MOSQUITO DIVERSITY AND FEBRILE ILLNESS IN KARAGWE AND KYERWA DISTRICTS, NORTH WESTERN 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

Mosquito-borne viruses cause emerging and re-emerging infections affecting humans and animals. These diseases present themselves mainly with fever. The present study aimed at determining socio-demographic and clinical characteristics among febrile patients, and detection of selected mosquito-borne viruses circulating in *Aedes aegypti* and *Ae. albopictus.* Using a hospital-based cross-sectional descriptive study design, in total 400 febrile patients were recruited after consenting into the present study. A structured questionnaire was administered to collect socio-demographic and clinical data. The results showed that most of the febrile patients (n=400) were aged between 20-29 years (25.25 %), followed by those aged 10-19 years (23.25 %), and only 13.15% were older than 50 years. The results show that fever (100 %) was the most common symptom reported, followed by headache (68.75 %), joint aches (67.75 %), seizures (63.50 %), vomiting (61.50 %) weakness in legs (59.50 %), laboured breathing (58.50%) and the least observed symptoms were abdominal pains (41.75 %), neck stiffness (33.75 %), and rashes (33 %). Screening 22 pools of *Ae. aegypti* and *Ae. albopictus* using reverse transcription polymerase chain reaction (RT-PCR) showed the presence of Flaviviruses, Bunyaviruses and *Alphaviruses*. Further screening of specific viruses in Aedes mosquitoes showed the presence of Chikungunya virus. Furthermore, the risk factors for mosquito-borne viral infections were investigated in the present study. The findings of this study show that 12.75 % of patients were in contact in forests and 79 % had been bitten by day-biting mosquitoes within three months prior to sampling. Only 28.75 % of febrile patients had malaria, indicating the widespread nature of febrile illness other than malaria. It can be concluded from the results of present study that Aedes mosquitoes are infected with Chikungunya virus and that interaction between humans and forests predisposes humans to mosquito bites.

DECLARATION

I, Edson Kinimi, 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.

_____ _____

Edson Kinimi **Date**

(MSc. Candidate)

The declaration is hereby confirmed;

_____ _____

Prof. Gerald Misinzo **Date**

(Supervisor)

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DEDICATION

My work is dedicated to my lovely Pastor Paula Bennett who prayed to pave the way for me to reach where I am today. I also dedicate my work to my lovely wife Ritha Rugalabamu for love, encouragement and support during my studies.

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LIST OF ABBREVIATIONS AND SYMBOLS

°C	degree celsius
CDC	Center of Disease Control and Prevention
CHIKV	chikungunya virus
DENV	dengue virus
DNA	deoxyribonucleic acid
EDTA	ethylenediaminetetraacetic acid
ELISA	enzyme-linked immunosorbent assay
HI	haemagglutination inhibition
ID	identification
IgG	immunoglobulin G
IgM	immunoglobulin M
ml	milliliter
NIMR	National Institute for Medical Research
nm	nanometer
NSs	nonstructural protein
ONNV	o'nyong nyong virus
OR	odds ratio
PBS	phosphate-buffered saline
PRNT	plaque reduction neutralization test
RNA	ribonucleic acid
RPM	revolutions per minute
RT-PCR	reverse transcriptase- polymerase chain reaction
RVF	Rift Valley fever
RVFV	Rift Valley fever virus

SACIDS	Southern African Centre for Infectious Diseases Surveillance
SUA	Sokoine University of Agriculture
USA	United States of America
WHO	World Health Organization
WNV	West Nile virus
YF	yellow fever
YFV	yellow fever virus
µl	microliter

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background Information

Mosquito-borne viruses compose a large group of zoonotic viruses that infect mosquitoes and have complex cycles (sylvatic and urban cycles) involving at least one non-human primary vertebrate host and one primary mosquito vector (Clifton and Noriega, 2012). The mosquito-borne viruses are commonly transmitted to humans mainly by the bite of mosquitoes (Gubler, 2004) and less frequently by ticks, which are maintained in endemic areas by horizontal transmission between vertebrates and blood-feeding insect vectors and then by vertical transmission among mosquitoes (Victor *et al*., 2002).

The mosquito-borne viruses are classified into the families *Togaviridae* (genus Alphavirus), *Flaviviridae* (genus Flavivirus), *Bunyaviridae* (genus Orthobunyavirus and Phlebovirus) and *Reoviridae* families (Kuno, 1998). Most of mosquito-borne viruses have a single-stranded RNA genome with spherical virions with a diameter ranging between 45-120 nm (Schneider and Higgs, 2008). These mosquito-borne viruses have become major global public health concern, with increasing incidence in recent decades as a result of the expansion of the vectors' geographic range, human encroachment into forests, global travel, unplanned urbanization and climate change (Weaver and Barrett, 2004). Although, mosquito-borne viruses cause serious pathology in humans and in animals, they have minimal impact on mosquito mortality. The mosquito immune system can control, but not clear, mosquito-borne viral infection. For this reason, infected insects can be vectors for life (Gautret *et al.,* 2010).

Furthermore, the only known DNA arthropod borne virus is African swine fever virus, classified into the family *Asfarviridae* and genus Asfivirus (Dixon *et al*., 2005). The

greatest genetic plasticity and higher mutation rates exhibited by RNA viruses allow them to accommodate a cycle of alternating replication in separate vertebrate and invertebrate hosts (Holland and Domingo, 1998). Mosquito-borne viruses circulate among wild animals, and cause disease after spill over transmission to humans and/or domestic animals that are incidental or dead-end hosts. Viruses such as dengue (DENV) and chikungunya virus (CHIKV) that have lost the requirement for enzootic amplification now produce extensive epidemics (Victor *et al.,* 2002).

Many mosquito-borne viruses that have evolved and diversified in the tropics have produced virulent and invasive strains that have caused major outbreaks at temperate latitudes (Samuel and Tyagi, 2006). The ability of these viruses to cause human disease depends on factors ranging from epidemiology to viral genetics. The present study aimed at determining the socio-demographic characteristics among febrile patients and to detect the presence of arboviruses transmitted by mosquitoes including DENV, yellow fever virus (YFV), Rift Valley fever (RVFV) and CHIKV in Aedes mosquitoes (Ayers *et al*., 2006).

1.2 Problem Statement and Justification

Mosquito-borne viral diseases pose a public health threat, as they cause large epidemics and result in increased financial costs that are associated with diagnosis and treatment. In addition, the mortality rates of several of these mosquito-borne viral diseases are considerable. Despite the worldwide distribution of mosquito- borne viruses, countries in tropical and subtropical areas with vast forest reserves and diverse fauna, represent environments that are more conducive to the ecology of mosquito-borne viruses. More than 38 percent of the territory (94.5 million hectares) in Tanzania consists of natural

ecosystems that provide ideal conditions for the development and dissemination of mosquito-borne viruses (Halstead, 2008).

Most of these infections are asymptomatic. The diagnosis of mosquito-borne viral diseases can be difficult due to the following factors, (i) a clinical similarity to other diseases including malaria (ii) the presence of clinically asymptomatic or oligosymptomatic disease (iii) difficult access to reference laboratories that can perform a differential diagnosis and (iv) phylogenetic cross reactions that can occur between serological tests, especially in endemic regions (Sharp *et al.,* 1995).

However, it is important to find out the vector infection rate as well as the density in an area to determine the transmission potential of mosquito-borne viruses. The present study aimed at determining socio-demographic and clinical characteristics among febrile patients, and to detect, through reverse transcriptase-polymerase chain reaction (RT-PCR), the presence of mosquito-borne viruses circulating in Aedes mosquitoes

1.3 Objectives

1.3.1 Overall objective

To determine socio-demographic and clinical characteristics among febrile patients and to detect the presence of mosquito-borne viruses in Aedes mosquitoes.

1.3.2 Specific objectives

i. To determine the socio-demographic and clinical characteristics among febrile patients attending health facilities in Karagwe and Kyerwa districts,
ii. To determine mosquito abundance in selected areas of Karagwe and Kyerwa districts and

iii. To investigate the presence of RVFV, YFV, CHIKV and DENV in *Ae. Aegypti* and *Ae. Albopictus* mosquitoes collected in Karagwe and Kyerwa districts.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Mosquito-borne Viruses

The viruses that are transmitted by mosquito (mosquito-borne viruses) belong to various families and genera of viruses and spread by mosquitoes (Jentes *et al*., 2010). In contrary, humans and animals are dead-end hosts because they do not produce significant viremia and do not contribute to the transmission cycle of mosquito-borne viruses (Kuno, 1998). Therefore the diseases of mosquito-borne virus exhibit wide range in severity from mild flu-like illness and fever to fatal encephalitis and meningitis.

Mosquito-borne viruses are classified according to antigenic relationships, morphology, and replication mechanisms (Gubler, 2004). However, clinically significant mosquito-borne viruses belong to the families and genera of *Togaviridae* (Alphavirus; toga is Latin for the garment and referring to the envelope of the virus), *Flaviviridae* (Flavivirus; "flavi" or "flavus," Latin for yellow and referring to yellow jaundice produced by the disease), *Bunyaviridae* (Bunyavirus; "bunya," referring to Bunyamwera, a locale in Western Uganda where the virus was isolated in 1943), and *Reoviridae* (Orbivirus; "reo," from respiratory enteric orphan virus and "orbi," Latin for ring and referring to ring-shaped capsomers). Some of these viruses predominantly cause encephalitis (Samuel and Tyagi, 2006).

2.1.1 Mosquito-borne viral diseases

2.1.2 Yellow fever

Yellow fever is a viral hemorrhagic fever which infects large number of people and causes considerable deaths globally per year (Tolle, 2009). The causative agent of yellow fever is

a mosquito-borne virus known as YFV from the Flavivirus genus of the family *Flaviviridae*. It is a single-stranded virus with a positive polarity RNA genome. Viral particles are 43 nm in size made up of a ribonucleoprotein core and a lipoprotein envelope (Reiter *et al*., 1998). The fundamental understanding is that there are only two genotypes of YFV in Africa, one represented by West African viruses and the other by Central and East African strains (Tolle, 2009).

Moreover, there are three different epidemiological patterns of yellow fever (YF) transmission: the sylvatic pattern, the urban cycle, and an intermediate cycle that bridges these two patterns (Tolle, 2009). The main vector of the urban cycle YF is the female *Ae. aegypti.* In the sylvatic cycle of yellow fever, monkeys and humans are the primary and accidental hosts, respectively. Humans become infected with sylvatic YF when bitten by the primary mosquito vector, *Ae. Africanus*, *Ae. bromeliae* or one of several other mosquito species (Gubler, 2004).

2.1.2.1 Clinical manifestation of yellow fever

In humans, the incubation period for the disease is generally three to six days after the bite from an infected mosquito (Thoisy *et al.,* 2004). The patient is infectious to mosquitoes for the first three to four days after the onset of symptoms (Nasidi *et al.,* 1989) clinical symptoms of yellow fever include a sudden onset of fever, headache, backache, general muscle pain, nausea, vomiting and bradycardia (Gubler, 2004).

In 2001 Reiter, reported that about 15 % of those infected develop a serious illness with acute, remission and toxic phases. The acute phase lasts about three days with the sudden onset of fever, headache, myalgia, nausea, and vomiting. Afterwards a remission phase for up to 24 hours is followed by a toxic phase of jaundice and vomiting in which

haemorrhaging of the gums and nose, hematuria, albuminuria and oliguria may occur. At least half of the individuals who reach the toxic phase do not survive and die between the seventh and tenth day after onset (Dutary and Leduc, 1981)

Malaria and YF may coexist in a region (Reiter, 2001), and malaria usually shows clinical symptoms nearly identical with those of the early stages of YF. During the acute febrile phase of an infection, there is little to distinguish the illness from a number of other febrile conditions such as typhoid fever, rickettsial infections, influenza, leptospirosis, viral hepatitis, infectious mononucleosis and other mosquito-borne viral fevers like dengue fever, Lassa fever and Chikungunya (Alison *et al*., 2000). The definitive diagnosis of YF is made by serology or virus isolation, which requires trained health care personnel, proper laboratory equipment, and special reagents for the interpretation of the test results (Gubler 2004).

2.1.2.2 Distribution of YF in East Africa

The distribution of YF in Africa is best understood in terms of vegetation zones which reflect rainfall patterns and determine the abundance and distribution of mosquito vectors and vertebrate hosts (Dutary *et al.,* 1989). The vegetation zones are equatorial rain forest, humid/semi-humid savannah and dry savannah. Typically, year-round enzootic YF transmission between monkeys and *Ae.africanus* occurs in the Equatorial rain forest (mainly sylvatic) zone (Beaty *et al*., 1980). Virus activity is generally at a low level and sporadic cases or focal outbreaks have been observed, in a manner similar to sylvatic YF in South America (Fontenille *et al*., 1997). Transmission is predominantly monkey-to monkey, and human infection is sporadic. The humid/semi-humid savannah with either monkey to- monkey or monkey-to-human transmission is a major area of risk especially during the rainy seasons where it is prone to repeated emergence of YF activity (Domingo

at el., 2011), which may occur at a high rate of transmission due to the presence of vector and host populations. Sylvatic Aedes (e.g., *Ae. furcifer, Ae. luteocephalus, Ae. vittatus*) reach very high densities during the rainy season, and are responsible for cyclic epizootics in monkey populations and epidemics with inter-human transmission (Deubel *et al.,* 1997). This zone is also known as the intermediate zone of transmission.

Vertical transmission in these mosquitoes assures virus survival and continuation of epizootic waves. It is in this vegetation zone that most epidemics of YF have occurred (Alison *et al*., 2000). In the dry savannah zones the sylvatic vector populations are too low or active for too short of a period to sustain an epizootic mainly human-to-human transmission occurs with the potential for epidemics (Domingo *et al.,* 2011). The virus may nonetheless be introduced into a cycle of inter-human transmission by *Ae. aegypti*, either if an epizootic extends from the humid savannah, or if infected individuals move to villages with the domestic vector in the dry savannah. If the virus is introduced into urban or very dry savannah regions where the human population stores water and lives in association with domestic *Ae. aegypti*, explosive outbreaks of *Ae. aegypti*-borne YF (urban-type transmission) may result (Monath and Cetron, 2002).

2.1.3 Dengue fever

"Dengue" is a Spanish word that means "fastidious or careful" and may refer to the gait of a person suffering from bone pain during dengue fever (Halstead, 2008). Dengue may also have its origin in the Swahli phrase "Kidenga pepo" that means "a sudden cramp like seizure caused by an evil spirit" (Guedes *et al*., 2010). It is an acute febrile disease caused by four closely related but antigenically distinct serotypes of dengue virus belonging to genus Flavivirus of the family *Flaviviridae.* There are four phylogenetically and

genetically distinct, but antigenically related serotypes classified as DENV-1, DENV-2, DENV-3 and DENV-4 (Mackenzie and Gubler, 2004).

2.1.3.1 Dengue virus structure

Dengue virion is a spherical particle with a diameter ranging between 50 nm and 60 nm diameter particles with a lipopolysaccharide envelope (Holmes and Twiddy, 2003). The dengue genome 11 kilo bases (kb) consisting of a single open reading frame that encodes the structural and none structural proteins include capsid (C), membrane (M), and envelope (E) (Mcbride and Bielefeldt-ohmann, 2000) while the non-structural proteins include NS1, NS2A, NS2B, NS3, NS4A, NS4B and NS5 (Halstead 2008). The viral particle consists of the RNA genome surrounded with C proteins and forms the inner core. The structural proteins E and M are surface proteins on the virion envelope and the conformations of these proteins are used to distinguish between immature and mature virus. The immature virus is referred to as “spiky” as M proteins bound to a precursor membrane protein (prM) form heterodimers with E proteins that appear as ‘spikes’ on the viral surfaces. In mature virions the soluble pr is cleaved from M protein by furin, anchoring the M proteins and causing the pr protein to be absent in the mature viral membrane (Pokidysheva and Zhang, 2006).

2.1.3.2 Dengue fever and dengue haemorrhagic fever

There are three types of dengue fever, namely classical dengue fever, dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS) (Gubler, 2004). The term “hemorrhagic fever” was first applied to illness in South-East Asia in the Philippines in 1953 (Sharp *et al*., 1995). Furthermore, dengue fever is seen in syndromes that are age-dependent, for which infants and children may have undifferentiated febrile illness or mild febrile disease with maculopapular rash (Mackenzie and Gluber, 2004). Older children and adults usually

have an overt illness characterized by fever, headache, myalgia, and gastrointestinal symptoms, often terminating with a macupapular rash (Gubler, 1998). Then the DHF or dengue shock syndrome proceeds through two stages. The illness begins with abrupt onset of fever accompanied by flu; during or shortly after the fall in temperature, the condition of the patient suddenly deteriorates, the skin becoming cold, the pulse rapid, and the patient becomes lethargic and restless (Guedes *et al*., 2010). In some children, the range of pulse pressure progressively narrows; the patient becomes hypotensive and if not treated, may die within four to six hours.

2.1.4 Rift Valley fever

Rift Valley fever (RVF) is a viral vector borne zoonotic disease caused by RVFV, a member of Phlebovirus genus in the family Bunyaviridae (Wilson *et al*., 2013). The Rift valley fever is zoonotic disease affects both animals and human and has been associated with epizootics and epidemics in different countries and occasions. Animals acquire disease through a bite of infected Aedes or Culex mosquito species. Transmission from animal to human is by direct contact with blood, body fluids or tissues of infected animal as well as the bite of infected mosquitoes. Currently there is no evidence of person to person transmission of RVFV (Flick and Bouloy, 2005).

2.1.4.1 Occurrence and clinical signs

The disease occurrences follow a trend of heavy rainfall leading to flooding, that provide environment for dormant eggs of Aedes and Culex mosquito species which are infected by RVFV to hatch. Then, they rapidly multiply and become prominent mosquitoes populations which results to transmission of virus to animals and from animals to humans (Wilson *et al*., 2013). The RVF disease largely affect domestic animals such as goats, sheep, cattle and camels and has been associated with abortion in pregnant animal and

high mortality in young animals (Wilson *et al*., 2013). Moreover, RVF virus has been demonstrated to affect a wide range among wild animals including buffalo, rhinoceros, kudu, impala, elephants, kongoni (Evans *et al.,* 2008). In human the RVFV causes influenza-like syndrome accompanied by fever and headache and occasionally leads to serious complications (Evans *et al.,* 2008). About 7-26 % of patients of RVF develop severe disease including haemorrhagic syndrome, encephalitis and death (Wilson *et al*., 2013). Also 5-20 % of RVF patients develop ocular complications including retinitis leading to scotomata and other visual disturbances (Wilson *et al*., 2013).

2.1.4.2 Distribution of Rift Valley fever

Since the isolation of Rift Valley fever virus in Naivasha, Kenya in 1931 the disease has remained endemic in many countries of Sub Saharan Africa with Social and environmental factors contributed to spread to other countries. The first episode of RVF outbreak outside Sub Saharan countries was reported in Egypt 1977 with 18,000 human cases and 600 deaths (Ahmad, 2000). By then, Tanzania had not reported Rift Valley fever cases until 1978, although epidemics were reported in neighbouring countries such as Kenya (1997/98), Malawi (1990) and Zambia (1993). The major outbreak of Rift Valley fever in Tanzania was detected among humans in January 2007 in Manyara region. In this outbreak, 309 human cases and 144 deaths were reported from twenty five districts of ten regions including Dodoma with socio-economic losses in communities (Swai and Schoonman, 2009).

The recurrence of RVF is now reported in many African countries, Kenya has reported ten episodes the current being in 2006, South Africa nine episodes the current ones in 2010, Egypt four episodes the current in 2003, Somalia four episodes the current in 2006 and Tanzania five episodes however the last two being well documented (Sindato *et al*., 2011)

Occurrence of large outbreaks of RVF in Egypt 1977, Kenya 1997-98 and other parts of Africa and outside Africa such as Saudi Arabia and in Yemen 2000 has increased the virological and entomological knowledge regarding the virus and vectors for RVF (Taylor *et al*.,2001).

2.1.4.3 Genome organization of Rift Valley Fever virus

RVF belong to Phlebovirus genus of the family *Bunyaviridae* (Sindato *et al*., 2011). RVFV genome consists of three negative senses, single-stranded RNA segments encoding structural and non-structural proteins (Wilson *et al*., 2013). The large segment (L) encodes the RNA-dependent RNA polymerase which is associated with the nucleocapsid protein (encoded by the small (S) segment) and genome segments to form ribonucleocapsids (Ahmad, 2000). The S-segment, which utilizes an ambisense coding strategy, additionally encodes a non-structural protein (NSs), which is the virulence factor of the virus that counteracts the host innate immune response. The medium (M) segment encodes two major envelope glycoproteins and two minor proteins, one of which is non-structural and may have anti-apoptotic properties (Wilson *et al*., 2013).

2.1.5 Chikungunya

Chikungunya virus (CHIKV) is an arthropod-borne virus that is transmitted by Aedes mosquitoes. It was first isolated in 1952 in the Makonde Plateau of the southern province of Tanzania (former Tanganyika) (Robinson, 1955). The word Chikungunya means bending in Makonde language, describing the clinical sign observed in patients due to musculoskeletal pains (Voss *et al*., 2010). The virus transmission cycle requires infection of female mosquitoes via a viremic blood meal taken from a susceptible vertebrate host and, following a suitable extrinsic incubation period, transmission to another vertebrate host during subsequent feeding (Solignat *et al*., 2009). After an incubation period, most

patients suffer from polyarthralgia and myalgia, with a significant impact on their quality of life (Powers *et al*., 2000). Chikungunya fever is characterised by a very high viremic load and concomitant abnormalities such as pronounced lymphopenia and moderate thrombocytopenia. The rate of asymptomatic cases is lower, and the percentage of infected patients requiring medical attention is higher, than in most other common arboviral infections. After the acute stage, some patients experienced relapse, persistent arthralgia or musculoskeletal pains (Enserink, 2007). Increase of age is the most obvious risk factor associated with severe disease or persistent symptoms in adults, whilst in pediatric populations; newborns have a higher risk of severe disease (Solignat *et al*., 2009).

2.1.5.1 Chikungunya virus

Chikungunya virus belongs to the Alphavirus genus of the family *Togaviridae* (Powers *et al*., 2000). CHIKV is a member virus of the Semliki Forest (Eurasia) virus antigenic complex, together with a number of other alphaviruses that are found in Africa (O'nyong–nyong virus (ONNV)), in South America (Mayaro virus) and in the Australia/Oceania region (Ross River virus (RRV)) that cause acute arthropathy in humans (Powers *et al*., 2000). The virion has an icosahedral capsid enclosed by a lipid envelope and a diameter of 60–70 nm. It is sensitive to desiccation and to temperatures greater than fifty eight degrees of Celsius (Powers and Logue, 2007).

2.1.5.2 Chikungunya virus structure and genomic organisation

The genome of Chikungunya virus is a single-stranded, positive sense, RNA molecule of 12 kb in length (Enserink, 2007). The genomic organisation is arranged as with others alphaviruses: 5'-nsP1-nsP2-nsP3-nsP4-junction region-C-E3-E2-6k-E1-poly (A)-3' with two open reading frames (ORFs). The 5' end of the genome has a 7-methylguanosine cap and there is a polyadenylation signal at the 3' end (Powers and Logue, 2007). The 5' ORF

is translated from genomic RNA and encodes four non-structural proteins (Powers *et al*., 2000). The 3' ORF is translated from a subgenomic 26 S RNA and encodes a polyprotein that is processed as the capsid protein (C), two surface envelope glycoproteins (E1 and E2) and two small peptides designated E3 and 6k (Voss *et al*., 2010).

The glycoproteins E1 and E2 are embedded in a heterodimeric form in the viral envelope and are responsible for virus attachment and membrane fusion (Voss *et al*., 2010). Virus fusion with the cell membrane is mediated by the E1 glycoprotein, a class II fusion proteins a process dependent on low-pH Acidic conditions induce a conformational change in the virus envelope proteins, dissociation of the E2-E1 heterodimers and formation of E1 homotrimers (Powers *et al*., 2000). The E1 trimmer is inserted into the target membrane via its hydrophobic fusion peptide and refolds to form a hairpin-like structure. Cholesterol is required for both cell membrane fusion and budding during Alphavirus infection (Solignat *et al*., 2009). A large number of more recent studies have been dedicated to the structural characterization of the envelope proteins of alphaviruses and to structural modifications that occur during fusion (Voss *et al*., 2010)

2.2 Seroprevalence of Mosquito-borne Viruses

Most populations in sub-Saharan Africa experience a great burden of disease due acute febrile illnesses (Campbell *et al.,* 2004). A greater percentage of these diseases are related to common diseases like malaria and typhoid fever, but recent studies have shown that they are misdiagnosed and a proportion of these illnesses could be caused by other pathogens not considered in the African medical diagnostic laboratories (Campbell *et al*., 2004). Detection of these viruses is hindered by poor diagnosis due to non-specific clinical presentation and limited surveillance that is being conducted in Africa (Moi *et al.,* 2010). In the 1980s, there was an increase of YFV cases in Africa (LaBeaud, 2008). CHIKV has

caused outbreaks from the 1960s to the 1980s and after 20 years, an outbreak was reported in the Congo in 2000 and subsequently in Eastern Africa and India in 2005-2006 (Pastorino *et al.,* 2004). Africa has also experienced outbreaks of dengue, Rift Valley, and O'nyong nyong diseases (Sang *et al.,* 2010).

There was a recent epidemic of CHIKV along the Indian Ocean Islands showing the ability of the virus to spread to new areas (Chretien *et al*., 2007).

2.3 Risk Factors Associated With Mosquito-borne Viral Infections

In recent decades there has been an increase in arbovirus outbreaks worldwide, especially in the sub-Saharan Africa. Arboviruses require blood sucking arthropods to spread from host to host (Alison *et al*., 2000). The worldwide increase in arbovirus activity is due to recent demographic or both changes in climate, human activities, epidemiology and viral genetics (Gubler, 2004). Majority of the recently recognized human viral diseases are zoonosis and due to the above changes, they switch the hosts and infect humans (Gubler, 2002). Some of the factors that cause sporadic outbreaks or clusters of human cases include environmental factors like human activities that enhance vector population densities (irrigation, heavy rains followed by floods, higher than normal temperatures, and formation of ecologic niches enabling the mass breeding of mosquitoes) allow the re-emergence of this mosquito-borne disease. For instance, global warming scenarios hypothesize warmer, more humid weather that may produce an increase in the distribution and abundance of mosquito vectors (Halstead, 2008). Other risk factors associated with arboviruses such as Crimean-Congo Hemorrhagic Fever (CCHVF) and RVFV include contact by trapping of animals, farming, slaughter, food preparation or veterinary work. Casual contacts for example neighbour keeping chickens or living in vicinity to a slaughterhouse are also risk factors. In addition, eating of raw fowl products or drinking

blood, butchering of sick animals, milking for pastoralists, handling of patients in acute phase of infection and tick or mosquito bites can expose one to infection (Gubler, 2002). Living in the river and lake basin areas also predisposes humans and animals to infection because of high vector abundance in the area.

2.4 Diagnosis of Mosquito-borne Viral Infections

Several methods can be used for diagnosis of mosquito-borne viruses from a variety of samples including vectors, wildlife, human and livestock. Serological tests, such as enzyme-linked immunosorbent assays (ELISA), may confirm the presence of Immunoglobulin M (IgM) and Immunoglobulin G (IgG) antibodies (Alison *et al*., 2000) specific to the virus in circulation. IgM antibody levels are highest within three to five weeks after the onset of illness and persist for about two months (Alison *et al*., 2000). The virus may be isolated from the blood during the first few days of infection through cell culture. Molecular methods such as reverse transcription-polymerase chain reaction (RT-PCR) may be used to detect the infecting viruses. Reverse transcription-polymerase chain reaction products from clinical samples may also be used for genotyping the virus, allowing comparisons across virus samples from various geographical sources (Kuno, 2000). Other methods that can be used for arboviruses diagnosis include hemagglutination-inhibition (HI) test used in qualitative and quantitative detection of IgG antibody, hemagglutination test used in the antigen titration and plaque reduction neutralization test (PRNT), a gold standard for virus diagnosis to rule out cross reactivity within a virus genus (Alison *et al*., 2000).

2.5 Distribution of Aedes as the Vector of Mosquito-borne Viruses

Generally *Ae. Aegypti* and *Ae. albopictus* distribution approximately corresponds to a winter isotherm of 10 °C (Gubler, 2004) and geographically it is distributed between

latitudes 35°N and 35°S. Although it has been reported from as far as 45°N, such invasions have occurred during warm weather, and the mosquitoes have not survived the cold season. Altitude is also a limiting factor for the distribution of *Ae. aegypti* and *Ae. albopictus* (Alison *et al*., 2000). Its distribution is normally restricted by an altitude of 1000 m as it has been reported at 2121 m in India, at 2200 m in Colombia, where the mean temperature is between 10 °C and 19 °C and at 2400 m in Eritrea (Alison *et al*., 2000).

This tropical mosquito is found in or around homes and breed in artificial containers like old automobile tires, buckets used for collection of rainwater, flower vases and trash in general. They also breed in containers used for water storage (drums) and even in septic tanks, thus breeding in large numbers near domesticated areas (Pokidysheva and Zhang, 2006). In fact, eggs of *Ae. Aegypti* and *Ae. Albopictus* are highly resistant to desiccation, which increases their chance of survival and is a cause of failure in its control and also they are an indoor resting mosquito that feeds on humans during the daytime (Gubler, 1998) and this biting activity of *Aedes* has two peaks, early morning for 2 to 3 hours after daybreak and in the afternoon for several hours before dark.

The female mosquito can act both as a biological and mechanical vector and can infect several people in a single blood meal due to its nervous feeding behavior known as disrupted feeding (Gubler, 1998). A slight movement can however disrupt the feeding process. The mosquito then returns to the same or a different person to continue feeding after some time therefore feeds on several people during a single blood meal. If this female mosquito takes a fresh viremic blood meal, (Clifton and Noriega, 2012), the proboscis is infected with dengue virus or other arboviruses and can transmit the virus even if the mosquito does not take blood meal thus acting as mechanical vector. Several members of the same family may become ill with dengue fever within a 24 to 36 hours

time frame suggesting that a single infective mosquito is responsible for their infection. These behaviors of Aedes make it an efficient epidemic vector of mosquito-borne viruses (Gubler, 1998).

2.5.1 Transmission of Mosquito-borne Viruses

In addition, there are two ways by which a mosquito can get infected. The first is by sucking blood from a mosquito-borne virus affected person in which the virus is transferred from human blood to the gut of the mosquito (Moi *et al.,* 2010). The normal incubation period of the virus in the mosquito is eight to ten days but also depends upon ambient temperature. In the gut wall the virus replicates resulting in infection of the salivary glands making the mosquito able to transmit the virus for life (Gubler, 2004). The second way of becoming infected is by vertical transmission in which the virus is transferred from a female mosquito to its offspring during egg transfer in oviduct and egg development (Gubler, 1998). It is also reported that the virus can be transferred from the male mosquito to its offspring through sperm during mating with an uninfected female (Domingo *et al.,* 2011). Vertical transmission is one of the strategies for mosquito-borne viruses for surviving adverse climatic conditions and is mainly observed in the summer months when mosquito-borne viral infections are low in the human population (Arunachalam *et al.,* 2008).

2.5.2 Mosquito Control

To reduce or prevent mosquito-borne viruses' transmission, environmental management and chemical control methods, including use of larvicides and adulticide space sprays, have been used with some effects (Gubler, 2004). Other methods of control are cleaning of stagnant water in containers that are near homes and public areas. Additionally, more effective vector control methods may provide additional reduction in viral transmission.

These include use of window curtains and water container covers treated with long-lasting insecticides, and control released larvicides that provide several months of application (Victor *et al.,* 2002).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Study Design

In the present study, an active surveillance was performed among patients presenting with fever in a hospital-based cross-sectional descriptive survey. Afterwards, a structured questionnaire was administered in order to collect information on socio-demographic and clinical characteristics of febrile patients. To augment the information about mosquito- borne viruses within sampling area, mosquitoes were included for screening the possible mosquito-borne viruses namely yellow fever, dengue, chikungunya and Rift Valley fever viruses.

3.2 Study Sites

The present study was carried out in Karagwe and Kyerwa districts of Kagera region. The two districts cover an area of 4 630 km^2 with 158 km^2 covered by water bodies. The districts border the Republic of Uganda in the North and the Republic of Rwanda in the West. In the South, they border the districts of Ngara and Biharamulo, while Muleba, Missenyi and Bukoba districts border these districts in the East. These two districts are characterized by mountain ranges, which are reported to have swampy valley bottoms and wetlands. The altitude ranges between 1500 - 1800 meters above sea level. The annual average temperature is 26 °C Rainfall distribution is bi-modal with peak rains between September and December and March and May. Most parts of Karagwe and Kyerwa districts receive average rainfall between 800 mm and 1000 mm annually. It has been reported by Tanzania National Bureau of Statistics (2013) that the population of Karagwe and Kyerwa districts was 653 046 people, according to the 2012 Tanzania National population and housing census. The dominant ethnic group are Nyambo, though a lot of

people are immigrants from neighbouring districts. There are also immigrants from neighbouring countries of Uganda, Rwanda, Democratic Republic of Congo and Burundi moving into Kagera region in search of pasture, agricultural land, labour, trade and settlement areas due to civil unrests in countries.

3.3 Study Population

The study population comprised all mosquitoes collected and persons (males and females) aged six months and above with a clinical case definition of acute febrile illness characterised by fever ≥ 38 °C upon arrival to the study health facilities.

3.4 Inclusion Criteria

All persons (males and females) aged six months and above, presenting to the clinic with high fever, who gave consent (≥ 18 years) or ascent (12 to 17 years) to participate in the study were included in the study. Consent was obtained from the parents or guardians for those children under 12 years of age. All minors were included in the study only if their parent/guardian provided consent and or if the minor assented to participate, if old enough to do so.

3.5 Exclusion Criteria

The patient was excluded from the study if he or she was less than 6 months of age or refused to give consent/assent for himself/herself or for the minors (aged less than twelve years) whose parents or guardians declined to give consent for their participation in the study. Children less than six months of age were excluded from the study because of the technical difficulties of drawing blood samples and immunological incompetence.

3.6 Sample Size

The sample size was estimated by the formula $n = Z^2SD^2/e^2$ for the unknown population as shown by Kothari (2004), where *n* = size of sample, *z* = standard variation at 95 % confidence level (1.96), SD = the standard deviation of population (0.35) and *e* = acceptable error (0.05).The required sample size per site was 188 ($n= (1.96)^2 (0.35)^2 / (0.05)^2$. Thus, a total of 1880 mosquitoes was the minimum required sample size for 10 sites where mosquitoes were collected. Human sampling was done at two sites and the minimum sample size was 376. The base sample size was further increased by 10 % to account for contingencies.

3.7 Questionnaire Administration

A structured questionnaire was administered to collect socio-demographic and clinical data. Upon recruitment of an eligible patient, the clinician documented the gender and age of the patient. In addition to that, the clinician asked questions on the current residence, duration of stay in the residence, travel history, occupation, contact and mode of contact with wild animals and/or mosquito bite frequencies, the reason for coming to hospital, clinical manifestations of the patient, signs of rashes and date of onset of disease. The clinician would then made a clinical diagnosis based on symptoms presented by the patient.

3.8 Sampling Procedure

3.8.1 Mosquito sampling

In this study, mosquitoes were trapped using a BG sentinel trap and battery powered aspirators (Bio gents Sentinel trap, Regensburg Germany). The indoor and outdoor mosquitoes were trapped early in the morning and dusk from different locations in Karagwe and Kyerwa districts. Mosquitoes were identified based on their morphology

using morphological identification keys with assistance from experienced entomologist. After identification mosquitoes were preserved in RNA later with exception of blood-fed mosquitoes which were placed on FTA cards (Sigma Aldrich, California, USA). Eventually, the mosquitoes were transported and maintained in a cold chain for laboratory analysis at Sokoine University of Agriculture in Morogoro.

3.9 Detection of Mosquito-borne Viruses in Aedes Mosquitoes

3.9.1 Pooling and Lysis of Aedes Mosquitoes

The *Ae. aegypti and Ae. albopictus* mosquitoes were collected from seven wards in Karagwe and Kyerwa districts, of which were used for the detection of Alphavirus, Bunyavirus and Flavivirus. Mosquitoes were pooled in groups of twenty two in which ten mosquitoes were pooled together in Eppendorf tubes. Thereafter, 300 µl of phosphate buffered saline (PBS) (Sigma Aldrich, california, USA) was added into each of Eppendorf tubes containing mosquitoes. Mosquitoes were afterwards crushed using a micropestal, followed by centrifugation at 10 000 rpm within three minutes to obtain cells-free supernatant.

3.9.2 RNA extraction

Ribonucleic acid was extracted from the homogenized lysates using QIAamp Viral RNA Kit (Qiagen, Hilden, Germany), according to the manufacturers' instructions. Briefly, 140 µl of mosquito lysate supernatant was put into an RNase-free Eppendorf tube followed with 560 µl of lysis buffer (AVL). The mixture was pulse vortexed for 15 seconds and incubated for ten minutes at room temperature (20 ^{o}C). Afterwards ethanol (98 %) was added followed by high speed centrifugation to pellet proteins. The supernatant was then transferred to QIAamp Mini Column. Furthermore 500 µl of washing buffer (AW1) was added in the QIAamp Mini Column and centrifuged at 8 000 rpm for one minute and the

tube containing filtrate was discarded. Then the QIAamp Mini Column was carefully opened and 500 µl of washing buffer (AW2) added and centrifuged at a full speed of 14 000 rpm for three minutes and accompanied with second repeat in a new two millilitre collection tube. Finally, 60 µl of elution buffer AVE equilibrated to room temperature was added into QIAamp Mini Column, incubated for one minute and centrifuged at 8000 rpm. The viral RNA in the eluate was stored at -20 °C and until cDNA synthesis.

3.9.3 The cDNA synthesis

Aliquots of extracted RNA were used for cDNA synthesis. RNA was converted into cDNA by using the Superscript III first strand synthesis system kit (Invitrogen, Carlsbad, and California, United States of America), containing random hexamers. Aliquot eight microlitre of RNA from each pool were transferred into PCR micro plates and RNA was heat denatured at 65 °C for five minutes. Plates were immediately incubated on melting ice to prevent renaturation of RNA.

Table 1: Mastermix for reverse transcription

Reagents	**Volume (µl)**
10x RT Buffer	2
25 Mm $MgCl_2$	4
0.1m DTT	2
RNase OUT	1
Superscript III RT	1
Total volume	10

Ten microliters of the mastermix were dispensed into each of the 96 wells of the PCR microplate. The plates were incubated at 25 °C for five minutes in order to maximize the annealing of the hexanucleotides to RNA targets. The plates were then incubated at 50 °C

for 50 minutes; 85 °C for five minutes and four degrees of Celsius hold temperature and finally the cDNAs were stored at -20 °C until PCR.

3.9.4 Viruses detection

Presence of viruses in the mosquitoes were detected by using VIR2052F/VIR2052 R, BCS82C/BCS332V and FU1/CFD2 primers designed from sequences of the NSP4, nucleocapsid protein and NS5 target gene respectively (Table 2). The Primer sequences were designed within the highly conserved regions of Alphavirus, Bunyavirus and Flavivirus and were found to amplify 138, 243 and 276 bp respectively. The samples which tested positive with genus primers were tested further with primers that target conserved genes in the specific viruses such as CHIKV, RVFV, YFV and DENV.

Table 2: Primers for selected mosquito-borne viruses

Virus	Gene/ protein target	Primer Name	Primer sequence 5'-> 3'	Position	Size(bp)
Dengue	Structural polyprotein	D1/D2	5'TCAATATGCTGAAACGCGCGAGAAACCG-3'	38-65	345
			5'TTGCACCAACAGTCAATGTCTTCAGGTTC-3'	455-483	345
Alphavirus	NSP4	VIR 2052 F/ VIR	5'-TGGCGCTATGATGAAATC TGGAATGTT-3'	6971-6997	138
		2052 R	5'TACGATGTTGTCGTCGCC GATGAA-3	7086-7109	138
Flavivirus	NS5	FU 1/ CFD 2	5'-TACAACATGATGGGAAAGAGA GAGAA-3')	9007-9032	276
			5'-GTGTCCCAGCCGGCGGTG TCATCAGC-3'	9308-9283	276
Bunyavirus	Nucleocapsid protein	BCS82C/ BCS332V	5'-ATGACTGAGTTGGAGTTT CATGATGTCGC-3'	86-114	243
			5'-TGTTCCTGTTGCCAGGAA AAT-3'	309-329	243
RVF	Glycoprotein M gene	RVF1/RVF2	5'-GACTACCAGTCAGCTCATTACC-3'	777-798	550
			5'-TGTGAACAATAGGCATTGG-3'	1309-1327	550
YF	polyprotein	CAG/YF	5'CGAGTTGCTAGGCAATAAACACATTTGGA-3'	43-71	1269
			5'-AATGCTCCCTTTCCCAAATA-3'	1293-1312	1269
CHIK	5'NTR	CHIK3F/CHIK3R	5'CACACGTAGCCTACCAGTTTC-3'	14-112	98
			5'GCTGTCAGCGTCTATGTCCAC-3'	14-112	98

Source: Ochieng *et al.,* 2013.

Table 3: Polymerase chain reaction mastermix

Reagents	Volume (µl)
PCR Mix	10
Forward primer	1
Reverse Primer	1
RNase free water	6
cDNA Template	2
Total volume	20

3.9.5 Analysis of PCR amplicons using agarose gel electrophoresis

The plastic gel trays were covered with masking tape and two 13-well combs placed in position and the tray placed on a flat surface. The agarose gel powder of 1.5 % was prepared by adding 5.25 g agarose to 350 ml 1xTAE (Serva Electrophoresis, Heidelberg, Germany) in a 500 ml conical flask. The solution was heated in a microwave until it become clear and transparent with no any traces of agarose particles. The Gel Red (Phenix Research Products, Candler, USA) was added to the solution and cooled under the tap water whilst rotating the conical flask. The semi-cooled solution at 50 $^{\circ}$C was added in the already prepared plastic casting gel tray and left for approximately 25 minutes. When set the combs were removed and placed in an electrophoresis tank containing 1xTAE buffer (Serva Electrophoresis, Heidelberg, Germany). Furthermore, PCR amplicons were loaded into the wells after mixed thoroughly with loading dye (Promega, Madison, USA) and the gel was run at 100 Volts for 40 minutes, (DNA is negative so goes toward the positive electrode). The PCR products were viewed under ultraviolet light (UV) using gel documentation system (EZ, Gel Doc, Bio Rad, USA) and a picture taken to compare the samples against a size standard to determine the size of targeted PCR amplicons.

3.10 Ethical Considerations

The study was approved by National Institute for Medical Research and National Health Research Ethics Review Committee, a Sub-Committee of the Medical Research Coordinating Committee, the National Institute for medical Research, Tanzania (Appendix 2). Consent was sought from all potential candidates and those meeting all inclusion criteria and willing to participate were recruited after signing a written informed consent. All information obtained in this study was kept confidential under lock and key in the principal investigators office. Questionnaire data and laboratory results were stored separately under unique study identity number without any biodata.

3.11 Data Management

Field data captured in the study questionnaires were stored securely and accessed only through a password. This data were linked to the laboratory results of the tests conducted on the mosquitoes and human samples. Hard copies of the results and data were kept in locked cupboards. No patient names were used. Unique ID numbers were used for sample identification in the laboratory. The ID numbers were printed in triplicates for the sample, questionnaire and consent form for easy sample traceability in case of a mix-up or incase of any patient follow-up required.

3.12 Data Analysis

The analysis of data was performed using Microsoft Office-Excel 2007 (Microsoft, USA) and Epi Info version 7.0.8.0 (CDC, Atlanta, USA). Data on proportions were compared using Chi Square. The factors considered included age of the patients, gender (female=2, male=1), contact with wild animals (no=2, yes=1), whether or not a subject had mosquito-bites (no=2, yes=1), and malarial status. Site was also controlled for by including site variable in the model. All tests were performed at five percent level of significance.

CHAPTER FOUR

4.0 RESULTS

4.1 Demographic and Clinical Characteristics of the Study Participants

In total, 400 febrile patients matching the inclusion criteria were involved in this study. The mean age for the patients was 28.76, the median age was 26 years and the range was one to 94 years. The results indicated that most of the subjects were in the age group between 20-29 years (25.25 %), followed by those aged 10-19 years (23.25 %), and only 13.15 % were at least 50 years old. On the other hand, the gender proportionality was found to be 248 (62 %) females and 152 (38 %) males.

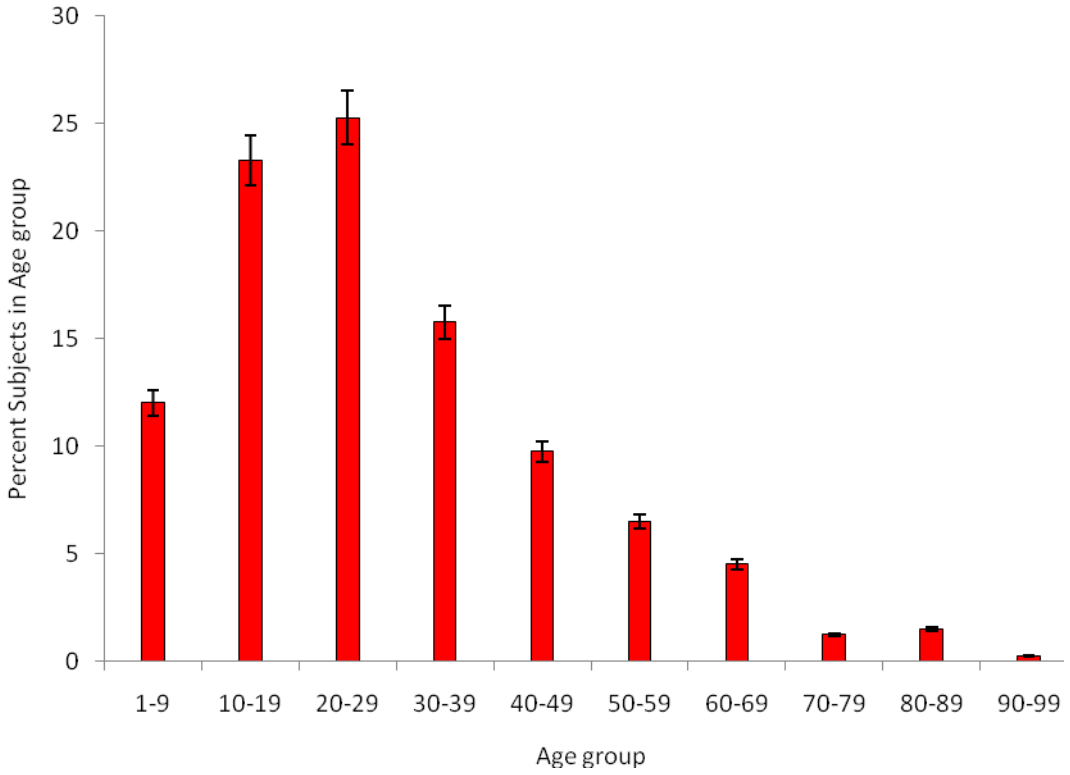

Figure 1: Age distribution of febrile study participants. Patients were enrolled at Nyakahanga and Kayanga hospitals in Karagwe and Kyerwa districts. Most of study participants were younger.

The febrile study participant's clinical temperatures ranged between 38 °C and 42 °C with an average of 39.04 °C. Clinical characteristics of the subjects are summarized in Table 3.The most common symptom reported among febrile patients enrolled included headache (68.75 %), followed with joint aches (67.75 %), seizures (63.50 %), Vomiting (61.50 %), leg weaknesses (59.50 %), laboured breathing (58.50 %) and the least observed symptoms were abdominal pains (41.75 %), neck stiffness (33.75 %), and rashes (33 %). Furthermore, only 28.75 % of febrile patients had malaria. The malaria status was investigated through malaria rapid diagnostic test.

Table 4: Clinical characteristics of febrile study participants in Karagwe and Kyerwa districts of Tanzania

Clinical Symptom	**Percentage Subject**		
	YES	**NO**	**UNKNOWN**
Fever	100.00	0.00	0.00
Headache	68.75	27.25	4.00
Nausea	55.25	39.75	5.00
Vomiting	61.50	38.50	0.00
Joint ache	67.75	31.75	0.50
Stiff neck	33.75	58.50	7.75
Seizures	63.50	35.75	0.75
Leg weaknesses	59.50	33.75	6.75
Rash	33.00	67.00	0.00
Abdominal pains	41.75	58.25	0.00
Pallor	54.25	45.75	0.00
Jaundice	56.50	43.50	0.00
Laboured breathing	58.50	41.50	0.00

4.2 Mosquito Abundance and Biting Propensity

4.2.1 Percentage abundance of collected mosquitoes

A total of 2137 mosquitoes were collected including 37.54 % *Culex quinquefasciatus*, 22.09 % *Mimomyia Spp*, 18.86 % *Anopheles gambiae,* 11.22 % *Culex pipiens*, 8.66 % *Ae.*

aegypti, and 1.6 % *Ae. Albopictus* (Fig. 3). It was estimated that *Aedes aegypti* abundance was relatively higher than *Aedes albopictus* by 84.10 %. This may suggest competition displacement among two species of Aedes mosquitoes.

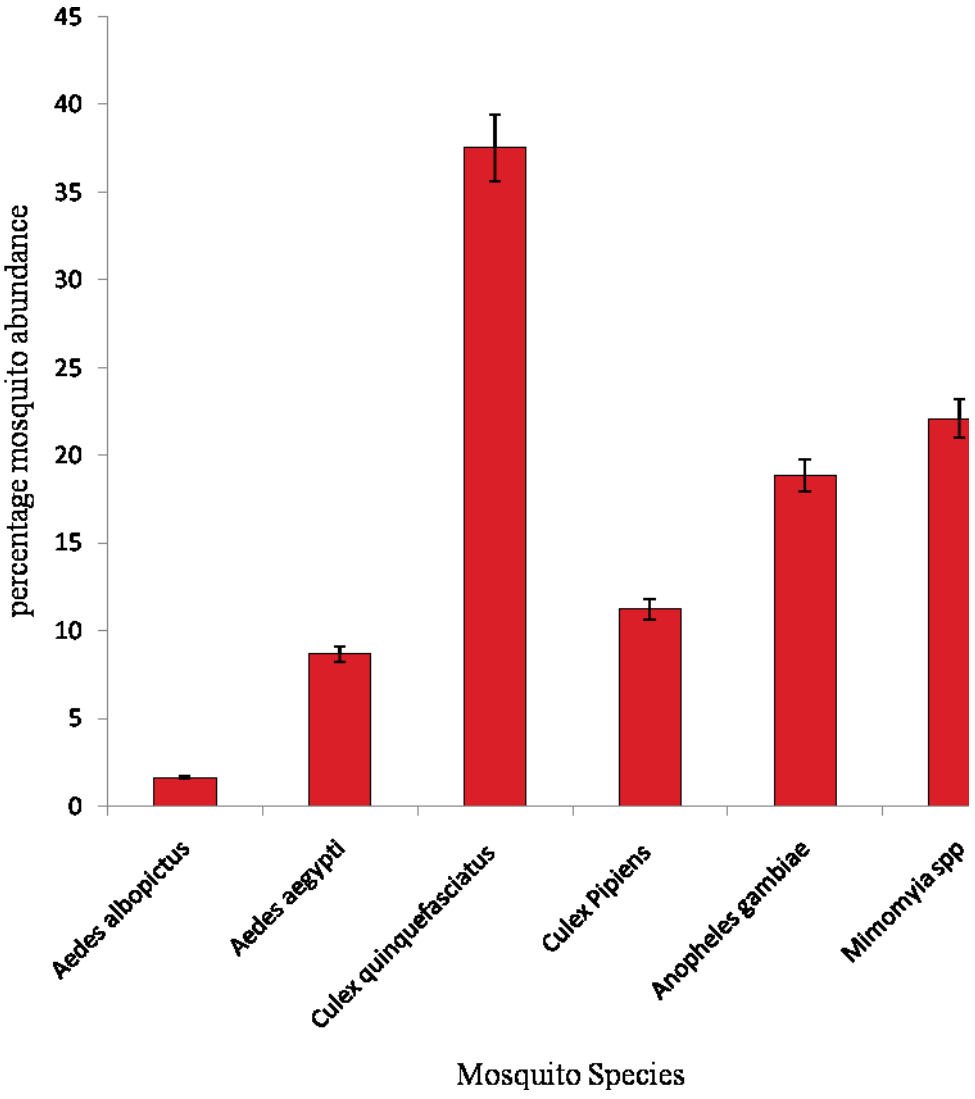

Figure 2: Mosquito species trapped in different sites in Karagwe and Kyerwa districts. A large number of mosquitoes were *Culex quinquefasciatus*. The least observed mosquitoes were *Ae. Albopictus*, this account for displacement among Aedes species.

4.2.2 Indoor and outdoor aedes mosquito

Mosquito breeding sites included old tyres, ornamental plant containers, pots, and vases. The outdoor water storage containers and vegetation formed another category of habitat including tree holes, leaves axils, flower bracts and fallen leaves. The first category of old tyres included all obsolete tyres that shown high density of Aedes mosquitoes sampled. Furthermore, the results indicated high percent of outdoor Aedes mosquitoes (77 %) compared to indoor Aedes mosquitoes (23 %).This accounts all outdoor potential breeding sites that favoured Aedes mosquitoes.

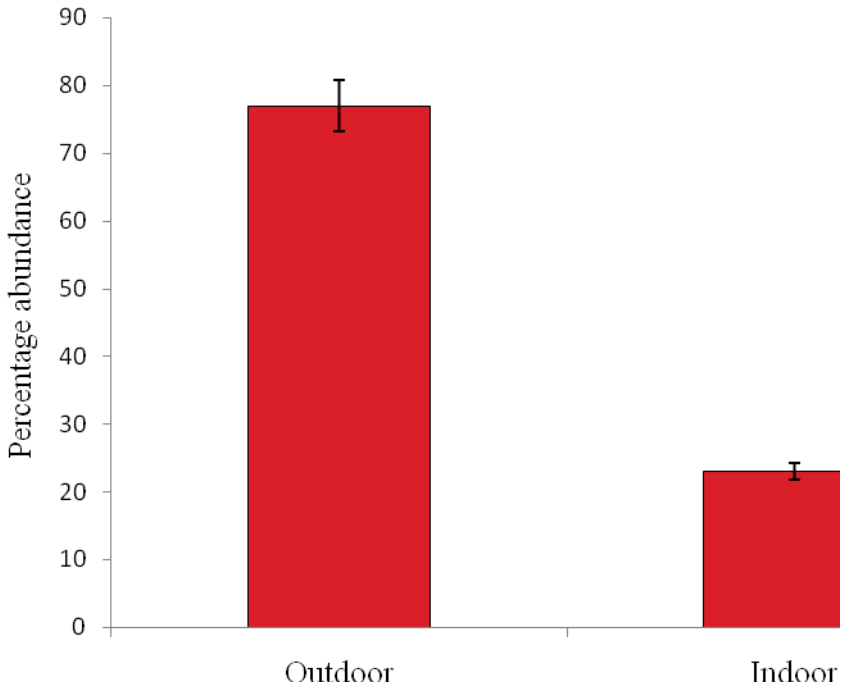

Figure 3: Percentage abundance of outdoor and indoor Aede*s* mosquitoes. The highest density of outdoor Aedes mosquitoes was collected around heap of old tyres.

4.2.3 Mosquito Biting Propensity

The study shows 51 (12.75 %) of patients had been in contact with wild animals, they had been in forest at most two months, 316 (79 %) they had been bitten by day biting

mosquitoes at most three months prior to sampling days. There were higher burden of febrile illness in humans and high frequency of day biting mosquitoes' propensity (79 %).

4.3 Detection of alphavirus, flavivirus and bunyavirus in mosquitoes by RT-PCR

The study went further to detect these specific mosquito-borne viruses, namely DENV, YFV, RVFV and CHIKV. It was found that most of mosquito pools were positive at genus level. After analysis the sample with virus specific primers, Chikungunya virus was positive in *Ae. aegypti* mosquito pools (Table 5).

Table 5: A total of 22 Aedes mosquito pools test positive and negative of Alphavirus, Flavivirus and Bunyavirus in Aedes mosquitoes by RT-PCR.

Mosquito pool	Alphavirus	Bunyavirus	Flavivirus	CHIKV	RVFV	DENV	YFV
1	-	-	-	-	-	-	-
2	-	-	+	-	-	-	-
3	-	-	+	-	-	-	-
4	-	-	+	-	-	-	-
5	+	-	+	+	-	-	-
6	+	-	+	-	-	-	-
7	+	-	+	-	-	-	-
8	+	-	-	-	-	-	-
9	+	-	+	+	-	-	-
10	+	-	+	+	-	-	-
11	-	-	-	-	-	-	-
12	-	+	-	-	-	-	-
13	-	-	-	-	-	-	-
14	-	-	+	-	-	-	-
15	-	+	+	-	-	-	-
16	-	-	+	-	-	-	-
17	-	-	+	-	-	-	-
18	+	-	+	-	-	-	-
19	+	-	+	+	-	-	-
20	-	-	+	-	-	-	-
21	+	-	+	-	-	-	-
22	+	-	-	-	-	-	-

The presence of Alphavirus, Bunyavirus and flavivirus in the mosquitoes were detected using VIR2052F/VIR2052R, BCS82C/BCS332V and FU1/CFD2 primers designed from sequences of the NSP4, nucleocapsid protein and NS5 target gene respectively. A reverse transcription polymerase chain reaction showed positive and negative amplifications on agarose gel electrophoresis as summarized in table five

CHAPTER FIVE

5.0 DISCUSSION

Mosquito-borne viruses are among the most important emerging and re-emerging infectious diseases facing the world and threatening public health. They are spread primarily by blood-sucking insects, like mosquitoes, ticks and sandflies (Gubler, 2002). Most mosquito-borne viruses that are of public health importance have been classified into three families namely; *Flaviviridae; Togaviridae and Bunyaviridae* (Gubler, 1998).

Transmission of disease is successful based on availability of reservoirs, amplifiers, susceptible vertebrate hosts. Furthermore, virus ability to develop high titre viremia for adequate duration of time to infect large number of mosquitoes that will then infect available susceptible hosts (Gubler, 2002). In addition, the changes in climate, ecology, demographics, land use patterns and increasing global travel have been linked to an upsurge in mosquito-borne viral disease. It is well known that there are no effective vaccines and therapeutic treatment for most mosquito-borne viral infections underscoring the need for active surveillance to monitor circulation in order to inform public health decision for early warning and response (Scott and Vasilakis, 2009). The present study investigated (i) socio-demographic and clinical characteristics among febrile patients attending district hospitals (ii) selected mosquito-borne viruses (iii) mosquito diversity and ecology in Karagwe and Kyerwa districts.

In this study, adult Culicine (Aedes and Culex) mosquitoes were mostly found around water bodies that were organically polluted by domestic waste. Similarly, Sattler (2005) and his research team in their study of habitat characterization and spatial distribution of

Anopheles species mosquito larvae in Dar es Salam, found out that mosquitoes of the Culicine group were abundantly breeding in organically polluted habitats.

In this study, the occurrence of large numbers of these mosquito species, especially Aedes and the Culex complex, is of public health significance due to their close proximity to humans. The reported past records of yellow fever, Rift Valley fever and more recently dengue fever in Tanzania is in any case associated to the presence of these mosquitoes (Ochieng *et al*., 2013). A considerable number of vector mosquitoes (Aedes and Culex genera) were collected from the vegetation habitats especially from banana and colocasia plants. The growth of vegetation cover provides shade for the oviposition and development of aquatic stages of mosquitoes. Moreover, adult mosquitoes also need shaded environment for biting and breeding purposes. These were similar factors considered more importance for control of potential mosquitoes responsible for mosquito-borne viral diseases (Gubler, 2002). However, the work in this study was conducted in part to identify potential mosquitoes for transmission of selected mosquito-borne viruses' genera. It was found out that were more positive amplifications of selected genera in Flavivirus, Bunyavirus and Alphavirus. There was only little positive amplification of observed using specific primers for specific viruses (Table 5).

Despite the bands amplifications by RT-PCR for specific virus the targeted bands were not obtained for DENV, YFV and RVFV. However, it was found that the positive amplifications for targeted Chikungunya virus 5'NTR in the Alphavirus genus was successfully noted. Chikungunya virus 5'NTR amplicons were similar to the study on mosquito-borne arboviral surveillance at selected sites in diverse ecological zones of Kenya (Lwande *et al*., 2012).

Moreover, socio-demographic and clinical characteristics of the febrile patients were found that fever was common among febrile illness patients and a higher burden of fever between patients aged 20-29 years. A similar result was documented in the study of changing epidemiology of yellow fever and dengue (Gubler, 2004). The middle age group 20-29 years was at a significantly higher burden febrile illness compared to the old age group (40+). This is an interesting finding because other studies have shown an increase in infection with age (Lwande *et al.,* 2012). This finding needs further investigation of the risk factors associated with infection among the younger age group not investigated in this study. This observation can, however, be attributed to the middle aged persons being most active in the society at school, colleges or engaged in formal and informal occupational activities such as grazing cattle, goats and sheep, hunting wild animals, fetching water and fishing. Therefore this study has shown that there were losses of manpower in the developmental programs for a reason that the highest percentage age groups in community working classes were presented with febrile illnesses and most of them being females (62 %).

CHAPTER SIX

6.0 CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

This study pointed out indispensable socio-demographic and clinical characteristics associated febrile illness such as age, fever, malarial status, contact with potential mosquitoes and wild animals. These parameters should be put into consideration when treating patients presenting febrile illness for proper diagnosis. This study confirms the occurrence of circulating Alphavirus, flavivirus and Bunyavirus in Aedes mosquito populations that may pose a serious public health concern in the study area. This study determined that age, site and contact with mosquitoes were risk factors for mosquito-borne viral infections.

6.2 Recommendations

For appropriate and concise diagnosis of infections among febrile patients. The medical clinicians should use clear case definitions based on the most common socio- demographic and clinical characteristics documented by this study while attending to patients presenting to the health facilities. These socio-demographic and clinical characteristics documented are high fever, headache, joint ache, contact pattern, residence, bleeding and travel history. This is supported with findings of this study that 100 % of patients sampled had fever and only 28.75 % had Malaria which makes 71.25 % of patients with fever of unknown origin. Nevertheless hospitals should be vested well and staff empowered to be able to deal with these infections. Infection control measures targeting the significant risk factors should be put in place to alleviate the burden of disease in these areas. The community should also be educated on the risks factors associated with these infections and possible prevention and control measures should be employed to stop transmission. Disease control and prevention strategies should be virus and site specific for effectiveness.

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APPENDICES

Appendix 1: List of socio-demographic and clinical characteristics of febrile illness patients in Karagwe and Kyerwa district

Patient ID.	Age	Sex	Headache	Nausea	Vomit	Jointache	Rash	Temp°C	MDT	Animal Contact	Site
NY001	6	2	1	1	1	1	1	39	Neg	2	Akishaka
NY002	38	2	1	1	1	1	1	40	Neg	2	Kafuro
NY003	80	1	1	1	1	1	2	41	Neg	2	Kibona
NY004	14	1	1	1	1	1	1	40	Pos	2	Chabalisa
NY005	4	2	1	3	1	1	1	39	Neg	2	Kayanga
NY006	6	1	1	3	1	2	2	39	Neg	2	O' shaka
NY007	23	2	1	1	2	1	1	38	Pos	1	Katanda
NY008	4	1	1	2	1	1	1	40	Pos	2	Rulalo
NY009	2	1	3	2	1	1	1	39	Pos	2	Rukole
NY0010	2	1	2	2	2	2	1	37	Neg	2	Irundu
NY0011	1	1	2	2	1	2	1	38	Neg	2	Rwabwere
NY0012	2	1	1	2	2	1	1	37	Neg	2	O' shaka
NY0013	1	2	3	2	1	1	1	39	Neg	2	Ruzinga
NY0014	5	2	1	2	2	2	2	38	Neg	2	Rukole
NY0015	18	1	2	1	2	2	2	41	Neg	2	Rwabwere
NY0016	7	2	1	2	2	2	2	39	Neg	2	Kijumbura
NY0017	42	2	1	1	1	1	2	37	Pos	2	Rwabwere
NY0018	1	1	3	3	1	1	2	39	Pos	2	Itera
NY0019	19	1	1	1	1	1	2	40	Neg	2	Miti
NY0020	20	2	1	1	1	1	2	38	Neg	2	Mato
NY0021	22	2	1	1	1	1	2	39	Neg	2	Cherunga
NY0022	19	2	2	1	1	1	1	41	Neg	2	Kashanda
NY0023	25	2	1	1	1	1	1	38	Neg	2	Rwambale
NY0024	14	1	2	2	2	2	2	40	Neg	2	Runyinya
NY0025	31	1	1	2	2	1	1	38	Neg	1	Rugera
NY0026	4	2	2	2	2	1	2	39	Neg	2	Kayanga
NY0027	10	1	2	2	2	2	2	38	Pos	2	O' shaka
NY0028	34	1	2	2	2	1	2	39	Neg	2	Rubya
NY0029	9	2	1	2	2	2	1	40	Neg	2	O' shaka
NY0030	14	2	2	2	2	2	1	38	Neg	2	Bukangara
NY0031	65	2	2	2	1	1	1	38	Pos	2	Kasheshe
NY0032	41	1	1	1	1	1	1	39	Neg	2	Bushangaro
NY0033	26	2	1	1	1	1	2	39	Neg	1	Chonyonyo
NY0034	29	2	1	1	2	2	2	40	Neg	2	Bisheshe
NY0035	29	1	1	1	1	1	1	41	Neg	2	Kamuli

NY0036	33	2	1	1	1	1	2	40	Neg	2	Bugene
NY0037	6	1	2	2	2	2	2	40	Neg	2	Kiruruma
NY0038	35	2	1	2	1	2	2	40	Neg	2	Nyabweziga
NY0039	30	1	1	1	1	1	2	40	Neg	2	Rukole
NY0040	34	2	1	1	1	1	2	40	Neg	2	Rwamgurusi
NY0041	43	2	1	1	1	2	1	38	Neg	2	Nyabwegila
NY0042	60	1	1	1	1	1	2	41	Neg	2	Bugene
NY0043	51	2	1	2	2	2	2	40	Neg	1	Nyakahanga
NY0044	65	2	1	1	1	1	1	40	Neg	2	Itera
NY0045	28	1	1	1	1	1	2	38	Neg	2	Nyakasimbi
NY0046	27	1	1	1	1	1	2	40	Pos	2	Ihanda
NY0047	25	1	1	2	1	1	2	39	Pos	2	Nyabwegila
NY0048	67	1	2	1	2	2	2	39	Neg	2	Cherunga
NY0049	16	1	3	3	2	2	2	40	Pos	2	Bweranyange
NY0050	27	2	2	2	2	1	2	38	Neg	2	Rwambale
NY0051	48	1	3	3	2	2	2	39	Neg	2	RUKAKA
NY0052	26	1	1	1	1	1	1	38	Neg	2	Bugene
NY0053	18	2	1	1	1	1	2	38	Neg	2	Chamchuzi
NY0054	17	1	1	1	1	1	2	40	Pos	2	Bisheshe
NY0055	7	2	1		1	1	1	39	Neg	2	Kikukuru
NY0056	51	1	1	1	1	1	2	41	Pos	2	Kijumbura
NY0057	29	1	1	1	1	2	2	40	Pos	2	Kayungu
NY0058	57	2	1	2	1	2	2	39	Neg	2	Nkwenda
NY0059	31	1	1	2	2	2	1	39	Neg	2	Kibondo
NY0060	28	1	2	1	2	1	1	38	Neg	1	Kibona
NY0061	16	2	1	1	2	2	1	37	Neg	2	Kafunjo
NY0062	9	2	1	2	1	1	2	38	Neg	2	Nyakahanga
NY0063	12	1	2	1	1	1	1	39	Neg	1	Rubale
NY0064	30	1	1	2	1	1	2	41	Pos	2	O' shaka
NY0065	23	2	2	2	2	1	2	40	Neg	2	Nyakahanga
NY0066	29	1	1	1	1	2	1	39	Neg	2	Mirambi
NY0067	32	1	1	1	1	1	2	38	Neg	2	Omukagando
NY0068	45	2	1	1	1	1	1	39	Pos	2	O' shaka
NY0069	28	2	1	2	2	1	2	39	Neg	2	Ihembe
NY0070	65	2	1	1	1	2	2	41	Neg	2	Rukuraijo
NY0071	5	1	1	2	2	1	2	39	Neg	2	Nyaishozi
NY0072	57	2	2	1	1	2	2	40	Neg	2	Ilala
NY0073	42	2	2	2	2	1	2	40	Neg	2	Chonyonyo
NY0074	27	2	1	1	1	1	1	39	Neg	2	Bujuruga
NY0075	61	2	1	1	1	1	2	41	Pos	2	Rugarama
NY0076	49	2	1	1	1	1	1	39	Neg	2	Kihanga
NY0077	6	1	2	2	1	2	2	40	Neg	2	Kalehe
NY0078	33	2	2	2	1	1	1	37	Neg	2	Rukole

NY0079	29	2	2	2	2	1	1	40	Neg	2	Misha
NY0080	52	1	1	2	1	1	1	39	Neg	2	Kamagambo
NY0081	9	2	1	2	1	2	1	38	Neg	2	Nyakahanga
NY0082	20	2	1	2	1	2	1	41	Pos	1	Ruzinga
NY0083	41	1	2	1	2	1	2	37	Neg	1	O' shaka
NY0084	35	2	1	1	1	2	1	37	Neg	2	Kashebe
NY0085	28	1	1	2	2	2	2	38	Neg	2	Kijumbura
NY0086	7	1	2	2	2	1	1	39	Neg	1	Chanyangabwa
NY0087	27	1	1	1	1	2	2	39	Neg	2	Kamagambo
NY0088	3	1	2	2	2	1	2	41	Neg	2	Omururama
NY0089	10	2	1	1	2	2	2	38	Neg	2	O'shaka
NY0090	7	2	1	1	2	2	1	39	Neg	2	O' shaka
NY0091	9	1	2	2	2	1	2	39	Neg	1	Kashebe
NY0092	25	1	2	2	2	1	1	41	Neg	2	Katembe
NY0093	17	2	1	2	1	2	1	38	Neg	2	Omkibinda
NY0094	18	2	2	2	1	2	2	38	Neg	2	Kamagambo
NY0095	23	2	1	1	1	1	2	39	Neg	2	Kaisho
NY0096	17	2	2	1	1	2	2	38	Pos	1	Bugene
NY0097	33	2	2	2	2	2	1	39	Neg	2	Ihanda
NY0098	24	1	1	1	2	1	2	38	Neg	2	Kagutu
NY0099	27	1	1	1	1	1	2	40	Neg	2	Lukare
NY00100	2	1	1	2	1	1	2	39	Neg	2	Omrusimbi
NY00101	20	2	1	1	1	1	2	37	Neg	2	Mabira
NY00102	32	2	1	1	1	1	2	39	Neg	2	Bugomora
NY00103	14	1	1	1	1	1	1	41	Pos	2	Ihanda
NY00104	28	2	1	1	1	1	2	40	Neg	1	Chonyonyo
NY00105	20	2	2	1	1	1	2	41	Pos	2	Itera
NY00106	36	1	1	1	1	1	2	38	Neg	2	Kayanga
NY00107	18	2	1	3	2	1	2	38	Neg	2	Kitega
NY00108	43	2	2	1	2	1	2	40	Neg	2	O'shaka
NY00109	61	2	2	1	1	1	1	39	Neg	2	Rwabwere
NY00110	43	2	1	2	2	1	2	38	Neg	2	Chanya
NY00111	56	2	1	1	1	1	2	38	Neg	2	Kitwechenkura
NY00112	22	2	1	1	1	1	1	37	Neg	2	Kiruruma
NY00113	63	2	1	1	1	1	2	38	Neg	2	Omururama
NY00114	28	2	1	1	1	1	2	39	Neg	2	Kamagambo
NY00115	50	2	1	2	2	2	1	37	Neg	2	Kayanga
NY00116	42	1	1	1	1	2	1	41	Pos	2	Ihanda
NY00117	45	2	1	2	2	2	2	38	Neg	2	Nyabwegira
NY00118	15	1	1	1	1	1	2	39	Neg	2	Bugene
NY00119	16	1	1	2	2	2	1	38	Neg	2	Bugene
NY00120	31	2	1	1	1	1	2	38	Neg	2	Kakerere
NY00121	33	2	1	1	2	2	2	41	Neg	2	Nyakatuntu

NY00122	12	1	2	1	1	1	1	39	Pos	2	Ihanda
NY00123	39	2	1	1	1	2	1	38	Neg	2	Nyakahanga
NY00124	18	2	1	2	2	1	2	40	Pos	2	Kalehe
NY00125	5	1	2	1	1	1	1	40	Pos	2	Kanongo
NY00126	41	2	1	1	2	1	1	38	Neg	2	Mkombozi
NY00127	12	1	2	1	2	2	2	38	Neg	2	Kibogoizi
NY00128	22	2	2	1	1	2	1	41	Neg	1	Omurusimbi
NY00129	32	1	1	1	1	2	2	39	Pos	2	O'shaka
NY00130	37	1	1	2	1	1	1	41	Neg	2	Nyabishenge
NY00131	50	2	1	2	2	2	2	39	Pos	1	Songambele
NY00132	55	1	1	1	1	1	1	41	Neg	2	Kayanga
NY00133	21	2	1	1	1	1	2	38	Neg	2	Rulalo
NY00134	16	1	1	2	2	1	2	39	Neg	2	Nyakahanga
NY00135	36	2	2	2	2	1	2	41	Neg	1	Kitwe
NY00136	67	2	1	1	1	1	2	39	Pos	1	Chanya
NY00137	30	2	1	1	1	1	2	38	Neg	2	Chonyonyo
NY00138	31	1	1	1	1	1	2	38	Neg	2	Chakalisa
NY00139	8	1	1	1	1	2	1	39	Pos	2	Kamuli
NY00140	34	2	1	1	1	2	2	40	Neg	1	Ihanda
NY00141	61	1	2	2	2	1	2	38	Neg	2	Ihanda
NY00142	31	2	2	2	2	2	1	39	Neg	2	Bugene
NY00143	1	2	1	1	2	2	2	38	Neg	2	Kitwe
NY00144	12	2	1	1	2	1	1	40	Neg	2	Ruzinga
NY00145	30	2	1	1	1	1	2	40	Pos	2	Irundu
NY00146	16	1	1	1	2	3	1	38	Neg	1	Bugene
NY00147	17	2	1	2	2	2	1	37	Neg	2	Ihanda
NY00148	18	1	1	1	1	1	1	36	Neg	1	Nyakaiga
NY00149	70	1	1	1	1	1	2	41	Neg	2	Chonyonyo
NY00150	12	1	1	2	1	2	1	37	Neg	1	Nyakahanga
NY00151	24	2	1	2	1	1	2	40	Neg	1	Bweranyange
NY00152	7	2	1	2	2	1	2	38	Neg	2	Kishao
NY00153	14	1	1	1	2	2	1	38	Neg	1	Kishao
NY00154	21	2	2	2	2	1	1	41	Neg	2	Omchikoma
NY00155	59	1	2	2	1	2	2	38	Neg	2	Nyakashenyi
NY00156	24	2	1	2	1	2	2	39	Neg	2	Nyakahanga
NY00157	22	2	2	2	1	1	2	39	Pos	2	O'shaka
NY00158	33	2	1	1	1	2	1	38	Neg	2	Kishojo
NY00159	35	2	2	1	1	2	1	39	Pos	1	Kinondoni
NY00160	12	1	2	1	1	1	2	37	Neg	2	Ihanda
NY00161	28	2	2	1	1	2	2	39	Neg	2	Nkwenda
NY00162	29	1	1	1	1	2	2	38	Pos	2	Chamarangi
NY00163	31	1	1	1	1	1	2	40	Neg	1	Nyakahanga
NY00164	47	1	1	1	1	2	2	40	Neg	2	Omkasambya

NY00165	23	1	1	1	1	1	1	40	Neg	2	Kakanja
NY00166	37	1	2	2	2	2	2	41	Neg	2	Kijumbura
NY00167	21	2	1	1	1	1	2	38	Neg	2	Rwere
NY00168	21	1	2	2	2	2	2	40	Neg	2	Kimuli
NY00169	13	1	1	1	1	1	2	40	Pos	2	O'shaka
NY00170	58	2	2	2	2	1	1	38	Neg	2	Kishao
NY00171	61	2	2	2	2	2	2	37	Pos	2	Ihanda
NY00172	26	2	1	2	2	2	2	37	Neg	2	Makazi
NY00173	40	2	1	1	1	1	2	39	Neg	2	Kayanga
NY00174	87	1	1	1	1	1	2	41	Pos	2	Kitwechenkura
NY00175	19	2	2	2	1	1	2	40	Pos	2	Kyerwa
NY00176	32	2	1	1	1	1	2	40	Pos	2	Chanya
NY00177	20	2	1	1	1	1	1	39	Pos	2	Mato
NY00178	12	1	2	2	2	2	2	39	Pos	1	Kihanga
NY00179	35	2	1	2	2	1	2	41	Neg	2	Rwabwere
NY00180	22	2	2	2	2	1	1	37	Neg	2	Chamchuzi
NY00181	1	2	1	2	1	1	2	36	Pos	2	Chonyonyo
NY00182	94	2	2	2	2	1	2	41	Neg	2	Katwe
NY00183	1	1	1	1	1	1	1	38	Pos	2	Rukili
NY00184	1	1	2	2	2	1	1	38	Pos	2	Rukili
NY00185	21	1	1	1	1	1	2	39	Neg	2	Rugera
NY00186	23	1	1	1	1	1	2	37	Neg	1	Rugarama
NY00187	26	1	1	1	1	1	2	38	Pos	2	Amtongole
NY00188	19	1	1	1	1	1	2	39	Pos	2	Karo
NY00189	38	1	1	1	1	1	1	39	Neg	2	Chonyonyo
NY00190	32	2	1	1	2	1	2	37	Neg	2	Bugene
NY00191	72	1	1	1	1	1	2	38	Neg	2	Kafuro
NY00192	24	1	1	1	1	2	2	39	Neg	2	Rukole
NY00193	3	1	2	2	2	1	1	38	Neg	2	Kayanga
NY00194	36	2	2	3	2	2	2	41	Neg	2	Chabuhora
NY00195	29	2	1	3	2	2	1	39	Neg	2	Kayanga
NY00196	42	2	3	2	1	2	1	37	Pos	2	Nkwenda
NY00197	18	2	2	3	2	2	2	37	Neg	2	Itera
NY00198	61	2	2	1	2	2	1	38	Neg	1	Kanoni
NY00199	32	1	1	1	2	2	2	41	Pos	2	Kayanga
NY00200	18	2	3	2	1	1	1	39	Neg	2	Rwabigaga
K001	18	2	3	1	1	2	2	36	Neg	2	Ndama
K002	6	2	1	2	2	1	1	39	Neg	2	Cherunga
K003	18	1	1	1	1	1	2	38	Neg	2	Ndama
K004	3	2	1	2	2	1	2	39	Neg	2	Ndama
K005	39	1	1	1	1	1	2	40	Pos	2	Kayanga
K006	82	1	1	1	1	1	1	39	Neg	1	Runyaga
K007	36	2	2	2	1	1	1	38	Neg	2	Kishao

K008	15	2	1	1	2	2	2	39	Neg	2	Kafunjo
K009	39	2	1	1	1	1	2	40	Neg	1	Kayanga
K0010	73	1	2	1	1	2	2	41	Pos	2	Kihanga
K0011	24	2	1	1	2	1	1	40	Neg	2	Kayanga
K0012	20	2	1	2	2	2	2	40	Neg	1	Kayanga
K0013	36	2	1	2	1	1	2	38	Neg	2	Kibweera
K0014	41	2	1	1	1	1	2	39	Neg	2	Kishojo
K0015	45	2	2	1	1	1	2	38	Neg	2	Kayanga
K0016	29	1	1	1	1	1	2	38	Neg	2	Kayanga
K0017	27	2	1	2	2	1	2	38	Neg	2	Chanika
K0018	49	2	2	2	1	1	2	38	Neg	2	Kayanga
K0019	43	2	1	2	2	1	1	38	Neg	1	Katanda
K0020	49	2	3	1	1	1	2	40	Pos	2	Kayanga
K0021	67	2	1	1	1	1	2	39	Neg	2	Kishao
K0022	14	2	1	1	1	2	2	38	Neg	1	Katwe
K0023	21	1	2	1	1	2	2	37	Neg	1	Ihanda
K0024	8	1	3	1	1	1	2	41	Pos	1	Ndama
K0025	12	2	1	2	2	1	2	38	Neg	2	Ndama
K0026	8	2	2	1	1	1	2	39	Neg	1	Ndama
K0027	58	2	1	2	1	1	2	38	Neg	1	Katwe
K0028	36	2	1	2	2	2	2	38	Neg	2	Bujuruga
K0029	43	2	1	1	2	1	2	39	Neg	2	Kanoni
K0030	60	2	2	1	2	1	2	39	Neg	2	Karaizo
K0031	29	2	1	1	1	1	2	39	Neg	2	Rwamugurusi
K0032	19	2	1	1	1	1	2	38	Neg	2	Kayanga
K0033	24	1	1	2	1	1	1	38	Neg	2	Rwambizi
K0034	19	2	2	2	1	1	1	38	Pos	2	Kayanga
K0035	55	2	1	2	2	1	1	39	Neg	2	Kishao
K0036	42	2	1	2	1	2	2	38	Neg	1	Karundu
K0037	37	1	1	2	2	1	2	38	Neg	2	O'shaka
K0038	22	2	2	2	2	1	2	39	Pos	2	Rwamugurusi
K0039	53	2	1	2	1	1	2	39	Pos	2	Kyerunga
K0040	67	2	1	1	1	1	2	38	Neg	1	Kishao
K0041	22	2	2	2	2	1	2	39	Pos	1	Kayanga
K0042	51	2	1	2	1	1	2	40	Pos	2	Kabingo
K0043	5	1	2	2	2	1	2	39	Pos	2	Miti
K0044	4	1	1	2	2	1	2	40	Pos	2	Ruzinga
K0045	29	1	1	2	1	1	2	38	Neg	2	Ndama
K0046	27	2	1	1	1	1	1	38	Neg	2	Nyabwegira
K0047	23	2	1	1	1	1	1	39	Neg	2	Kayanga
K0048	13	2	1	2	1	1	2	39	Pos	2	Kayanga
K0049	28	2	1	1	1	2	2	39	Neg	2	Kayanga
K0050	53	2	1	1	1	1	2	39	Pos	2	Kayanga

K0051	27	1	1	1	2	1	2	39	Neg	2	Kayanga
K0052	64	2	1	2	2	2	2	39	Neg	2	Ihanda
K0053	50	2	1	1	1	2	1	41	Pos	2	Ndama
K0054	12	1	1	1	1	1	2	39	Neg	2	Ndama
K0055	24	2	1	1	1	2	1	38	Pos	1	Kayanga
K0056	46	2	1	1	1	1	1	38	Pos	2	Kayanga
K0057	11	2	1	2	2	1	2	38	Neg	2	Kyerunga
K0058	29	2	1	1	2	1	2	39	Pos	2	O' korongo
K0059	28	2	2	1	1	2	2	39	Pos	2	Kayanga
K0060	17	2	1	1	2	1	2	39	Neg	2	Kayanga
K0061	52	2	2	1	2	2	2	40	Pos	2	Runyaga
K0062	40	2	1	2	2	1	2	38	Neg	2	Rwambale
K0063	75	2	1	1	2	1	2	38	Neg	2	Kibona
K0064	11	1	1	1	1	1	1	40	Neg	2	Kayanga
K0065	29	2	1	3	2	2	2	38	Neg	2	Kishojo
K0066	27	2	1	1	1	1	1	39	Pos	2	Kayanga
K0067	24	2	3	2	1	2	2	39	Neg	2	Ndama
K0068	19	2	1	1	1	1	2	38	Neg	2	Ndama
K0069	26	2	1	2	1	2	2	37	Neg	2	Kihanga
K0070	33	2	1	2	2	2	2	39	Neg	2	Rwambale
K0071	21	2	1	1	1	1	2	39	Pos	2	Ndama
K0072	26	2	1	2	1	1	2	39	Pos	2	Runyaga
K0073	19	1	1	2	2	1	2	38	Neg	2	Miti
K0074	18	2	2	2	2	1	2	39	Pos	2	Kayanga
K0075	35	2	1	1	1	1	2	40	Pos	1	Kayanga
K0076	34	2	3	1	1	2	1	41	Pos	2	Katanda
K0077	20	2	1	1	1	2	2	40	Neg	1	Bweyaja
K0078	10	1	1	1	1	1	2	38	Neg	2	Bweyaja
K0079	28	1	1	1	1	1	2	39	Neg	2	Kayanga
K0080	31	2	1	1	1	1	1	39	Neg	2	Kayanga
K0081	43	2	1	1	1	1	1	38	Neg	2	Kayanga
K0082	18	1	1	1	2	1	2	40	Neg	2	Kayanga
K0083	18	2	2	2	1	2	2	41	Pos	2	Kayanga
K0084	21	2	1	2	2	1	2	41	Pos	2	Ndama
K0085	36	2	1	3	2	1	2	39	Neg	2	Kayanga
K0086	35	1	1	1	1	2	2	40	Neg	2	Kayanga
K0087	20	2	1	1	1	1	2	37	Neg	2	Katembe
K0088	39	2	1	2	1	2	2	39	Neg	2	Magereza
K0089	34	1	1	2	1	1	2	41	Neg	2	Rwamugurusi
K0090	35	2	2	1	2	1	2	38	Neg	2	Kihanga
K0091	14	2	1	1	1	1	1	39	Pos	2	Kishao
K0092	31	2	2	2	1	1	1	40	Neg	2	Rwambaizi
K0093	18	2	1	2	1	1	2	39	Neg	2	Kayanga

K0094	15	2	1	2	1	2	2	41	Pos	1	Kayanga
K0095	6	2	1	1	1	1	1	39	Pos	2	Kishojo
K0096	50	2	1	2	2	2	2	39	Pos	2	Kishojo
K0097	16	2	1	1	2	1	2	38	Pos	2	Ndama
K0098	23	1	2	1	1	1	2	40	Neg	2	Miti
K0099	78	2	1	2	2	1	2	38	Neg	2	Bujuruga
K00100	69	1	1	2	2	1	2	39	Pos	2	Nyakayanja
K00101	28	2	2	1	2	2	1	38	Pos	2	Ndama
K00102	22	2	1	1	1	1	2	39	Pos	2	Kayanga
K00103	16	1	1	1	1	1	2	39	Pos	2	Lukaka
K00104	19	1	1	2	2	1	2	40	Pos	2	Ndama
K00105	15	2	1	1	1	1	2	40	Pos	2	Kayanga
K00106	19	1	1	1	1	1	2	39	Neg	2	Kayanga
K00107	20	2	1	2	1	1	2	39	Pos	2	Kayanga
K00108	45	2	1	1	1	1	2	39	Neg	1	Runyaga
K00109	40	1	1	3	2	2	2	39	Pos	2	Miti
K00110	13	2	1	1	1	1	2	38	Neg	1	Rukajange
K00111	34	2	1	1	1	1	2	38	Neg	2	Kayanga
K00112	44	2	1	2	2	1	2	38	Pos	2	Miti
K00113	58	2	2	1	1	2	2	39	Neg	1	Kagutu
K00114	27	2	2	1	2	1	2	38	Neg	2	Rwamugurusi
K00115	5	1	1	1	1	1	2	41	Pos	2	Kayanga
K00116	25	2	2	2	2	1	1	41	Pos	2	Kagutu
K00117	16	2	2	1	1	1	2	40	Neg	2	Kakanja
K00118	85	2	2	3	2	2	1	39	Neg	2	Rwambaizi
K00119	7	1	1	2	2	2	2	38	Neg	2	Kayanga
K00120	16	1	2	2	1	2	2	39	Neg	2	Ndama
K00121	8	1	2	1	1	2	2	41	Pos	2	Masese
K00122	19	2	1	3	2	1	1	41	Pos	2	Miti
K00123	25	2	1	2	1	1	2	40	Pos	2	Rukaka
K00124	14	2	2	2	1	1	2	40	Pos	1	Kayanga
K00125	23	1	1	2	1	1	2	41	Pos	2	Runyaga
K00126	28	2	1	1	1	1	2	41	Neg	2	Bugene
K00127	19	2	1	1	1	1	2	39	Neg	2	Nyarugando
K00128	16	2	1	1	2	3	1	39	Neg	2	Kayanga
K00129	19	2	1	1	1	1	1	39	Neg	2	Kayanga
K00130	82	1	1	1	1	1	2	40	Neg	2	Kayanga
K00131	29	1	1	2	1	1	2	40	Neg	2	Kayanga
K00132	11	2	1	2	2	1	2	40	Pos	2	Rwambale
K00133	34	2	1	3	2	1	2	39	Neg	2	Runyaga
K00134	16	2	2	1	1	1	2	40	Pos	2	Kayanga
K00135	6	2	1	2	2	1	2	39	Neg	2	Kayanga
K00136	8	2	1	2	2	2	2	41	Neg	2	Kayanga

K00137	12	1	2	2	2	1	2	39	Neg	2	Ndama
K00138	15	2	3	1	1	2	2	40	Neg	2	Rugera
K00139	12	1	2	1	1	2	2	38	Neg	2	Ihanda
K00140	14	1	1	1	1	1	1	38	Neg	2	Kakiro
K00141	23	1	2	3	2	1	1	38	Neg	2	Miti
K00142	18	2	1	2	1	1	2	38	Neg	2	Kakiro
K00143	11	1	1	1	1	2	1	41	Pos	2	Rwambaizi
K00144	29	2	2	2	1	2	2	38	Neg	2	Kayanga
K00145	18	2	2	3	1	2	1	42	Pos	1	Miti
K00146	25	2	1	1	1	1	2	41	Neg	1	Nsheshe
K00147	27	2	3	2	1	1	2	41	Pos	2	Rwamugurusi
K00148	18	1	1	1	2	2	2	40	Pos	2	Rugera
K00149	15	1	1	1	1	1	2	40	Neg	2	Ndama
K00150	20	2	2	2	2	1	1	37	Neg	2	Kayanga
K00151	54	2	1	1	2	2	2	41	Neg	2	Katanda
K00152	31	2	1	2	2	1	1	40	Neg	2	Kayanga
K00153	45	1	1	1	1	2	1	37	Neg	2	Kayanga
K00154	24	1	2	2	1	1	1	41	Pos	2	Kalukwanzi
K00155	15	1	2	1	1	1	2	38	Pos	2	Kayanga
K00156	48	1	1	1	2	2	1	38	Neg	2	Ndama
K00157	38	2	2	2	2	1	1	37	Pos	2	Kayanga
K00158	55	2	2	2	1	1	1	39	Neg	2	Kayanga
K00159	20	2	2	2	2	2	1	38	Neg	2	Kayanga
K00160	50	1	1	1	2	2	2	38	Neg	2	Kayanga
K00161	17	2	2	2	2	1	1	38	Neg	2	Ndama
K00162	45	1	2	1	1	1	2	40	Neg	2	Kayanga
K00163	19	1	1	1	2	2	2	38	Neg	2	Kayanga
K00164	20	2	1	2	2	2	1	40	Neg	1	Kayanga
K00165	25	2	2	2	2	1	1	38	Neg	2	Kayanga
K00166	30	1	1	1	1	1	2	38	Neg	2	Kayanga
K00167	47	1	1	1	1	2	1	41	Neg	2	Kayanga
K00168	25	2	2	2	1	1	2	38	Neg	2	Kayanga
K00169	35	2	1	1	2	1	1	39	Neg	2	Iteera
K00170	10	1	1	1	1	1	1	41	Neg	2	Kakanja
K00171	52	2	1	1	1	1	2	39	Pos	2	Ndama
K00172	40	2	2	2	2	1	2	39	Neg	2	Bujuruga
K00173	35	1	1	1	1	2	1	39	Neg	2	Kayanga
K00174	31	2	1	2	2	1	1	40	Neg	2	Kishao
K00175	46	2	1	1	1	1	2	39	Neg	2	Runyaga
K00176	41	2	1	1	1	1	2	39	Pos	2	Kayanga
K00177	14	1	1	1	1	1	1	40	Neg	2	Karaizo
K00178	42	2	2	2	2	1	2	40	Neg	2	Nyabwegila
K00179	35	1	2	2	1	2	2	41	Pos	2	Nyabwegila

K00180	15	2	1	1	1	2	2	40	Neg	2	Kayanga
K00181	5	2	1	2	2	1	1	39	Neg	2	Omugakorongo
K00182	12	1	1	1	1	1	2	39	Neg	2	Kishao
K00183	16	1	1	1	1	1	2	39	Pos	2	Kamagambo
K00184	12	2	1	1	2	1	1	38	Neg	2	Ihanda
K00185	45	2	1	1	1	1	1	40	Pos	2	Miti
K00186	2	2	2	2	1	2	1	38	Neg	2	Kanyabureza
K00187	58	2	1	1	1	1	2	39	Pos	2	Kayanga
K00188	42	1	2	2	2	1	2	39	Neg	2	Kihanga
K00189	26	2	1	2	1	1	2	41	Neg	2	Rukole
K00190	55	1	1	1	1	1	2	41	Pos	2	Rugera
K00191	24	1	1	1	1	1	2	40	Pos	2	Nyabwegila
K00192	21	2	1	1	1	1	2	39	Neg	2	Kayanga
K00193	12	2	2	2	2	1	2	41	Pos	2	Rushe
K00194	30	1	1	2	1	1	2	39	Neg	2	Kayanga
K00195	81	2	2	3	2	1	2	39	Neg	2	Omugakorongo
K00196	14	1	2	2	1	1	2	38	Neg	2	Katoma
K00197	25	2	3	3	2	2	1	38	Neg	2	Rwamugurusi
K00198	60	2	1	1	2	2	1	40	Neg	2	Nyabwegila
K00199	1	2	3	3	1	2	2	41	Pos	2	Kayanga
K00200	34	2	1	1	2	1	2	39	Neg	2	Kishao

* 1 = Yes/Male; 2 = No/Famale, 3 = unknown

Appendix 2: Ethical clearance certificate. Approved by National Institute for Medical Research and National Health Research Ethics Review Committee in Tanzania.

THE UNITED REPUBLIC OF TANZANIA

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Ministry of Health and Social Welfare
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16th April 2015

Mr Edson Kinimi
Sokoine University of Agriculture
Faculty of Veterinary Medicine
Department of Veterinary Microbiology and Parasitology
P O Box 3019, MOROGORO

CLEARANCE CERTIFICATE FOR CONDUCTING MEDICAL RESEARCH IN TANZANIA

This is to certify that the research entitled: Serological and molecular epidemiology of mosquito borne viruses in Karagwe and Kyerwa, Tanzania, (Kinimi E *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 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 & Social Welfare 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. NIMR Act No. 23 of 1979, PART III Section 10(2).
5. Sites: Karagwe and Kyerwa Districts, Kagera Region.

Approval is for one year: 16th April 2015 to 15th April 2016.

Name: Dr Mwelecele N Malecela

Signature
CHAIRPERSON
MEDICAL RESEARCH
COORDINATING COMMITTEE

Name: Dr Margaret E Mhando

Signature
Ag CHIEF MEDICAL OFFICER
MINISTRY OF HEALTH, SOCIAL WELFARE

CC: RMO
DED
DMO