and are classified as biological, environmental (physical), chemical control and integrated mosquito management approach (Raman *et al.*, 2016; Gottlieb, 2018).

2.8.1 Biological control

Protozoa, fungi, bacteria and viruses pathogenic to mosquito larvae have also been considered as biological control agents (Gottlieb, 2018). Example *Bacillus thuringiensis israelensis* (Bti) and *Bacillus sphaericus* (Bs) are naturally occurring bacteria used extensively as biological control agents against mosquito larvae that produce spores with endotoxin (Kamareddine, 2012). The toxin hydrolyzes the epithelial cells of the gut of mosquito larvae leading to death within 24 hours. In Tanzania, Malaria control interventions are using Bti and Bs produced by the Tanzania Biotech Product Limited (TBPL) in controlling mosquitoes in selected urban areas (MOHSW, 2014). Other commonly used biological agents or predators of mosquitoes are fishes, dragonflies, birds, aquatic bugs, beetles, tadpoles, flatworms, nematodes, copepods and bats (Kamareddine, 2012; Gottlieb, 2018).

Genetic control methods, sterile-male release techniques are being studied in some countries for getting a high proportion of infertile insemination (Alphey, 2010). Mosquitoes that are resistant to infection with human diseases such as malaria are released for replacing the naturally susceptible ones. Susceptible genes to malaria, for example, in the mosquito population will be "diluted" by the males with refractoriness

genes. These control methods are still at the experimental stage (Alphey, 2010; Isaacs *et al.*, 2012).

2.8.2 Chemical control

This involve the use of insecticides and synthetic larvicides (Gottlieb, 2018). Larvicides are applied to the water where mosquito larvae develop or to water that may provide a habitat for mosquitoes (Gottlieb, 2018). Mineral oils, insect-growth regulators and some organophosphates such as temephos and malathion are used as mosquito larvicides in many countries (Raman *et al.*, 2016). Larviciding is used to prevent mosquito larvae from hatching and becoming adults when source reduction is not feasible (Rydzanicz *et al.*, 2009). Because it is impossible to eliminate all breeding habitats or to control all mosquitoes before they become adults, adulticiding or the control of adult mosquitoes through the application of adulticides is used to control adult mosquitoes (Rydzanicz *et al.*, 2009). Adulticide/insecticide has a knockdown effect and is applied to surfaces where adult mosquito will rest or in the air where it flies (Gottlieb, 2018). Insecticides with residual effect are applied to the interior surfaces of walls (indoor residual spraying) for killing indoor-resting adult mosquitoes such as the endophagic malaria vectors (Raman *et al.*, 2016). Space spraying of insecticidal droplets in the air to kill adult mosquitoes tend to kill all flying insects in the sprayed areas and this is recommended in emergencies when an outbreak of mosquito-borne disease is already in progress or may occur and a substantial reduction in the mosquito population has to be achieved rapidly (Raman *et al.*, 2016). Different species are active at different times of night and are susceptible to different adulticides, surveillance data are extremely important for effective control of adult mosquitoes (Gottlieb, 2018).

2.8.3 Environmental control

Habitat modification such as harborage alteration and source reduction can be used for mosquito control (Gottlieb, 2018; Foster and Walker, 2019). Harborage alteration renders the sites unsuitable for resting of adult mosquitoes and source reduction changes the larval habitat so that mosquito oviposition, hatching and larval development are prevented (Foster and Walker, 2019). Accessibility of water to adult mosquito can be altered or eliminated by ditching, draining, covering and filling. Shredding of disused tires, proper disposal of water containers, alteration of the flow rate of water, disturbance of water surface, removal of shelters such as vegetation and refuse in water bodies, etc. can interfere with the breeding of mosquitoes (Gottlieb, 2018). Larval habitats vary in size. Some of the water bodies cannot be covered, filled or drained because of ecological or technical reasons. In addition, it may be too costly to drain or fill the water bodies (Raman *et al.,* 2016). Converting sloping edges of ponds/pools with exposure of muddy areas to almost vertical banks with deep water (impoundment) can reduce the breeding of Aedes mosquitoes (Gottlieb, 2018). Increase sunlight on the water by trimming overhanging vegetation prevents the breeding of mosquitoes that prefer shaded habitats. Removal of rooted and floating vegetation reduces the breeding of mosquitoes such as the species of Mansonia that require plants to obtain their oxygen supplies. Grass carp are used purposely in the pond for removing aquatic vegetation that provides shelters for the mosquito larvae and pupae (Gottlieb, 2018).

2.8.4 Integrated mosquito control

This is a comprehensive approach of managing mosquito populations that uses various techniques from biological, chemical and physical control methods to reduce mosquito numbers while maintaining a quality environment (Gottlieb, 2018).

2.9 Dengue Virus Taxonomy

Dengue viruses belong to the genus Flavivirus and family *Flaviviridae (Kuhn et al., 2002)*. DENV causes Dengue fever, Dengue hemorrhagic fever and Dengue shock syndrome in humans worldwide (Kuhn *et al.,* 2002). The Dengue virion is a spherical particle with a diameter ranging between 50 and 60 nm with a lipopolysaccharide envelope (Kuhn *et al.,* 2002). Dengue has a single stranded positive-sense RNA of about 11 kilobases (kb) which contains 3 structural genes and 7 non-structural genes (Kuhn *et al.,* 2002). The RNA genome lacks a poly-A tail at 3'. The genome contains a single open reading frame (ORF) flanked by two untranslated regions (5' and 3'UTRs). The 5' and 3' terminal RNA sequences of the genome form large stem-loop structures known as stem-loop A (SLA) and stem-loop (SL), respectively, both essential for viral replication. Structural gene codes for core protein (capsid), membrane-associated protein and envelope protein. Non-structural gene codes for non-structural proteins such as NS1, NS2a, NS2b, NS3, NS4a, NS4b and NS5 which play a role in viral replication. NS5 is the RNA dependent RNA polymerase (Figure 2) (Kuhn *et al.,* 2002*;* Guzman *et al.,* 2010).
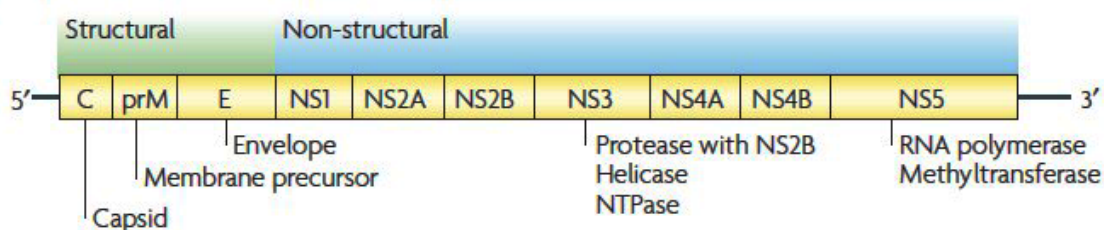
Figure 2: Genome structure of Dengue virus. Translation of viral RNA yields a single polypeptide that is co-translationally processed by viral and cellular proteases, generating three structural proteins (Capsid, Membrane precursor and Envelope) and at least seven non-structural (NS) proteins. Source: Whitehead *et al.,* 2007.

There are four serotypes of DENV (1-4) (WHO, 2016) transmitted by Aedes (Stegomyia) mosquitoes, primarily *Ae. aegypti* and to a lesser degree *Ae. albopictus.*

2.10 Transmission of DENV

Two distinct DENV transmission cycles are recognized (Figure 3): endemic or epidemic cycle and sylvatic or zoonotic cycle. Endemic and epidemic cycles involve the human host and viruses are transmitted by *Ae. aegypti, Ae. albopictus* and other mosquitoes as secondary vectors (Wang *et al.,* 2000; Bhatt *et al.,* 2013). The sylvatic transmission cycle involves monkeys and several different Aedes mosquitoes identified in Asia and West Africa (Holmes and Twiddy, 2003). Aedes mosquito gets infected by sucking blood from a person with Dengue in which the virus is transferred from human blood to the gut of the mosquito (Ivoire *et al.,* 2010; Cheng *et al.,* 2016). The viruses subsequently establish an infection in the mosquito's epithelial cells by overcoming the physical and immune barrier of the epithelia before spreading into the mosquito haemocoel. The viruses subsequently infect other tissues, such as the salivary glands, ovaries and neural systems (Cheng *et al.,* 2016). Thus, the infected mosquito becomes competent to transmit the viruses to naive hosts through blood-feeding for life (Chan and Johansson, 2012; Cheng *et al.,* 2016; Wu *et al.,* 2019). The incubation period of DENV in the Aedes mosquito is eight to ten days (Chan and Johansson, 2012). In human, symptoms of Dengue fever usually begin four to six days after infection, last for up to 10 days and may include high fever, severe headaches, orbital pain, severe joint and muscle pain, nausea vomiting and skin rash (WHO, 2016).

Also, DENV can be transferred from a female mosquito to its offspring (transovarial transmission) during egg transfer in the oviduct and egg development (Gubler, 1998; Espinosa *et al.,* 2014; Ferreira-De-Lima and Lima-Camara, 2018) or from the male mosquito to its offspring through sperm during mating with an uninfected female (Espinosa *et al.,* 2014; Ferreira-De-Lima and Lima-Camara, 2018).

The life span of adult *Ae. aegypti* is two to four weeks depending on conditions (Foster and Walker, 2019). This have implication in spread of the disease as particular mosquito may have time to transmit viruses to many people in its lifetime (Foster and Walker, 2019). In addition, their eggs can remain viable for about a year in a dry state, which allows re-emergence of mosquito after a cold winter or dry spell, which makes prevention and control to difficult (Foster and Walker, 2019).
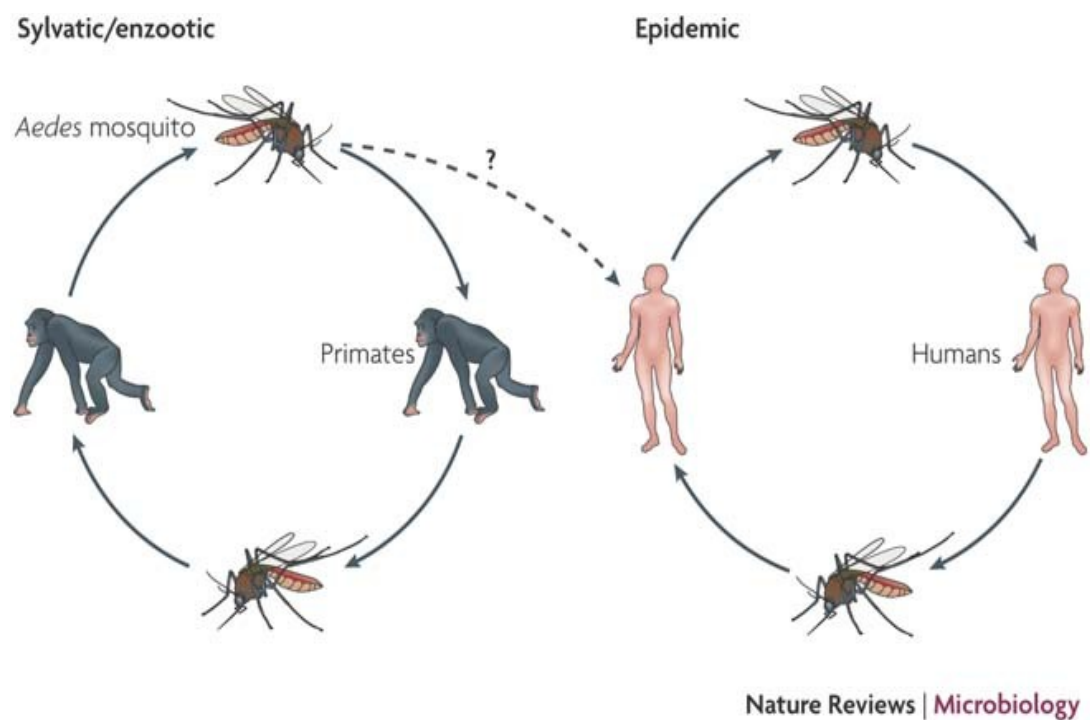
Figure 3: Dengue virus transmission cycles. There are two DENV transmission cycles, (i) Sylvatic/enzootic occurring in forests and (ii) Epidemic cycle that occur in urban areas. Source: (Whitehead et al., 2007)

2.11 Epidemiology of Dengue

Dengue is the most rapidly spreading mosquito-borne viral disease in the world (Messina *et al.,* 2019). Roughly, 53% of the global population live in areas that are suitable for dengue transmission, with the vast majority in Asia, followed by Africa and the Americas

(Messina *et al.,* 2019) causing major social and economic consequences (Shepard *et al*., 2016).

During the past 50 years, sporadic cases of Dengue fever have been reported in Africa with laboratory-confirmed cases reported in 15 countries (Were, 2012). In East Africa, Dengue has been reported in Kenya, the United Republic of Tanzania, Somalia and Djibouti (Rodier *et al.,* 1996; Kanesa-thasan *et al.,* 1998; Hertz *et al.,* 2012; Chipwaza *et al*., 2014). In Tanzania, several Dengue outbreaks have been reported since 2010 (WHO, 2019). In a recent 2019 Dengue outbreak, Tanzania reported 6917 cases and 13 deaths (WHO, 2019).

2.12 Diagnosis of Dengue

Several methods can be used for the diagnosis of Dengue from a variety of samples from vectors and humans. These include serological tests, virus isolation and molecular techniques (Tang and Ooi, 2012; Raafat *et al*., 2019). Serological tests, such as enzyme-linked immunosorbent assays (ELISA), detects the presence of immunoglobulin M (IgM) and Immunoglobulin G (IgG) antibodies specific to the virus in circulation. Immunoglobulin M antibody levels are highest within three to five weeks after the onset of illness and persist for about two months (Raafat *et al*., 2019). The virus may be isolated from mosquitoes or blood during the first few days of infection through cell culture together with hemagglutination-inhibition (HI) test, hemagglutination (HA) and plaque reduction neutralization test (PRNT) (Tang and Oodi, 2012; Raafat *et al*., 2019). Molecular methods such as reverse transcription polymerase chain reaction (RT-PCR) (Lanciotti *et al.,* 1992), nested RT-PCR methods, quantitative RT-PCR (qRT-PCR), multiplex PCR based microarray assay and reverse transcription loop-mediated isothermal amplification (RT-LAMP) (Raafat *et al*., 2019) are available. When

complemented by next-generation sequencing technologies, molecular methods may be used to detect and genotype the infecting virus, allowing comparisons across virus samples from various geographical sources (Tang and Ooi, 2012).

2.13 DENV Clinical Management and Control

Regardless of the negative impact of Dengue in public health, no antiviral therapies are available and there is only one licensed vaccine, Dengvaxia® manufactured by Sanofi Pasteur that is only available in a few countries (Dighe *et al*., 2019). Preventing and control of Dengue virus transmission depends entirely in controlling the mosquito vectors (Dighe *et al.,* 2019). In summary, to reduce or prevent Dengue and any other mosquito-borne viruses' transmission, environmental management and chemical control methods, including use of larvicides and adulticide, have been used with some effects (Srinivas, 2015; Naseem *et al*., 2016). Environmental management is another effective mosquito control measure (Srinivas, 2015; Naseem *et al*., 2016).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Study Area and Design

This cross-sectional entomological study was carried out in Kinondoni district of Dar es Salaam, Tanzania. Kinondoni is the northernmost of the three districts in Dar es Salaam. It is found at latitude 6° 42' 43" S and longitude 39° 07' 54" E. To the east, it is bordered with the Indian Ocean, to the north, and west the Pwani region and to the south Ilala district. It has a total area of 531 km² with a population size of about 1 775 049 and population density of 3343 inhabitants per km² (URT, 2013). The climate is generally hot and humid with small seasonal and daily variations in temperature. The mean annual temperature and the annual rainfall is about 30°C and 1100mm, respectively. The relative humidity is generally high and range between 65 and 96% for a year (Ndetto and Matzarakis, 2013).

This district was selected purposely based on reported Dengue outbreak in recent years (Vairo *et al.,* 2016; Mboera *et al.,* 2016). Adult and immature mosquito sampling was conducted in Mikocheni, Mzimuni and Mwananyamala wards. The wards were selected based on some ecological and demographical characteristics such as adult mosquito and larvae habitats, the topography, vegetation and area of high human density. In each ward at least three streets were randomly selected as sampling sites. The selected streets were Regent Estate, Mikocheni A, TPDC, Idrisa, Mwinyimkuu, Mtambani, Msisiri A, Msisiri B and Kopa (Fig. 4). Selection of sentinel sites for setting mosquito traps considered the areas perceived to have mosquitoes and the practicality of obtaining permission from owners or head of a household to access private property, setting and collecting traps.
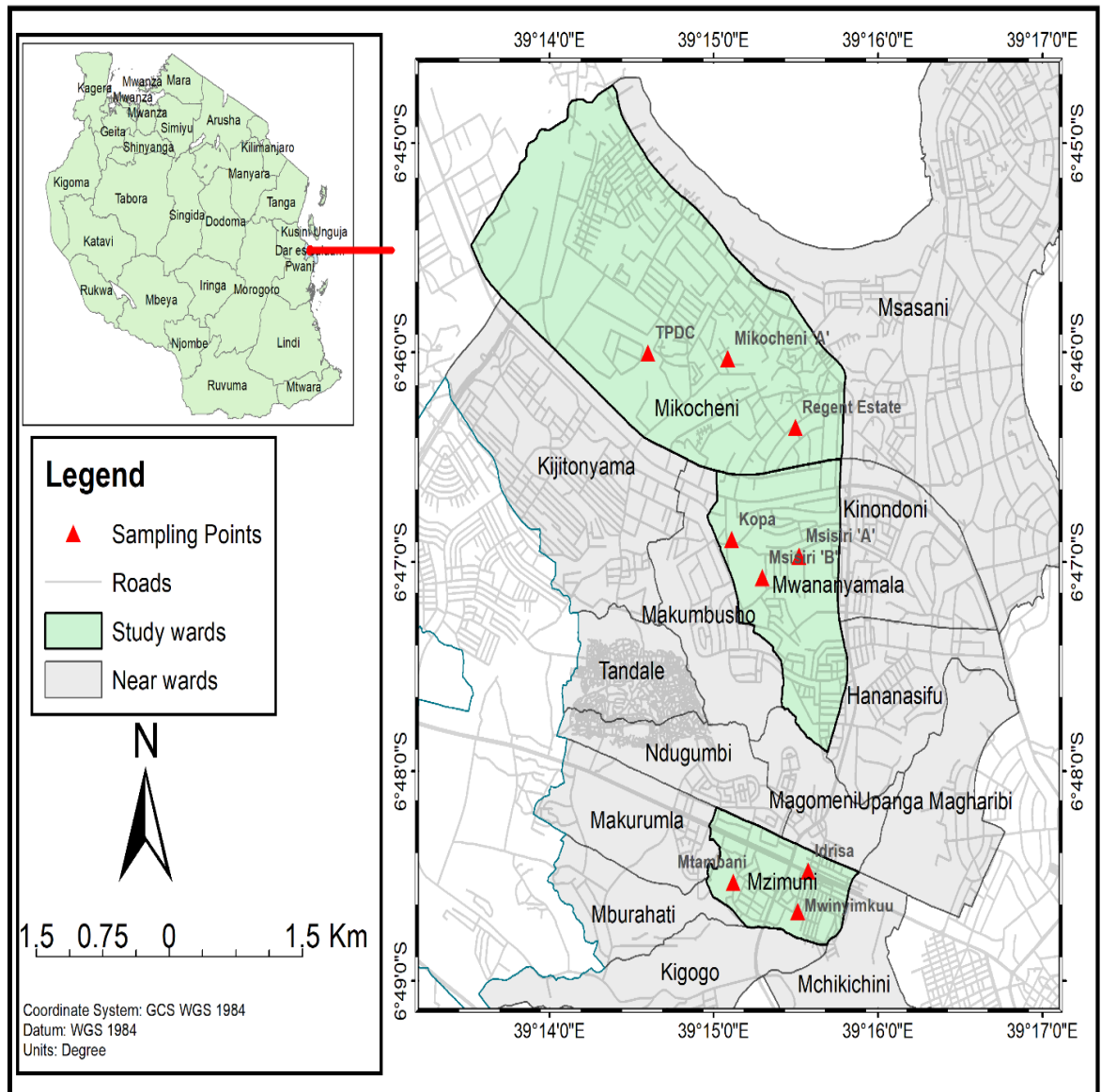
Figure 4: Map of Kinondoni district showing the three study wards

3.2 Sampling Strategies

The present study was a cross-sectional entomological study conducted involving sampling both adult mosquitoes, larvae and pupae. Mosquito sampling was conducted between December 2019 and January 2020. All ecological data were recorded using adult mosquito and larvae sampling forms (Appendices 1 and 2).

3.2.1 Sampling adult mosquitoes

Adult mosquitoes were collected outdoor using carbon dioxide-propane powered Mosquito Magnet Liberty Plus traps (American Biophysics Corporation, Rhode Island, USA) as

described by Mboera *et al*. (2015). Three traps per ward were set at three different sentinel sites (Outdoor and open spaces) and left to operate for 24 hours from 6:00 hours and mosquitoes were collected in the morning 6:00 hours of each sampling day for three consecutive days. A manual aspirator was used to collect adult mosquitoes from trap collecting bag into paper cups closed with netting materials. Mosquito specimens were transported to an insectary at Muhimbili University of Health and Allied Sciences (MUHAS) for identification.

3.2.2 Sampling mosquito larvae or pupae and rearing

Each household visited was inspected for water-holding containers. All water-holding containers found in selected houses or sites were examined for the presence of mosquito larvae and pupae. A container found with at least a larvae or pupae was considered positive for the presence of mosquito immature stages. Larvae and pupae found were collected into a small bowl using standard plastic dippers and transferred into a labelled water-filled Whirl-Pak plastic bag using Pasteur pipette as described by Mboera *et al.* (2015). Each container was scored for container type, approximate container volume, water volume within the container and water type or state. All data were entered in immature mosquitoes survey form (Appendix 2).

Mosquito larvae and pupae sampled from different habitats were transported in a cool box to MUHAS insectary for rearing and eventual morphological identification of emerged adults. The larvae were fed on Whiskas® cat food and maintained at $26 \pm 2^\circ C$ and relative humidity of $82 \pm 10\%$. Pupae were collected each morning and evening and transferred to netting cages ($30 \times 30 \times 30$ cm). Emergent adults were knocked down in the freezer at -20 ºC for 15 minutes and identified.

3.3 Identification of Mosquitoes

Mosquitoes were identified to species level as much as possible morphologically using standard mosquito taxonomic keys (Huang, 2001). Female Aedes mosquitoes were pooled into labeled 1.5 ml cryovial in groups of 20 mosquitoes according to their species, location, whether captured or reared and stored in liquid nitrogen. After collection, all collected specimens stored in liquid nitrogen were transported to SACIDS Laboratory at the College of Veterinary Medicine and Biomedical Sciences, Sokoine University of Agriculture in Morogoro and stored at -80 °C until laboratory analysis for detection of DENV using RT-PCR.

3.4 Detection of DENV in Mosquitoes

3.4.1 Extraction of viral RNA

Viral ribonucleic acid (RNA) was extracted from 140 µL of mosquito-homogenised lysate using QIAamp RNA mini kit (Qiagen, Hilden, Germany), according to the manufacturer's instructions. The quality of RNA was evaluated using a NanoDrop ND1000 spectrophotometer (Biochrom LTD, Cambridge, England). The purified RNA was immediately stored in aliquots at -20 °C until used to avoid freeze-thawing cycles that could damage RNA stability.

3.4.2 Conventional RT-PCR

One-step reverse transcription polymerase chain reaction (RT-PCR) was done using a SuperScript III Platinum/Taq DNA polymerase kit (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions using D1 (TCAATATGCTGAAACGCGCGAGAAACCG) and D2 (TTGCACCAACAGTCAATGTCTTCAGGTTC) primers targeting structural polyprotein gene with expected amplicon size of 511 bp as described by Lanciotti *et al.* (1992). PCR

was performed in a 25 µL reaction containing 12.5 µL of 2x reaction mix, 1 µL of SuperScript III RT/Platinum Taq mix, 0.5 µL of 10 μM sense primer (D1), 0.5 µL of 10 μM anti-sense primer (D2), 0.5 µL magnesium salt (Invitrogen, Carlsbad, CA, USA), 4 µL of RNA template and 6 µL of nuclease-free water. Reverse-transcription reaction was performed in a single cycle at 48 ºC for 30 minutes, followed by 1 cycle of initial denaturation at 94 ºC for 2 minutes. Thermocycling conditions were; 35 cycles with denaturation at 94 ºC for 15 seconds, annealing at 55 ºC for 30 seconds and elongation at 68 ºC for 1 minute and a final extension at 68 ºC for 5 minutes.

3.4.3 Visualization of RT-PCR amplicons

1.5% agarose gel was prepared by adding 1.5g of Agarose powder to 100 ml of 1x TAE buffer (Serva Electrophoresis, Heidelberg, Germany) in a 500 ml conical flask. The mixture was then heated in a microwave until became clear and transparent. The solution was allowed to cool to about 50 ºC at which 3µL of Gel red (Phenix, Research product, Candler, USA) was added and mixed. After mixing, the mixture was poured into already prepared plastic gel casting tray (with wells comb in place) placed on a flat surface and left to solidify for about 25 minutes. Solidified gel was removed from casting tray and placed into an electrophoresis tank containing 1x TAE buffer (Serva Electrophoresis, Heldelberg, Germany). 5 µL of each RT-PCR amplicon was mixed with 4µL of loading dye (Promega, Midson, USA) and loaded into respective wells along with 100 bp DNA ladder (Bio-Rad Laboratories, Hercules, CA, USA) at a first well and controls (Negative and positive) at two wells after samples. After loading, electrophoresis was set to run at 100 volts for 40 minutes. After finishing, agarose gel was taken out of electrophoresis tank into Gel doc EZ Imager System (Bio-Rad Laboratories, Hercules, CA, USA) for visualization. Picture was taken and the results evaluated with reference to positive, negative control and expected amplicon size of about 511 bp. Lastly, agarose gel was disposed.

3.5 Data Analysis

Data management and analysis were performed using Microsoft Excel 2016 and R version 3.5.3. All data were entered in duplicates into Microsoft Excel, cleaned and evaluated against the original forms. The proportion of mosquito by genera and species was calculated using Microsoft Excel 2016. Dengue transmission levels were assessed by (i) House (premises) 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. From these indices, potential risk areas were estimated based on WHO criteria (WHO, 1971).

3.6 Ethical Consideration

Ethical approval was sought from the National Health Research Ethics Committee (NatHREC) of the National Institute of Medical Research (NIMR), Tanzania (Appendix 3). Permission to conduct this research was granted by Sokoine University of Agriculture (Appendix 4). Permission to get into household premises was sought from the head of the household and written consent was obtained from household heads (Appendix 5). The confidentiality of the study participants was strictly observed.

CHAPTER FOUR

4.0 RESULTS

4.1 Mosquito Species Composition and Abundance

A total of 2001 adult mosquitoes were collected (Table 1). *Culex quinquefasciatus* was the most abundant mosquito species accounting for 53.12% (1063) of the total mosquitoes collected. Mansonia species and *Aedes aegypti* accounted for about 23.64% (473) and 23.24% (465) of all mosquitoes, respectively. *Ae. aegypti* and *Cx. quinquefasciatus* were found in all wards but the majority of *Ae. aegypti* were found in Mikocheni ward, the majority of *Cx. quinquefasciatus* were found in Mwananyamala while Mansonia species mosquitoes were found in Mwananyamala only (Figure 5). In all species and all wards, female mosquitoes had a higher count compared to male mosquitoes. Generally, in Kinondoni district, of 465 *Ae. Aegypti* collected, 51.8% were female; of 1062 *Cx. quinquefasciatus* collected, 69.6% were female and of 473 Mansonia species mosquitoes collected, 92.6% were female. A total of 1750 *Ae. Aegypti* mosquitoes were hatched from larvae and pupae samples and reared in an insectary.

Table 1: Abundance of adult mosquito collected by mosquito magnet traps

Ward	Mosquito species	No. mosquitoes	% of the total collected
Mikocheni	*Aedes aegypti*	284	44.9
	Culex quinquefasciatus	348	55.1
	Subtotal	**632**	**31.6**
Mwananyamala	*Aedes aegypti*	69	6.2
	Culex quinquefasciatus	568	51.2
	Mansonia mosquitoes	473	42.6
	Subtotal	**1110**	**55.5**
Mzimuni	*Aedes aegypti*	112	43.2
	Culex quinquefasciatus	147	56.8
	Subtotal	**259**	**12.9**
	Total	**2001**	
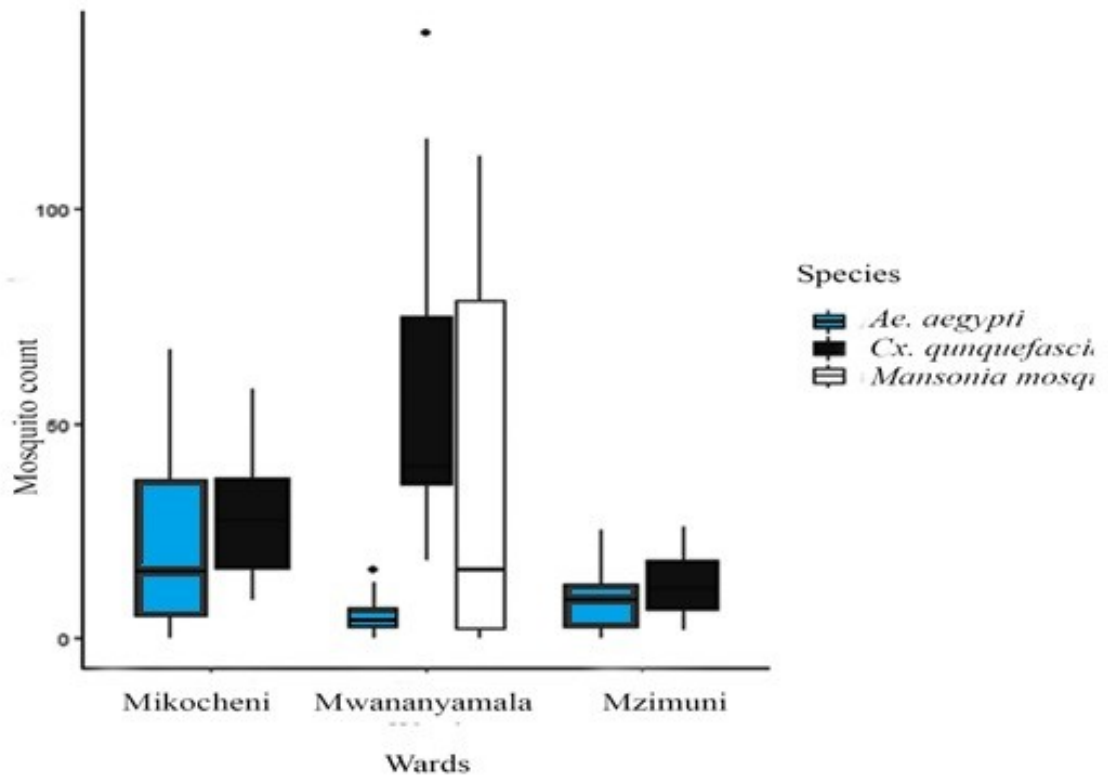
Figure 5: Distribution of the number of female mosquitoes by species and ward

4.2 Aedes Mosquito Breeding Sites

A total of 176 houses were inspected for the presence of potential *Aedes* mosquito breeding sites around outdoor premises. Of these, 132 (75%) houses had water-holding containers or vessels in their premises and 97 (55.1%) houses had water-holding containers with at least *Aedes* larvae or pupae. Mikocheni ward had the largest proportion (85%) of households with water-holding containers, followed by Mzimuni (75.9%) and Mwananyamala (63.8%). The most common breeding containers for the *Aedes* mosquitoes included used car tires and flower pots. Other breeding sites included plastic water tanks, small plastic containers, cement water tanks, plastic drums and plastic water containers (Table 2 and Figure 6).

Table 2: Containers with at least a larvae or pupae in outdoor premises of Mikocheni, Mwananyamala and Mzimuni wards of Kinondoni district, Dar es Salaam, Tanzania

Container type	Number of container visits (+ve)			
	Mikocheni	Mwananyamala	Mzimuni	Total
Cement water tank	3 (2)	1 (1)	1 (1)	5 (4)
Flower pot	11 (7)	4 (3)	6 (6)	21 (16)
Plastic water container	5 (3)	2 (0)	10 (3)	17 (6)
Plastic drum	3 (2)	4 (3)	3 (1)	10 (6)
Plastic water tank	7 (7)	4 (2)	4 (0)	15 (9)
Used car tire	45 (38)	19 (18)	45 (38)	109 (94)
Small plastic container	4 (4)	5 (4)	6 (4)	15 (12)
Water bucket	19 (8)	26 (12)	40 (5)	85 (25)
Water bottle	9 (4)	5 (0)	2 (0)	16 (4)
Others	17 (15)	11 (4)	12 (7)	40 (26)
Grand Total	**123 (90)**	**81 (46)**	**129 (65)**	**333 (201)**

Figure 6:   Aedes mosquito breeding habitats identified during a larvae survey in Kinondoni district, (A) car dump, (B) discarded car tire and (C) water buckets.

4.3 Aedes Indices

The overall *Aedes* House index of Kinondoni district was 55.1%. Mikocheni had the highest HI (71.7%) followed by Mwananyamala (48.3%) and Mzimuni (44.8). The overall *Aedes* container index was 60.4%. Mikocheni had the highest CI (73.2%) followed by Mwananyamala (56.8%) and Mzimuni (50.4%). The overall *Aedes* Breteaux index was 114.2 and it was highest in Mikocheni (150) followed by Mzimuni (112.1) and Mwananyamala (79.3) (Table 3 and Figure7).
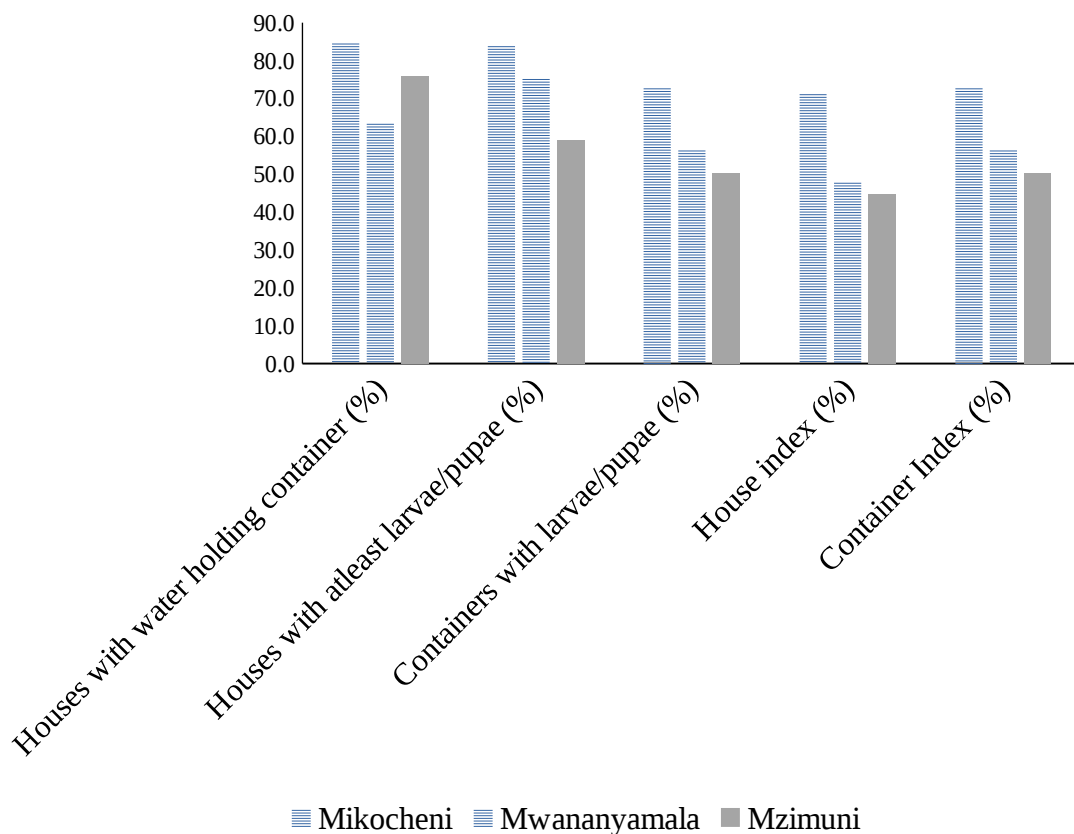
Figure 7: Summary of Aedes larvae survey in Kinondoni district by wards

4.4 DENV Infection Rates

All pools of mosquitoes, field trapped and reared, tested negative for DENV using RT-PCR (Figure 8).
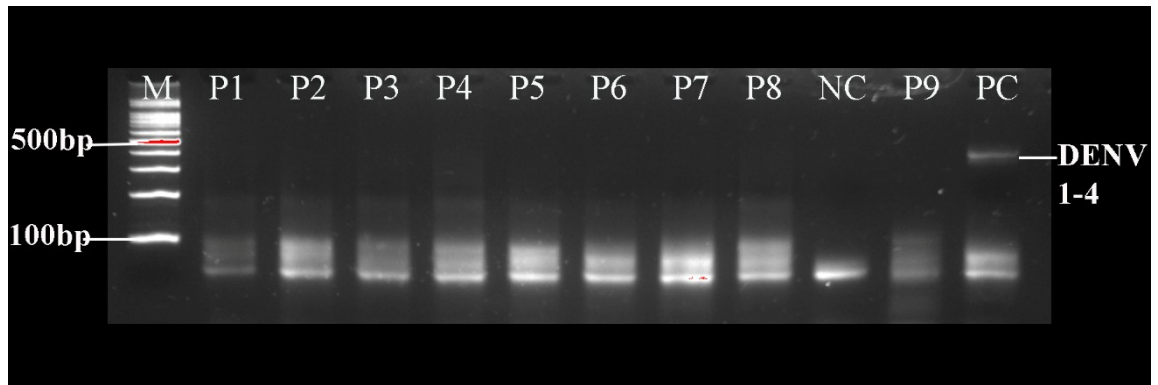
Figure 8: Gel electrophoresis of RT-PCR products of Dengue virus in female *Aedes aegypti* mosquitoes. M is a 100bp DNA ladder, PC-positive control, NC-negative control and P1-10 are mosquito pools (samples).

Table 3: Number (%) of houses surveyed, with water holding containers and Aedes indices by ward in Kinondoni district, Dar es Salaam

Ward	No. of house surveyed	No. (%) houses with water-holding containers	No. (%) housed with larvae/pupae	No. containers inspected	No. (%) containers with larvae/pupae	House index (%)	Container index (%)	Breteaux index
Mikocheni	60	51 (85%)	43 (71.7%)	123	90 (73.2%)	71.7	73.2	150
Mwananyamala	58	37 (63.8%)	28 (48.3%)	81	46 (56.8%)	48.3	56.8	79.3
Mzimuni	58	44 (75.9%)	26 (44.8%)	129	65 (50.4%)	44.8	50.4	112.1
Overall	**176**	**132 (75%)**	**97 (54.0%)**	**333**	**201 (60.4%)**	**55.1**	**60.4**	**114.2**

5.0 DISCUSSION

The results of the present study showed that mosquito species collected in Kinondoni were *Culex quinquefasciatus*, *Aedes aegypti* and *Mansonia* species. Mosquito abundances differed among sentinel sites with *Cx quinquefasciatus* being the most abundant mosquito species accounting for over half of the mosquitoes. *Aedes aegypti* accounted for about a quarter of the mosquitoes collected. The majority of *Ae.aegypti* were collected

from Mikocheni ward while the majority of *Cx quinquefasciatus* were collected from Mwananyamala. This difference is likely to be attributed to ecological and anthropogenic factors (Derraik and Slaney, 2007; Yee *et al.*, 2010).

The findings of this study are similar to what was reported by Mboera *et al.* (2015; 2016) except for the presence of *Mansonia* mosquitoes. While in their study they found five mosquito species in the same district, the low number of species in our study could be due to difference in the sampling method used, mosquito sampling sites and time of the year. As it has been reported, we found that mosquito species composition and abundance in the sampled wards were not homogeneous. The occurrence of *Cx. quinquefasciatus* as the most abundant mosquito species in Kinondoni could be explained by sewage drainage systems, presence of unplanned settlements, uncontrolled population growth that affect the level of environmental pollutions (Chavasse *et al.,* 1995; Govella *et al.,* 2011). The significant presence of *Ae. aegypti,* approaching a quarter of the total host-seeking mosquitoes caught in sentinel sites of Kinondoni district is similar to what was reported in 2015 (Mboera *et al.,* 2015). Findings of the presence of *Ae. aegypti* in Kinondoni can be mostly linked to human activities, mismanagement of containers, mismanagement of backyard, flowers and home gardens, presence of old tires that can be attributed to lack of environmental hygiene.

Generally, the occurrence of large numbers of *Cx. quinquefasciatus* and *Ae. aegypti* in this study is of public health significance due to their ability to transmit lymphatic filariasis and viral diseases, respectively. Previous reports of Yellow fever, Rift Valley fever, Chikungunya and more recently

several Dengue fever outbreaks in Tanzania are associated to the presence of *Aedes* mosquitoes (Ochieng *et al.,* 2013; Mboera *et al.,* 2015; Mboera *et al.,* 2016; Hertz *et al.,* 2016; Mathias *et al.,* 2017; Kinimi *et al.,* 2018; Patrick *et al.,* 2018; Mselemu *et al.,* 2020; WHO, 2019).

In this study, more than half of inspected houses had water-holding containers positive for *Aedes* mosquito larvae and or pupae. The most common breeding containers or vessels for the *Aedes* mosquitoes were used car tires and flower pots. A large number of water-holding containers with mosquito immature stages found outdoors in Kinondoni district explained the mosquito species composition and abundance reported in this study. Similar to these findings, a study by Mboera *et al*. (2016) found that water-holding containers left outdoors have been harboring Aedes immature stages in Dar es Salaam. Additionally, *Ae. aegypti* has been reported as a common outdoor breeding mosquito even in rural areas or small towns of Tanzania. A study by Kahamba *et al*. (2020) in Ifakara found that more than 27% of the water holding containers outside houses were infested with *Ae. aegypti* while indoor there was practically no breeding in containers. Also, Trpis (1972) reported that a part of the *Ae. aegypti* population in East Africa is maintained in some biotypes such as automobile dumps and coral rock holes by continuous breeding.

About three-quarters of houses in Kinondoni had water-holding containers potential for mosquito breeding. The highest proportion of houses with water-holding containers was observed at Mikocheni ward. These proportions could be explained by either lack of awareness on mosquito breeding habitats, poverty to some people in study areas such as Mikocheni A street and insufficiency water supply that makes most households to depend on rainwater or stock enough water in containers for future use. Inappropriate disposal of used tires, mismanagement of home gardens and flower

ports contributed highly to mosquito breeding sites in the district. Community awareness on potential mosquito breeding habitats should be assessed while vector control interventions targeting mosquito immature stages should consider both indoor and outdoor water-holding containers as potential breeding habitats in Kinondoni district. A previous study in Zanzibar also reported shortage of water supply as a reason to why people keep water for a considerable time thereby creating *Ae. aegypti* habitats (Saleh *et al*., 2018).

The overall house and container indices for Kinondoni district were higher. Container productivity varied by ward and according to urbanization degree. A previous study during the long rains reported slightly lower house index (35.4%) but higher container index (65.2%) in Kinondoni district (Mboera *et al.,* 2016). Another study in Ifakara, Tanzania reported lower larvae indices (container index 14.6% and house index 6.6%) than those found in this study (Kahamba *et al*., 2020). Despite the fact that this study was carried out during a short rainy season, the higher Aedes mosquito indices are likely to be contributed by the tendency of residents of Kinondoni district to store water in their households and availability of man-made water containing equipment around their houses. All these influence the presence of *Aedes* mosquitoes. The Breteaux indices in the three wards of Kinondoni district were extremely high. Higher Aedes indices, BI, in particular, indicate geographical units being at high risk for Dengue transmission (Sanchez *et al*., 2006). Findings of this study are similar to a study in northern Ghana where house indices were found to range from an average of 55.9 to 88.3 and Breateaux index from 72.4 to 180.9 during a dry season (Appawu *et al*., 2010).

None of the mosquito pools tested positive for DENV infection by conventional RT-PCR using universal primers. This finding is contrary to findings of a previous study which reported infection rates of about 6.8% in Kinondoni district (Mboera *et al.,* 2016). Longitudinal studies employing various sampling techniques are recommended to provide a better picture of DENV transmission in Dar es Salaam. Although the DENV was not detected in Aedes mosquitoes, the abundance of Aedes mosquitoes and the high Breteaux indices in Kinondoni district signifies that the district is one of the risk areas for DENV, mosquito control interventions are therefore recommended to prevent future outbreaks.

CHAPTER SIX

6.0 CONCLUSION AND RECOMMENDATIONS

6.1 Conclusion

Generally, Kinondoni district continues to be at risk of transmission of Dengue as *Aedes aegypti*, a DENV vector was observed to be present. Although DENV was not detected in mosquito vectors, the presence of potential breeding sites around Kinondoni district and higher Aedes indices (HI, CI and BI) put Kinondoni at risk of DENV transmission. The best option in the prevention of Dengue remains to be through mosquito control.

6.2 Recommendations

i. Vector control interventions specifically integrated mosquito control approaches should be directed towards the elimination of breeding sites and adult mosquitoes.

ii. Large surveillance studies on mosquito vectors of Dengue in Tanzania are recommended to give a wider picture of Dengue and its vectors in Tanzania including Dar es Salaam.

iii.   Environmental management and manipulation in controlling mosquitoes should be emphasized and on proper disposal of used car tires as the majority of Aedes immature stages were observed and collected in car tires.

iv.   Molecular techniques with high detection sensitivity and specificity such as real time PCR and direct sequencing using next generation sequencing platforms are recommended in surveillance studies on arboviruses so as to increase the chance of detecting viruses in case they are circulating in mosquitoes in particular time and space.

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APPENDICES

Appendix 1: Adult mosquito collection form

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II: ADULT MOSQUITO COLLECTION FORM-KINONDONI, DAR ES SALAAM

Street: _____ Date: _____

ector: _____ Longitude: _____

t: None/light/Heavy/Showers/Storm (Tick)

attle/sheep/goats/donkeys/pigs in the compound (Tick)

ecies	Female (F) mosquito abdominal status				Male (M) mosquitoes
	Unfed (UF)	Fed (F)	Half gravid (HG)	Gravid (G)	

ools: _____

emale mosquito per pool: _____

Appendix 2: Immature mosquito survey form

MMATURE MOSQUITO SURVEY FORM- KINONDONI, DAR ES SALAAM

Street: _____ Name of collector: _____

_____ Longitude: _____

Type of breeding site	Volume of bleeding container	Volume of water within container	Type of water 1=CLEAN 2=POLLUTED	Presence of eggs: 1=YES 2=NO	Presence of larvae 1=YES 2=NO	Presence of pupae 1=YES 2=NO	Mosquito species 1=Anopheline 2=Culicine 3=Aedine	Any other remarks

Appendix 3: Ethical clearance certificate for conducting research in Tanzania from National Health Research Ethics committee of the National Institute for Medical Research

THE UNITED REPUBLIC
OF TANZANIA

National Institute for Medical Research
3 Barack Obama Drive
P.O. Box 9653
11101 Dar es Salaam
Tel: 255 22 2121400
Fax: 255 22 2121360
E-mail: nimrethics@gmail.com

Ministry of Health, Community
Development, Gender, Elderly & Children
University of Dodoma, College of
Business Studies and Law
Building No. 11
P.O. Box 743
40478 Dodoma

NIMR/HQ/R.8a/Vol. IX/3278

2nd December 2019

Baraka L Ngingo,
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: "Mosquito species composition, abundance and transmission risk of dengue in Kinondoni district, Dar es Salaam Tanzania" (Ngingo BL. 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: Dar es Salaam region.

Approval is valid for one year: 2nd December 2019 to 1st December 2020.

Name: Prof. Yunus Daud Mgaya

Name: Prof. Muhammad Bakari Kambi

Appendix 4: Clearance permit for conducting research in Tanzania from SUA

CLEARANCE PERMIT FOR CONDUCTING RESEARCH IN TANZANIA

SOKOINE UNIVERSITY OF AGRICULTURE
OFFICE OF THE VICE-CHANCELLOR
P.O. Box 3000 CHUO KIKUU, MOROGORO, TANZANIA
Phone: 255-023-2640006/7/8/9, Direct VC: 2640015; Fax: 2640021;
Email: vc@sua.ac.tz;

Our Ref. SUA/ADM/R.1/8/441   **Date:** 8th October, 2019

The Regional Administrative Secretary,
Dar es Salaam Region,
P.O. Box 5429,
DAR ES SALAAM.

Re: UNIVERSITY STAFF, STUDENTS AND RESEARCHERS CLEARANCE

The Sokoine University of Agriculture was established by University Act No. 7 of 2005 and SUA Charter, 2007 which became operational on 1st January 2007 repealing Act No. 6 of 1984. One of the mission objectives of the university·is to generate and apply knowledge through research. For this reason the staff and researchers undertake research activities from time to time.

To facilitate the research function, the Vice Chancellor of the Sokoine University of Agriculture (SUA) is empowered to issue research clearance to staff, students, research associate and researchers of SUA on behalf of the Tanzania Commission for Science and Technology.

The purpose of this letter is to introduce to you **Mr. Baraka Laurian Ngingo** a bonafide **MSc. (One Health Molecular Biology)** student with Registration number **MOH/D/2018/0041** of SUA. By this letter **Mr. Baraka Laurian Ngingo** has been granted clearance to conduct research in the country. The title of the research in question is **"Mosquito species composition, abundance and Transmission Risk of Dengue in Kinondoni District, Dar es Salaam, Tanzania".**

The period for which this permission has been granted is from **November, 2019** to **April, 2020**. The research will be conducted in **Kinondoni District.**

Should some of these areas/institutions/offices be restricted, you are requested to kindly advice the researcher(s) on alternative areas/institutions/ offices which could be visited. In case you may require further information on

Appendix 5: Informed consent statement

SOKOINE UNIVERSITY OF AGRICULTURE (SUA)
SACIDS Foundation for One Health
College of Veterinary Medicine and Biomedical Sciences
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

MOSQUITO SPECIES COMPOSITION, ABUNDANCE AND TRANSMISSION RISK OF DENGUE IN KINONDONI DISTRICT, DAR ES SALAAM, TANZANIA

The following statements will be read to head of household to be visited for mosquito sampling.

My name is …………………………, and I am from the Sokoine University of Agriculture. We are carrying out a study to determine Mosquito species composition, abundance and transmission risk of dengue in Kinondoni district, Dar es salaam, Tanzania. We planned not to visit all households and open sites in this area, we selected few as representatives.

Your house or site is one of those selected to for this survey, if you accept, information and samples collected from your house or site and several others will be analysed to get the general picture of this disease (Dengue and its vectors). Our survey involves collecting adult mosquitoes and its immature stages found in houses and outside compounds and sending to laboratory for further analysis. We request visit and collect mosquitoes if found in your house or site as explained. You are free to accept or not accept our request. Participation is confidential and the risk of disclosure of the personal data will be minimized

Do you accept our request?

YES
NO

Participant Signature _____ Participant thumbprint

NB: *If you have any questions regarding this research, you may ask the research staff or contact Mr. Baraka L Ngingo, Sokoine University of Agriculture, P.O. Box 3297, Chuo Kikuu, Morogoro;Telephone: +255 683671003; E-mail: baraka.ngingo@sacids.org.*