

SURVIVAL RATES AND BLOOD MEAL PATTERNS OF *Aedes aegypti* AND *Aedes simpsoni* MOSQUITOES IN KERIO VALLEY AND RABAI ARBOVIRUS ECOLOGIES, KENYA

WINNIE W. KAMAU (BSc.)

A RESEARCH THESIS SUBMITTED IN PARTIAL FULFILMENT OF THE REQUIREMENT FOR THE AWARD OF THE DEGREE OF MASTER OF SCIENCE IN INFECTIOUS DISEASES (MEDICAL PARASITOLOGY) IN THE SCHOOL OF HEALTH SCIENCES, KENYATTA UNIVERSITY

SEPTEMBER, 2022

DECLARATION

This thesis is my original work and has not been presented for the award of a degree or any other award in any other University.

Name: Winnie W. Kamau

REG. No: P150/38587/2017

Dept: Medical Laboratory Science


Signature  **Date** 19/09/2022

Supervisors:

This thesis has been presented for examination with our approval as the University supervisors.


1. Dr. Nelson C. Menza

Department of Medical Laboratory Science, Kenyatta University

Signature:  **Date:** 19/9/2022

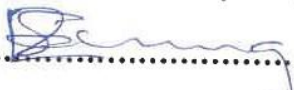
2. Dr. David P. Tchouassi

International Centre of Insect Physiology and Ecology (*icipe*)

Signature:  **Date:** 19/09/2022

3. Prof. Rosemary Sang

International Centre of Insect Physiology and Ecology (*icipe*)

Signature:  **Date:** 19th Sept 2022

ACKNOWLEDGEMENT

It is with gratitude, I appreciate Kenyatta University graduate school for the approval of this study and the opportunity granted to undertake this research project at International Center of Insect Physiology and Ecology (*icipe*) through Dissertation Research Internship Programme (DRIP), under the capacity building and institutional development, human health theme. I am also grateful for my supervisors; Prof. Rosemary Sang, Dr. David Tchouassi and Dr. Nelson Menza for the support, mentorship and excellent supervision. Special thanks to the staffs and colleagues for the resolute support.

TABLE OF CONTENTS

DECLARATION.....	Error! Bookmark not defined.
ACKNOWLEDGEMENT.....	iii
LIST OF TABLES	vii
LIST OF FIGURES	viii
LIST OF ABBREVIATIONS AND ACRONYMS	ix
ABSTRACT.....	x
CHAPTER ONE	1
1.0 INTRODUCTION.....	1
1.1 Background Information.....	1
1.2 Statement of the Problem.....	4
1.3 Justification.....	5
1.4 Research Questions.....	6
1.5 Objectives	6
1.5.1 General Objective	6
1.5.2 Specific Objectives	6
1.6 Significance of the Study.....	7
CHAPTER TWO	8
2.0 LITERATURE REVIEW	8
2.1 Global distribution and burden of dengue and yellow fever.....	8
2.2 Transmission cycles of dengue and yellow fever viruses.....	10
2.3 Concept of vectorial capacity, blood feeding and survival rates	11

2.4 Blood meal patterns and significance in pathogen risk assessment.....	12
2.6 Survival rates of mosquito	14
2.6.1 Methods of estimating mosquito age	14
2.7 Blood meal analysis of mosquitoes.....	16
2.8 Distribution and genetic forms of <i>Ae. aegypti</i> and <i>Ae. simpsoni</i> mosquitoes.....	17
CHAPTER THREE	19
3.0 MATERIAL AND METHODS	19
3.1 Study area.....	19
3.2 Study design.....	19
3.3 Mosquito retrieval and processing	19
3.4 Laboratory Analysis.....	20
3.4.1 Mosquitoes morphological identification	20
3.4.2 Determination of parity rates of <i>Ae. aegypti</i> and <i>Ae. simpsoni sl</i> mosquitoes.....	21
3.4.3 Estimate of mosquito daily survival and longevity.....	21
3.4.4 Determination of blood meal sources from <i>Ae. aegypti</i> and <i>Ae. simpsoni</i>	22
3.4.5 Molecular Identification of Blood-Fed <i>Ae. aegypti</i> and <i>Ae. simpsoni sl</i>	23
3.5 Genetic and Phylogenetic Analysis	25
3.6 Data Analysis	25
CHAPTER FOUR.....	27
4.0 RESULTS	27
4.1 Mosquitoes retrieved from Kerio valley and Rabai	27

4.2 Parity rates of <i>Ae. aegypti</i> and <i>Ae. simpsoni</i> sl.	27
4.3 Survival rates and longevity of <i>Ae. aegypti</i> and <i>Ae. simpsoni</i> sl mosquitoes	28
4.4 Blood meal patterns of <i>Ae. aegypti</i> and <i>Ae. simpsoni</i> sl	29
4.5 Human blood feeding habits among the genetic forms of <i>Ae. aegypti</i> and subspecies of <i>Ae. simpsoni</i> sl.	31
CHAPTER FIVE	34
5.0 DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS	34
5.1 Discussion.....	34
5.1.1 Parity rates of <i>Ae. aegypti</i> and <i>Ae. simpsoni</i>	34
5.1.3 Survival rates and longevity of <i>Ae. aegypti</i> and <i>Ae. simpsoni</i> sl mosquitoes	35
5.1.4 Blood meal patterns of <i>Ae. aegypti</i> and <i>Ae. simpsoni</i> sl	36
5.1.5 Human blood feeding habits among the genetic forms of <i>Ae. aegypti</i> and subspecies of <i>Ae. simpsoni</i> sl.	37
5.2 Study Limitations.....	39
5.3 Conclusions.....	40
5.4 Recommendation	40
5.4.1 Recommendations for further research	41
APPENDICES	50
Appendix. I: Graduate School Research Approval.....	50
Appendix. II: Publication.....	51

LIST OF TABLES

Table 1: Summary of primers used in this study	246
Table 2: Daily survival rates and life expectancy (longevity) of <i>Ae. aegypti</i> and <i>Ae. simpsoni</i> <i>sl</i> in Rabai and Kerio Valley.....	30

LIST OF FIGURES

Figure 1: A map showing global distribution of Dengue fever (Source: WHO 2019)	8
Figure 2: A map showing global distribution of yellow fever (Source: https://www.gs.international/yellow-fever)	9
Figure 3: A Map showing sampling locations in Rift Valley a history of Yellow fever and coastal Kenya an endemic for Dengue (Source: QGIS 3 software).....	20
Figure 4: A presentation of tracheolar skeins under microscopy at 40X magnification.	21
Figure 5: Variation in parity rates estimated for <i>Ae. aegypti</i> and <i>Ae. simpsoni s.l.</i> collected at different periods in Kerio Valley (KV) and Rabai.	28
Figure 6: Blood meal patterns of <i>Aedes aegypti</i> in Kerio Valley and Rabai.	30
Figure 7: Blood meal patterns of <i>Aedes simpsoni sl</i> in Kerio Valley and Rabai.	31
Figure 8: A Maximum likelihood phylogenetic tree of 41 nucleotide mosquitoes COI gene sequences using Tamura-Nei model.	32
Figure 9: A Maximum likelihood phylogenetic tree of 46 nucleotide mosquitoes, ITS2 sequences using Tamura-Nei model.	33

LIST OF ABBREVIATIONS AND ACRONYMS

BG	Bio Agent
CO1	Cytochrome C Oxidase subunit 1
DENV	Dengue Virus
EIP	Extrinsic Incubation Period
ELISA	Enzyme Linked Immunosorbent Assay
GC	Gonadotrophic Cycle
HBI	Human Blood Index
IgG	Immunoglobulin G
ITS	Internal Transcribed Spacer 2
NHPs	Non- Human Primates
PCR	Polymerase Chain Reaction
PR	Parity Rate
Ro	Reproductive number
TAE	Tri- Acetate- EDTA
VC	Vector Capacity
WHO	World Health Organization
YFV	Yellow Fever Virus

ABSTRACT

Understanding the vectorial capacity for arboviruses transmission can allow for improved prediction and of arboviral disease outbreaks and control. Like other vector-borne diseases, transmission of arboviruses is influenced by vector bionomic traits including age structure and vector feeding habits. The current study investigated the survival rates, blood meal patterns and the human blood feeding habits in field collected populations of *Aedes aegypti* and *Aedes simpsoni* mosquitoes, which are vectors of dengue virus (DENV) and yellow fever virus (YFV), respectively, in East Africa. Adult female mosquitoes analysed were trapped during the day using CO₂-baited BG Sentinel traps from peri-urban Rabai, Kilifi County (dengue-endemic) and rural Kerio Valley, Baringo County (with a history of yellow fever outbreak) during the period between August 2019 to February 2020. The mean parity rates following dissection and microscopic examination of ovarian tracheoles was high for *Ae. simpsoni* (85% (n=539) that did not vary between the trapping periods, while in *Ae. aegypti* was 74.9% (n=735) but varied between the trapping periods. Assuming a 3-day gonotrophic cycle, these translated to a high daily survival rate and longevity ranging between 15.8-19.7 days and 7.7-12.4 days in *Ae. simpsoni* and *Ae. aegypti*, respectively. Analysis of blood fed cohorts through DNA sequencing of the 12S mitochondrial rRNA fragment showed a diverse host feeding range for *Ae. aegypti* with estimated human blood index (HBI) of 0.53. HBI did not vary between mitochondrial lineages indicative of domestic and forest genetic forms of *Ae. aegypti*. The genetic forms of *Ae. aegypti* were determined by PCR of a *cox1* gene fragment and then sequencing followed by phylogenetic reconstruction. Similarly, *Ae. simpsoni* complex also exhibited a broad host feeding range, with *Ae. bromeliae* being the most predominant sub-species as determined using Internal Transcribed Spacer 2 (ITS2) PCR sequences, which exhibited a low HBI (0.18 and 0.33 in Rabai and Kerio Valley, respectively). Phylogenetic analysis also suggested the presence of a species which is yet to be described within the *Ae. simpsoni* complex, demonstrating human blood feeding tendency. The species diversity in the *Ae. simpsoni* complex may well be greatly higher than earlier thought, which requires more studies. Overall, both species exhibited high survival/longevity that could lead to high vectorial capacity for YFV and DENV transmission. Additionally, the low human blood meals of both *Ae. aegypti* and *Ae. simpsoni* cohorts indicated a high capacity for zoonotic transmission of other pathogen and therefore a need for continued efforts to control these vectors. These findings demonstrated the applicability to include other bionomic parameters such as vector competence, which defines vectorial capacity, for an effective understanding of spread and recurrence risk of these arboviruses. In addition to enhance cost effectiveness interventions (e.g. vaccines) and prediction of diseases occurrence, there is urgency to generate surveillance information of vector population founded on genotype analyses.

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background Information

Dengue (DEN) and Yellow fever (YF) are re-emerging important arboviral diseases worldwide, DEN infecting about 390M people per year (Smith *et al.*, 2004; WHO, 2015; Atieno, 2017). Globally there has been increase of DEN reported cases between year 2013-2019, from 8 Million to 60 M with 10,000 deaths and about 50-100 Million cases occurs in tropical and sub-tropical countries, every year (Garcia-rejon *et al.*, 2018 ; WHO 2019), incurring a global illness burden of ~\$8.9 Billion annually (Gwee *et al.*, 2021). Despite availability of an effective YF human vaccine, it is estimated that 200,000 YF cases occur annually with about 30,000 deaths worldwide, with about >500 Million persons at risk of infections, mostly from South America and sub-Saharan Africa (WHO, 2014; Kraemer *et al.*, 2017).

In the last two decades YF outbreaks have been experienced in east Africa including; Kenya in 1992-1995 (Reiter *et al.*, 1998); Uganda in 2011, 2016, 2019, Ethiopia in 2012–2014, 2018, Sudan in 2012 and South Sudan 2020 (WHO, 2015, 2016 ; InterHealth Worldwide, 2016). In addition, Angola and Democratic Republic of Congo (DRC) outbreaks in 2015-2016, with imports to Kenya (WHO, 2015), revealed the possible spread and risk of YF in the region. These examples indicate that these vector-borne diseases continue to pose public health challenges and afflicting humans (Weetman *et al.*, 2018).

The *Ae. simpsoni* and *Ae. aegypti* consist of sub-siblings/sub-species that exhibit varying degrees of geographic and reproductive isolation and tend to vary in vectoring abilities (Mukwaya *et al.*, 2000; Hadinegoro *et al.*, 2012). For instance, two genetic forms of *Ae.*

aegypti have been described; the domestic *Ae. aegypti aegypti* and the forest form *Ae. aegypti formosus* (Powell & Tabachnick, 2013). *Aedes simpsoni* consists of about 10 sub-species including the known YF vector *Ae. bromeliae* (Mukwaya *et al.*, 2000).

In spite of the expanding epidemiology of these diseases, important knowledge gaps exist in our understanding of the transmission ecology, dynamics and bionomic role of geographic populations of these known mosquito vectors. Biological traits of these mosquito vectors such as survival rate, genetic diversity, competence, abundance and blood feeding habits are important driving factors of arbovirus transmission and spread (San Martín *et al.*, 2010; Hugo *et al.*, 2014). Surveilling these aspects of mosquito vectors are particularly crucial for risk assessment and guiding vector control or cost-effective vaccinations (e.g. YF). However, geographical transmission and re-emergence of these arboviruses are also modulated by climatic conditions such as rainfall and favourable temperature and human driving activities e.g. travel, land use, urbanisation and global trade (Joyce *et al.*, 2018).

The bionomic trait of the vector relating to blood feeding controls opportunities for infection and transmission (Ladeau *et al.*, 2015). It is also a critical parameter used in modelling and control of arboviruses through assessment of vector feeding preference and the way they change over time and space (Kilpatrick *et al.*, 2006). This trait among *Ae. aegypti* and *Ae. simpsoni*, and their genetic forms remain poorly characterized for wild populations in East Africa, and Kenya in particular. According to the few studies, an anthropophilic *Ae. aegypti* habitat near human and typically blood feed on humans (up to 99%) in Thailand (Harrington, 2005) and in Australia (Stephenson *et al.*, 2019), Nonetheless, it has been found to blood feed on other wild and domestic animals such as cat and dogs in South Texas (Olson *et al.*, 2020) and Senegal (Diallo *et al.*, 2013).

In East Africa, West Pokot Kenya, *Ae. aegypti* exhibited multiple host blood feeding including humans, but majorly feeding on livestock including; rock hyraxes, cattle, goats and sheep, (Chepkorir *et al.*, 2018) with similar zoophilic tendency observed in Kisumu, Kenya (Agha *et al.*, 2019), and about 17% feeding on cattle both in Kisumu and Mombasa (Agha *et al.*, 2019). The feeding behaviour of this species has genetic basis; *Ae. aegypti aegypti* is reported to be more anthropophilic and *Ae. aegypti formosus* zoophilic (Huber *et al.*, 2008). Unlike *Ae. aegypti*, the data on blood feeding behaviour in *Ae. simpsoni* is limited. *Ae. bromeliae* a dominant sub-species among *Ae. simpsoni* complex, breeds near domestic area and is highly an anthropophilic while the sister species *Ae. lili* is zoophilic (according to blood feeding preference) and breeds in forest (Mukwaya *et al.*, 2000 ; Walter *et al.*, 2014).

The vector survival rate (longevity) is another bionomic trait of importance in pathogen transmission. It is one of the most sensitive determinants of a vector population's capacity for pathogen transmission (Cook *et al.*, 2013). In the transmission cycle of a pathogen, an infected female mosquito after taking a blood meal must survive the extrinsic incubation period (EIP) of the pathogen before transmission can occur. The increase in the survival/longevity of female adult mosquito linearly increases the vectorial capacity, which explains the incidence of arboviruses and the potential risk of exposure to the infected vector and therefore allows an effective measures in control of vectors (Ndoen *et al.*, 2012) . The survival ability of *Ae. aegypti* species has been assessed in the recent years globally, such as in Asia (Reinhold & Lazzari, 2018) and central Vietnam (Hugo *et al.*, 2014) displaying a seasonal variation in survivorship, with relatively high survival rates during rainy seasons. Such data is limited or non-existent as for *Ae. simpsoni* in East Africa, Kenya in particular. Enhanced understanding of these entomological risk factors responsible for the re-emerge of

YF and DEN outbreak risks remains a key basis for intervention and preventive strategies against repeated outbreaks of these diseases.

1.2 Statement of the Problem

Yellow fever virus (YF) and Dengue virus (DEN) are *flaviviruses* of medical importance globally. Yellow fever is a public health threat to 34 countries in Africa and 13 countries in central and southern America (Garcia-rejon *et al.*, 2018). Since the first outbreak reported in Kenya (Rift Valley) in 1992-1995 ; Sentinel Surveillance for Yellow Fever in Kenya in 1995 eastern Africa demonstrated an increasing frequency of YF outbreaks including; Uganda, Ethiopia, Sudan, South Sudan and Angola, spreading to Kenya and Congo (WHO, 2015, 2016 ; InterHealth Worldwide, 2016). Over two decades, the number of cases are increasing in east Africa despite the availability of an effective vaccine (WHO, 2016), possibly due to poor health systems, increased urbanization, and low immunity against YF (Neiderud, 2015).

In addition, DEN is a global health threat, affecting over 100 countries (Gloria-soria *et al.*, 2016), including; America, Eastern Mediterranean, South-East Asia and Western Pacific and Africa. In eastern Africa, frequent outbreaks of DEN have continued to occur including; Tanzania, Somalia, Djibouti, Eritrea, Sudan, Ethiopia and Kenya (Malik *et al.*, 2011; de Laval *et al.*, 2012; Bosa *et al.*, 2014; Mwanyika *et al.*, 2021; WHO, 2021). Owing to the fact that there is no effective vaccine against DEN or an effective drug (Londono-Renteria *et al.*, 2016), entomological vector control strategies remains the only solution against the increasing outbreaks.

The expanding epidemiology of these diseases occurs against the poor understanding of their transmission dynamics, ecology, including bionomic role of geographic populations of

known vectors. Survival rate and Blood feeding habits provides opportunities for vector infection and efficient transmission (Kramer & Ebel, 2003; Tchouassi *et al.*, 2020). Little information is in existence regarding these bionomic traits among *Ae. aegypti* and *Ae. simpsoni* and their genetic forms in Kenya particularly in Kerio Valley and Rabai. These knowledge gaps limits the capacity to accurately monitor and respond to arboviral infections, outbreaks, and timely predict the spread. There is therefore, urgent need to understand transmission drivers of DENV and YFV, crucial to address on a better and cost effective preventive strategy.

1.3 Justification

Owing to recurring outbreaks of YF and DEN in the East African region (LaDeau *et al.*, 2015;WHO, 2015, 2016; Atieno, 2017), high fatality rates (Huhtamo *et al.*, 2013) as well as the established distribution of vectors *Ae. simpsoni* and *Ae. aegypti* (Getachew *et al.*, 2015) there is a risk of continued recurrence of these diseases. This is further exacerbated by human activities that potentiate transmission across wider geographical regions (WHO, 2015).The highlighted risks necessitate all possible efforts towards mitigating re-emergence and severity of future outbreaks, owing to, Kerio Valley has a history of YF and Rabai Kenya, a DEN endemic area.

The effectiveness of transmission of arboviral diseases is reliant on various bionomic traits intrinsic to the vector (Ladeau *et al.*, 2015). For the YF and DEN vectors of Kerio valley and Rabai Kenya, these traits remain poorly characterized. It is therefore necessary to characterize these traits among the locally established populations of these vectors. In addition, genetic variability has been reported in various mosquito vectors. However, it is not clear whether the existence of such variability contributes to or influence important bionomic traits relevant to

pathogen transmission. It is thus of interest to determine the level of genetic diversity among the local populations of DEN and YF vectors and whether such diversity affects traits such as blood feeding and distribution of their subspecies.

1.4 Research Questions

- i. What are the survival rates of *Ae. aegypti* and *Ae. simpsoni* mosquitoes in Kerio Valley and Rabai areas?
- ii. What are the blood feeding patterns of *Ae. aegypti* and *Ae. simpsoni* in Kerio Valley and Rabai areas?
- iii. What are the human blood feeding habits among the genetic forms of *Ae. aegypti* and subspecies of *Ae. simpsoni* in Kerio Valley and Rabai areas?

1.5 Objectives

1.5.1 General Objective

To determine the survival rates and blood meal patterns of *Aedes aegypti* and *Aedes simpsoni* mosquitoes collected from Kerio Valley and Rabai arbovirus ecologies in Kenya.

1.5.2 Specific Objectives

- i. To determine the survival rates of *Ae. aegypti* and *Ae. simpsoni* mosquitoes in Kerio Valley and Rabai areas.
- ii. To determine the blood feeding patterns of *Ae. aegypti* and *Ae. simpsoni* in Kerio Valley and Rabai areas.
- iii. To determine the human blood feeding habits among the genetic forms of *Ae. aegypti* and subspecies of *Ae. simpsoni* in Kerio Valley and Rabai areas.

1.6 Significance of the Study

This study unravels the on survival rates and blood meal patterns of *Ae. aegypti* and *Ae. simpsoni* mosquitoes in two areas (Kerio valley and Rabai). In addition, the study assesses how these parameters vary among their genetic forms. These two bionomic traits are among the important indicators of the vectorial capacity, as they provide the opportunities for effective vector infections and transmission. A clear understanding of these parameters, may help in modeling transmission essential in diseases risk predictions and an urgent warning.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Global distribution and burden of dengue and yellow fever

Aedes aegypti and *Ae. simpsoni* mosquitoes are associated with the transmission of arboviruses including dengue and yellow fever viruses, associated with the diseases (dengue and yellow fever) of global public health threat. Dengue occurs globally both in epidemic and endemic transmission cycles, implicated with principle vector *Ae. aegypti* majorly in east Africa (Bhatt *et al.*, 2013) and *Aedes albopictus* as a secondary vector in Asia and European region (WHO 2021). Dengue is estimated to infect 50 million people yearly, globally affecting over 100 countries (Gloria-soria *et al.*, 2016). These include both subtropical and temperate climate areas such as America, Eastern Mediterranean, South-East Asia and Western Pacific and Africa (Ngoi *et al.*, 2016; Garcia-rejon *et al.*, 2018).

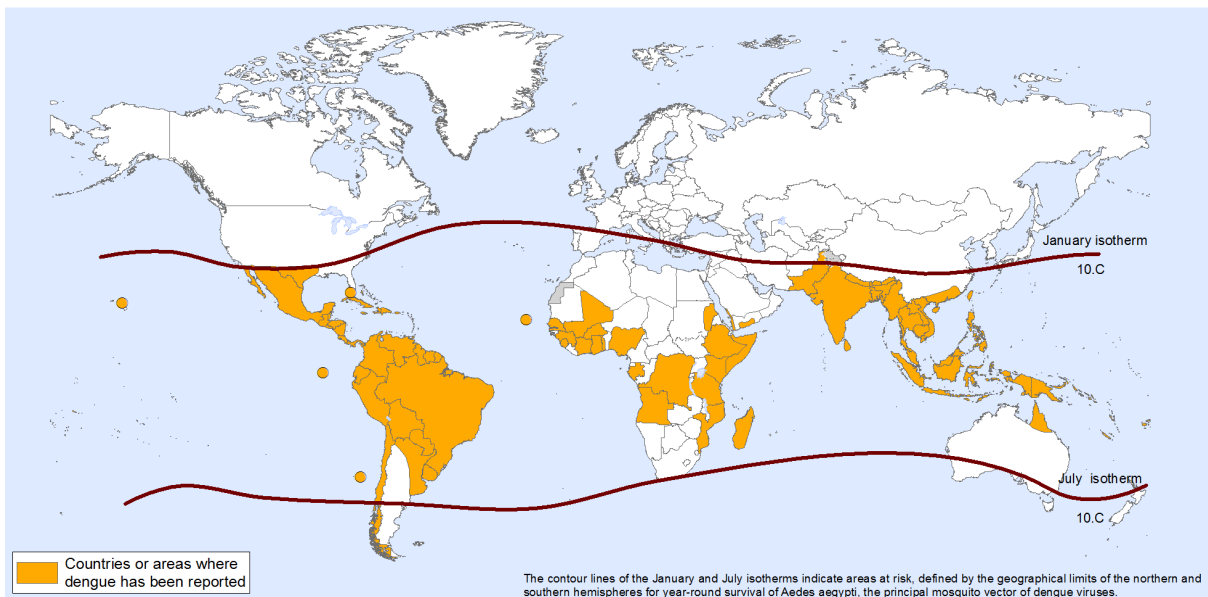


Figure 1: A map showing global distribution of Dengue fever (Source: WHO 2019)

In Africa, since the first outbreak in Zanzibar between the years 1823-1870, outbreaks have been experienced in other countries including Kenya, Tanzania, Somalia, Sudan, Eritrea and Djibouti (Simo *et al.*, 2019). Unlike YF, DEN outbreaks have been rising sporadically in east Africa, Kenya in particular since it was first reported in the coastal region in 1982 (Ngoi *et*

al., 2016) with subsequent outbreaks reported between again 2013-2014 and 2017-2018 along the Kenyan coastline (LaDeau *et al.*, 2015; Atieno, 2017). In addition, a severe outbreak was reported during 2011 in Mandera (north eastern Kenya) that resulted in more than 2100 reported cases (Huhtamo *et al.*, 2013). The increasing outbreaks of dengue in coastal Kenya have implicated the transmission of DEN-1, 2 and 3 serotypes and the increasing distribution of the primary vector *Ae. aegypti* in the region (Konongoi *et al.*, 2016). Dengue infection is asymptomatic with 50% of infected cases presenting flu-like symptoms. Other symptoms may include, headache, rashes and myalgia (Simo *et al.*, 2019). There is no effective human vaccine or drug against dengue fever; to mitigate the transmission, monitoring and controlling of disease vector remains the major approach.

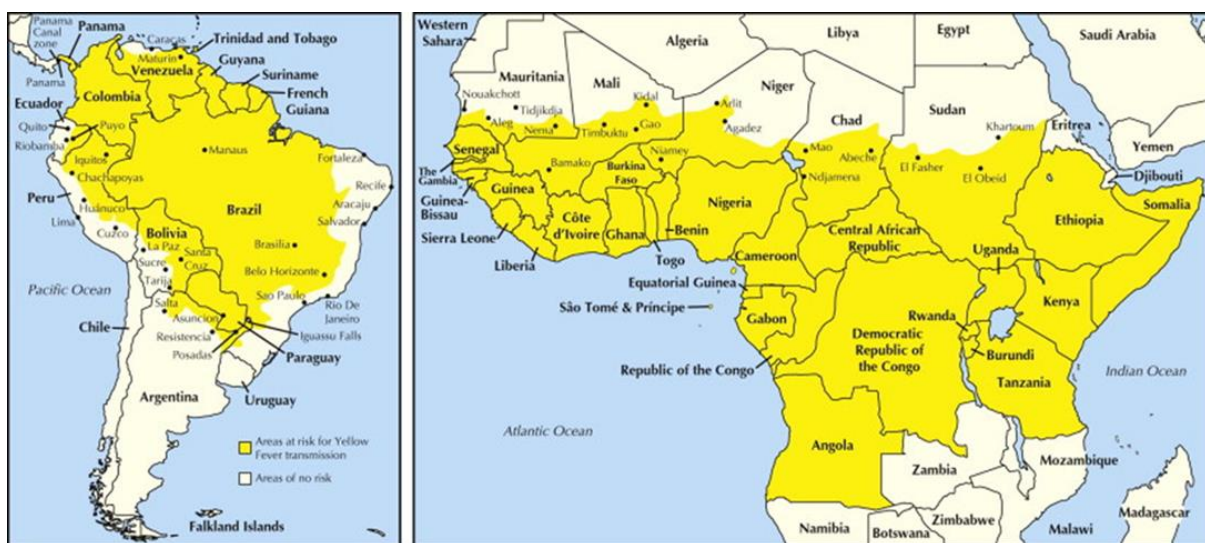


Figure 2: A map showing global distribution of yellow fever (Source: <https://www.gs.international/yellow-fever>)

Yellow fever virus is another *Flavivirus* in east Africa and it is endemic to tropical regions of Africa, central and southern American countries (Garcia-rejon *et al.*, 2018). YF infections exhibit non-specific symptoms, severely leading to high fever, jaundice, hemorrhaging and nausea, potentially leading to death. In spite of availability of an effective vaccine (live

attenuated 17D vaccine), the burden of this disease is estimated at 200,00 cases annually with 30,000 deaths globally, majority occurring in Africa, with annually estimated 84,000 to 170,000 cases and 29,000 deaths (Garske *et al.*, 2014).

2.2 Transmission cycles of dengue and yellow fever viruses

Yellow fever (YF) has three transmission cycles in Africa (urban, intermediate or rural, and the sylvatic or jungle), with each cycle involving a specific set of vertebrate hosts and vectors (Garske *et al.*, 2014). The sylvatic cycle (jungle) involves transmission of the virus between non-human primates (NHPs), mediated by tree-hole breeding vectors (*Ae. africanus* in Africa and *Haemagogus spp* in America); however, humans can become infected when they encroach into the forests through human activities (Garske *et al.*, 2014). The urban cycle involves human as the reservoir host, and transmission of the virus by domestic *Ae. aegypti*. Unlike sylvatic, urban transmission cycles are rare, but fatal if occur, which make it difficult to control. Such an outbreak recently occurred in Africa, in Angola and Democratic Republic of Congo (DRC) resulting to > 300 mortalities and > 900 reported cases (with imports to Kenya); (WHO, 2015). These outbreaks generally occur due to both dense urban population and high *Ae. aegypti* vectors. An intermediate YF cycle also occur (rural transmission cycles) in Africa involving both human and NHPs, vectored by domestic and peri-domestic *Aedes* mosquito species, including; *Ae. metallicus*, *Ae. simpsoni S.I* and *Ae. vittatus* (Gaythorpe *et al.*, 2021). Dengue virus also originates from sylvatic cycles involving NHPs as reservoir hosts; however, in urban epidemic cycles, human is implicated as the main reservoir. Both endemic and epidemic DEN cycles occur, epidemic cycles arising in urban/peri-urban environments vectored by *Aedes aegypti* in eastern Africa (Bhatt *et al.*, 2013).

2.3 Concept of vectorial capacity, blood feeding and survival rates

Vectorial capacity allows an examination of the average rate at which infective mosquitoes initiate infection to a single host. It usually includes the feeding habits, time required for pathogen to fully develop (extrinsic incubation period) and the survival of vector species (Anderson & Rico-Hesse, 2006).

Vectorial capacity (VC) is calculated as indicated in the equation,

$$VC = \frac{[ma^2bp^n]}{-\ln(p)}$$

Where; M = vector density

a = probability of daily feed

p = probability of daily survival of vector

n = length of extrinsic incubation period (EIP)

The variation of vectorial capacity thus, has an impact on basic reproductive number (Maciel-De-Freitas *et al.*, 2008). Reproductive number (Ro) indicates the expectation of number of hosts that can be infected by only a single host introduced into uninfected population. Therefore, a value of Ro greater than one shows that the number of infected by a pathogen increases and the value of Ro less than one shows a decrease of people infected by the pathogen (Maciel-De-Freitas *et al.*, 2008). The understanding of the Ro and VC in arboviruses endemic areas generate measures to disease control and awareness of disease transmission. In addition, survival and human blood feeding are two of the most sensitive parameters in VC estimation.

For pathogen transmission, infected female mosquitoes after taking a blood meal must survive the EIP of pathogen for an effective transmission (David *et al.*, 2009). Female

mosquitoes life cycle therefore, indicates the survival rate as an important factor in analysing the stability of female mosquito oviposition, population and pathogen transmission risk (Hugo *et al.*, 2014). This life cycle however, maybe affected by the environmental factors both in their ecosystem and habitats, which includes temperature, humidity and sunlight (Getachew *et al.*, 2015). These factors may thus, affect the survival rates of the female mosquito.

2.4 Blood meal patterns and significance in pathogen risk assessment

In pathogen transmission, an adult female mosquito vector requires a blood meal by first biting on an infected hosts and later the recipient host (Stephenson *et al.*, 2019). The host selection of a mosquito is influenced by both extrinsic and intrinsic factors. The host abundance, chemicals released by hosts, the defensive mechanisms of the host and biomass are some of the extrinsic factors that guide host seeking behaviours among mosquito vectors (Stephenson *et al.*, 2019). Other environmental factors include variation of climatic conditions such as relative humidity which influences the availability or unavailability of hosts in their habitants (Joyce *et al.*, 2018). Different mosquito species intrinsically prefer certain hosts as blood meal sources. Mosquito blood feeding patterns play an important role in determination of vectorial capacity (VC), allowing the identification of the host selection and biting rate among mosquitoes. This provides informative insights on vector borne pathogens in epidemiological studies (Cebri, 2020). Knowledge on the human biting preference among mosquitoes allows an estimation of the reproduction rate (R_0).

In sub-Saharan Africa, an anthropophilic *Ae. aegypti* has shown to circulate both in peri-domestic and sylvatic cycles which involves human and wild animals (Sylla *et al.*, 2009). Female *Ae. aegypti* has been found to have a high human-blood feeding preference (up to

99%) both in rural and urban areas (Harrington, 2005; Faraji *et al.*, 2014; Sivan *et al.*, 2015). In Thailand it has been found to prefer feeding on human blood (99%) while less than 1% fed on cats, dogs, rats, and birds (Harrington, 2005). However, a recent study in East Africa in West Pokot County, Kenya, *Ae. aegypti* exhibited a low human blood feeding preference, with most feeding on rock hyrax (79%), goats (9%), cattle (4%), human (3%) and 2% on hippopotamus and lizard (Chepkorir *et al.*, 2018) with similar zoophilic tendency in Kisumu, Kenya (Agha *et al.*, 2019). The disparity trends of the feeding habits of *Ae. aegypti*, may be associated with the variety of factors including; difference ecological study site, method of sample collection and the distribution of the genetic forms (*Ae. aegypti aegypti* and *Ae. aegypti formosus*).

2.5 Species identification methods

Reliable identification of mosquito vectors is a crucial component of disease surveillance and implementation of vector control strategies. Species level identification can be accomplished routinely morphologically or using molecular techniques. Morphological identification involving the use of taxonomic keys and microscopy is based in examination of external features of adult or larval specimens. For instance, the two forms of *Ae. aegypti* can be discriminated as adults based on scale pattern of the wings being dark in the domestic form and pale in the forest ecotype (McAvin *et al.*, 2005). However, these features are labile depending on the breeding area and difficult to discern.

Aedes simpsoni mosquitoes belong to a complex where individual sibling species cannot be reliably identified based on morphology alone. These challenges necessitate the use of molecular techniques to supplement morphological identify to elucidate taxonomic status of vector species including potential genetic variability within species. Molecular

characterization or genetic barcoding is most frequently used in identification of mosquito up to species or sub-species level. Common genetic markers include DNA barcoding of the cytochrome *c* oxidase subunit 1(CO1) gene in mitochondrial genome region and internal transcribed spacer 2 (ITS2) located at in the ribosomal genome region (Bennett *et al.*, 2015b). Although a 860 bp barcode region of mitochondrial *CO1* gene has been successfully and effectively utilized in discrimination of closely related species (Paupy *et al.*, 2012), the use of CO1 mitochondrial gene has shown the ability of pseudo gene introgression which limit species discrimination. The region of (ITS2) fast evolve, and was found to be a reliable marker to discriminate some closely related mosquito species including *Anopheles* complexes and *Ae. simpsoni S I* (Tchouassi *et al.*, 2014; Beebe, 2018; Ogola *et al.*, 2019). Nested PCR-based method is another method developed to distinguish *Ae. simpsoni S I* sub-siblings; *Ae. bromeliae*, *Ae. lillii* and *Ae. simpsoni spp* based on the ITS region (Bennett *et al.*, 2015b). Other genetic regions used are *cox2* in the mitochondrial region and D3 segment located in nuclear ribosomal region (McAvin *et al.*, 2005).

2.6 Survival rates of mosquito

2.6.1 Methods of estimating mosquito age

The chronological and physiological age structure of female mosquitoes have been evaluated using different methods. One of the methods includes the mark-release-recapture method (M. Trpis *et al.*, 1971; Muir & Kay, 1998). The mark-release-recapture is a quick and simple method of determining the age of mosquitoes which involves marking of laboratory reared mosquitoes (within 24 -72 hrs after adult emerge) using florescent pigments, before releasing to the field. Later, within 12-18hrs the marked and unmarked mosquitoes are collected to determine the age structure (Trpis *et al.*, 1971; Muir & Kay, 1998). Using this method, the age of *Ae. aegypti* has been estimated in Australia, with the mean survival rate of 19 days for

female and 14 days for males (Muir & Kay, 1998) and in Shauri Moyo village (Rabai, Kenya) with mean survival rates of 10.7 and 5.8 days in males and female respectively (Trips and Hauserman *et al.*, 1995). Nevertheless, this method is laborious and difficult to conduct in some areas, and since it relies on laboratory reared mosquitoes, it may not accurately represent wild mosquito populations (Joy *et al.*, 2012).

The use of reproduction system of a female mosquito has provided a reliable and logistically easier technique to determine the physiological age structure of female mosquito (Ndoen *et al.*, 2012). The technique involves the dissection and examination of adult female mosquito ovaries. The ovarian tracheae are then analysed to determine the female parity status either parous or nulliparous. The parous female mosquitoes, is characterised by uncoiled endings of tracheoles skein, observed under a microscope. This indicates that the female mosquitoes, have taken a blood meal and laid eggs at least once while nulliparous studied under a microscope have folded and coiled tracheoles skeins (Ndoen *et al.*, 2012). To determine the physiological age structure, the approach adopts assumptions including; the mortality rates of the mosquito populations are equal in all ages, the emigration and immigrations are equal and gonadotrophic estimation ranging 3-4 days depending on the mosquito species (Arum *et al.*, 2016). This technique has successfully and broadly been used globally in areas such as West Timor, Central Java (Ndoen *et al.*, 2012) and Eastern African (Arum *et al.*, 2016; Tchouassi *et al.*, 2020). Nonetheless this method may be unsuitable in analysing large mosquito sample size.

Molecular based approaches including; cuticle rings counting to represent the mosquito daily growth of layers at the skeletal apodemes, transcriptomic profiling, mosquito protein spectrometric analysis are some of the methods used as an alternative of ovary- based

approach. However, the molecular based approaches are expensive and requires a high trained personal making it unsuitable for field collected mosquitoes. Use of mid- infrared spectroscopy approach is another alternative approaches used in estimating mosquito age (Babayan *et al.*, 2019). The infrared spectroscopy analyze the difference in structural and chemical composition in the cuticle, which changes between the species and during mosquito life cycle. These allows prediction of survival and mosquito speciation using machine-learning analysis. Although this approach is not broadly used, according to (Babayan *et al.*, 2019), the approach is suitable to large number of mosquito analysis, both in laboratory and field collected mosquitoes and cost effective to entomological surveillance compared to molecular based approaches.

2.7 Blood meal analysis of mosquitoes

Different techniques have been employed to identify the vertebrate blood meal sources of field collected mosquitoes. These techniques include serological methods such as ELISA (Enzyme Linked Immunosorbent Assay), gel diffusion, precipitin test and molecular techniques (Cebri, 2020). The identification of blood meals via serological methods involves the exposure of immunoglobulin G (IgG) in blood conjugated against the host species (Harrington, 2005 ; Sivan *et al.*, 2015). However, the technique faces a challenge of the availability of serum proteins and antisera against some other targeted species (Cebri, 2020).

To increase the specificity of the host identification, molecular techniques are used, which entail the use of specific or universal primers in the amplification of target sequences (Kent, 2009). DNA sequencing is one of the simplest, specific and ultimate molecular techniques for mosquito vectors which feed on a broad range of unknown vertebrate hosts. This include use of a single marker and Sanger sequencing technique to identify single blood feed host.

However, for a broad assessment of mosquito vector blood meals, multiple markers and high-throughput sequencing may be used to identify multiple feeds on different hosts (Muturi *et al.*, 2020 ; Logue *et al.*, 2016). Sequencing approaches are heavily reliant on availability of properly annotated sequence databases and search algorithms/tools for specimen identification usually given as percent identities and/or similarities (Kent, 2009). In addition, sequencing and biological databases enable the inference of evolutionary relatedness through phylogenetic reconstruction analyses. Other molecular methods include real time PCR, PCR-restriction fragment length polymorphism (PCR-RFLM) and high-resolution melting (HRM) analyses on *cyt b* and 16S ribosomal RNA genes PCR products (Omondi *et al.*, 2015).

2.8 Distribution and genetic forms of *Ae. aegypti* and *Ae. simpsoni* mosquitoes

Ae. aegypti is found in tropical and subtropical areas worldwide, as well as temperate areas with high latitude (Gloria-Soria *et al.*, 2018). Human activities such as global trade and human movements have facilitated the spread of *Ae. aegypti* from one continent to another (Joyce *et al.*, 2018), originally from Africa to America and Asia (Gloria-soria *et al.*, 2018). Two subspecies of *Ae. aegypti* are well defined: the sylvan *Ae. aegypti formosus* and the domestic *Ae. aegypti aegypti* (Powell & Tabachnick, 2013). Despite their morphology not reflecting the ecological distinction, the two subspecies have been described according to their geographical distribution (Gloria-soria *et al.*, 2016). The domestic form is more anthropophilic, breeding nearer to human habitats such as in water holding containers (Neira *et al.*, 2014). *Aedes aegypti formosus* prefers non-human blood feeding and breeds in non-human areas including; natural breeding sites such as tree holes (Huber *et al.*, 2008).

Aedes (Stegomyia) simpsoni is a species complex (i.e. morphologically identical as adults) consisting of at least ten species, of which three species have originally been described; *Ae.*

simpsoni, *Ae. bromeliae* and *Ae. lili* (Mukwaya *et al.*, 2000). These species are genetically different, and exhibit variation in biting behaviour, oviposition or breeding site preferences and geographic distribution. Among the *Ae. simpsoni* complex, *Ae. bromeliae* is known to be anthropophilic and attracted to human bait while *Ae. lili* is zoophilic and does not bite humans (Mukwaya *et al.*, 2000). This explains why *Ae. bromeliae* is the known yellow fever vector within the complex (Mukwaya *et al.*, 2000). *Aedes bromeliae* breed in domestic areas while *Ae. lili* are confined to forest areas (Walter *et al.*, 2014). Although unreliable, morphological identification key has been illustrated to differentiate *Ae. simpsoni*, by use of the pale coloured scales at the mid and fore tarsomeres and manifestation of tooth of the mid-tarsal claws (Huang *et al.*, 1986). A reliable identification of adult forms of *Ae. simpsoni* complex using genetic markers, is fundamental due to presence of anthropophilic *Ae. bromeliae*, a vector of importance in transmission of yellow fever virus to humans and other primate (Bennett *et al.*, 2015a).

CHAPTER THREE

3.0 MATERIAL AND METHODS

3.1 Study area

This study utilised adult female mosquito samples of the species *Aedes aegypti* and *Aedes simpsoni* sensu lato (s.l.) that had been collected from Kerio Valley (11.4099° N, 41.2809°E) and Rabai (3.9454° S, 39.5588° E). Kerio Valley is located in Rift Valley while Rabai is located in coastal Kenya, approximately 25 km from Mombasa town (Figure. 3). Kerio Valley has a history of Yellow fever outbreak (*Sentinel Surveillance for Yellow Fever in Kenya*, 1993 to 1995, 1996) while Rabai in Kilifi County is endemic for dengue fever (Ngoi *et al.*, 2016).

3.2 Study design

The study adopted an experimental study design using archived stored mosquitoes collected from Rabai and Kerio valley areas.

3.3 Mosquito retrieval and processing

The study used archived freezer stored mosquitoes that had been collected as part of an arboviral surveillance project. The mosquitoes were trapped using CO₂-baited BG Sentinel traps set during the day (6:30 am – 18:00), for at least 8 consecutive days at different time points, after the rains. These periods included: Kerio Valley (13th to 28th November, 2019), Rabai (28th August to 4th September 2019) and Rabai (6th to 17th February 2020). This trap is suitable for trapping these *Stegomyia* mosquito species which are active during the day (Agha *et al.*, 2017). After trapping the mosquito samples were transported in liquid Nitrogen to International Centre of Insect Physiology and Ecology (Emerging Infectious Diseases laboratory) Nairobi.

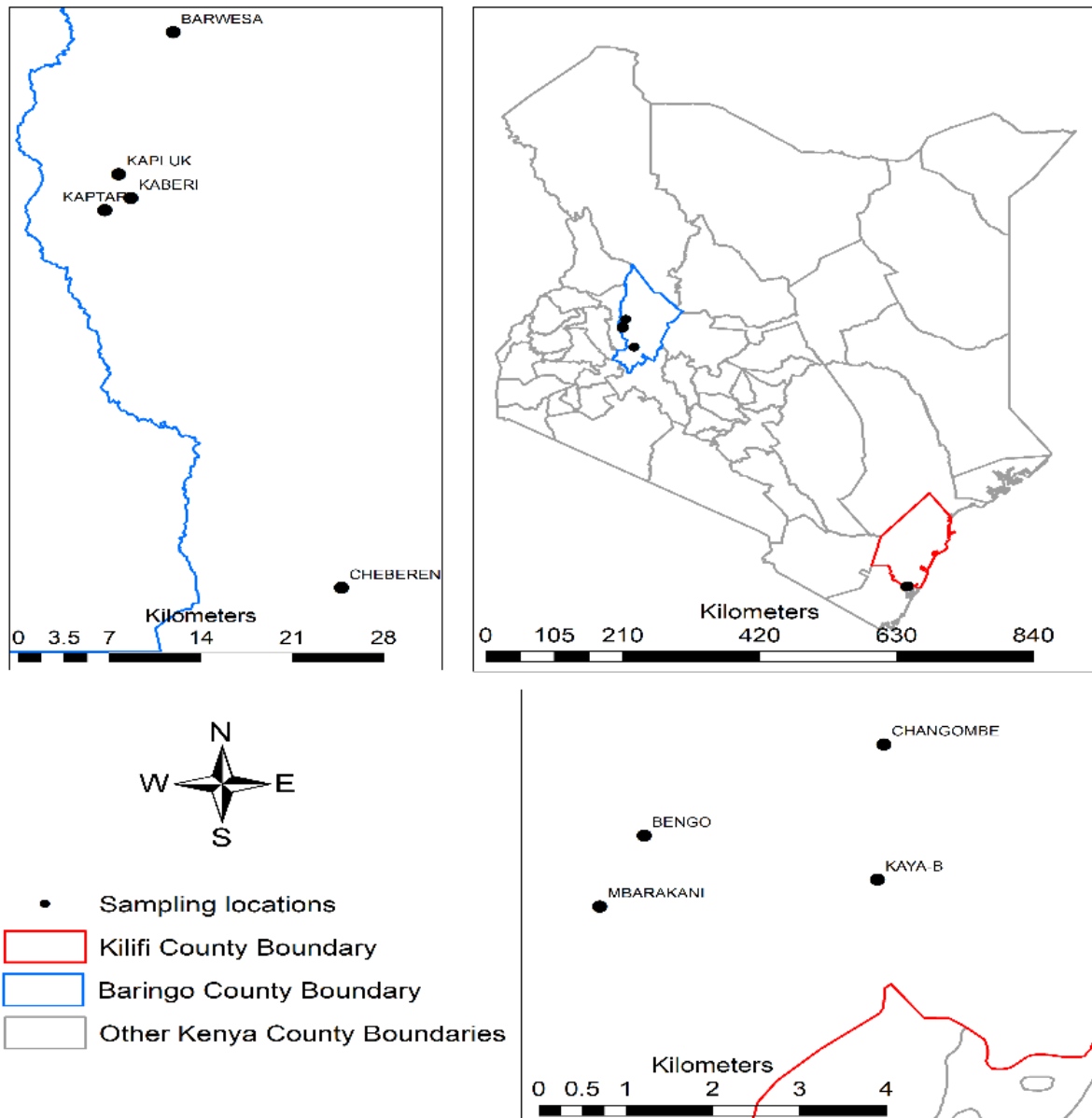


Figure 3: A Map showing sampling locations in Rift Valley a history of Yellow fever and coastal Kenya an endemic for Dengue (Source: QGIS 3 software).

3.4 Laboratory Analysis

3.4.1 Mosquitoes morphological identification

All the mosquito samples collected from the field were retrieved from liquid nitrogen and morphologically identified to species level, according to (Edwards, 1941) and were stored at -80 °C freezers.

3.4.2 Determination of parity rates of *Ae. aegypti* and *Ae. simpsoni* sl mosquitoes

A total of 1,008 adult female *Ae. aegypti* and 570 *Ae. simpsoni* sl mosquitoes were retrieved from -80 °C freezers, and each allowed to thaw for 10 minutes, by placing on petri dish on ice. The ovaries from each mosquito was extracted and dissected under a stereomicroscope on 2/3 drop of saline on a sterile microscope slide. After drying out, parity was scored via microscopy at 40X magnification by observing for the presence or absence of coiled tracheolar skeins or dilation of the avarioles (Detinova, 1962; Tchouassi *et al.*, 2020). Specimens with dilated tracheolar skeins were classified as parous, an indication of an earlier blood feeding and egg production or nulliparous with presence of coiled tracheolar skeins indicating mosquitoes without any previous blood feed and egg production. The thorax and legs from each dissected sample were preserved individually and stored at -80°C for further molecular analysis.

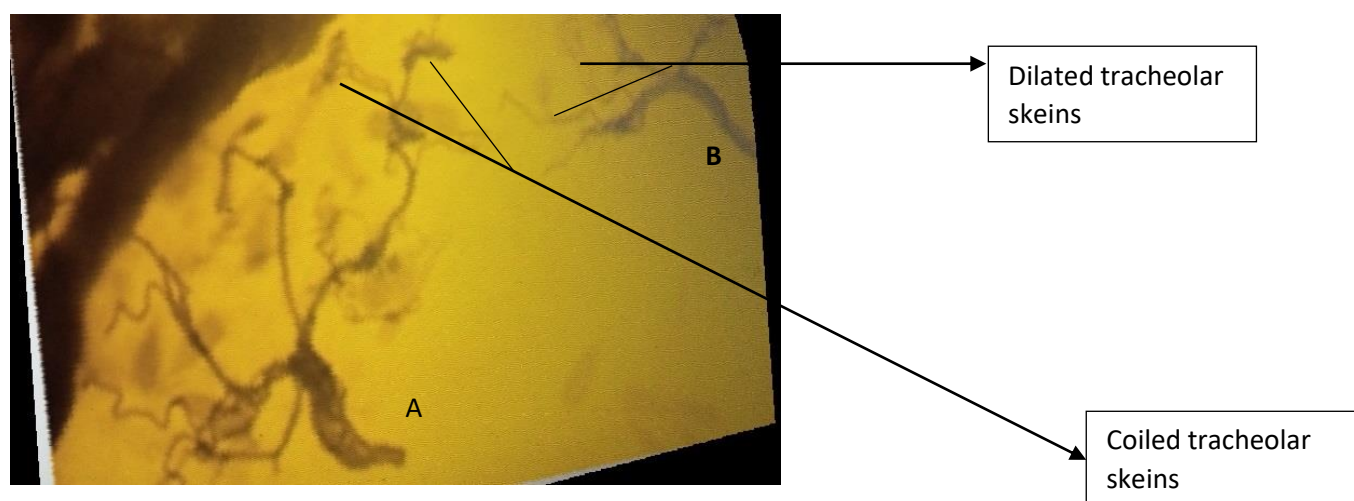


Figure 4: A presentation of tracheolar skeins under microscopy at 40X magnification. (A) Indicates Nulliparous and (B) Parous.

3.4.3 Estimate of mosquito daily survival and longevity

Generated data on parous rates from the different sampling periods for each area were used to estimate the daily survival rate for both mosquito species as described previously (Davidson,

1954). Using formula, $P^n = M$, where p is the daily survival rate, n the gonotrophic cycle, which was assumed as 3 days according to (Tchouassi *et al.*, 2020) and M is the parity rate. Lastly, using $1/lnp$ formula, the longevity in days was estimated, where P represented estimated survival rate (Davidson, 1954).

3.4.4 Determination of blood meal sources from *Ae. aegypti* and *Ae. simpsoni*

Preserved blood-fed specimens (individually at -80°C freezer) encountered during trapping in both areas were processed to identify the host meal sources. The blood-fed specimens were retrieved at -80°C freezers and abdomen was separated from the thorax or head using a sterile scalpel. To avoid contamination, the scalpel was disinfected between each sample using 70% ethanol applied on cotton wool. The head/thorax was preserved frozen for further molecular analysis while the abdomen (with blood) was processed for DNA extraction using DNeasy Blood and Tissue Kit (Bioline, Germany) according to the manufacturer's instructions. To identify the blood meal sources of the engorged mosquitoes, the extracted DNA was used as the template in Polymerase chain reaction (PCR), to amplify a 500 bp of the 12S mitochondrial rRNA gene using established 12S3F and 12S5FR primers (Table 1) as described previously (Roca *et al.*, 2004); Tchouassi *et al.*, 2020).

The PCR was done using MyTaq HS Mix kit (Bioline, Germany), in amplification reaction volume of 10 μl , which consisted of 2 μl of 2xMytaq HS mix polymerase, 10 M of each primer, 0.2 μl of Mytaq polymerase and 1 μl the template DNA (~20ng). Thermal cycling conditions were 95°C for 3 min followed by 40 cycles at 95°C for 20 s, 59°C for 30 s and 72°C for 30 s and 72°C for 7 min. PCR products were then run on 1.2% agarose gel electrophoresis in Tri- Acetate- EDTA (TAE) stained with Ethidium bromide against a 100 bp DNA ladder (Fisher Scientific, UK) and gel visualised in UV trans illuminator. The

amplicons were purified using the Sure Clean Plus kit (Meridian Bioscience) and sequencing were outsourced to Microgen (Tchouassi *et al.*, 2020).

3.4.5 Molecular Identification of Blood-Fed *Ae. aegypti* and *Ae. simpsoni* sl

The remaining portion (head/thorax) of each mosquito used for host blood meal determination was processed to identify the sibling species (*Ae. simpsoni* sl) or genetic forms (*Ae. aegypti*). First, DNA was extracted using an in-house sodium dodecyl sulcate method adapted from (Adams *et al.*, 2008) with slight modifications. Briefly, each specimen was homogenized for 20 s using 4 to 5, 2-mm zirconium beads in 50 μ l PBS in a Mini-beadbeater-16 (BioSpecs inc USA). A 300 μ l of Cell Lysate Buffer (CLB) consisting of 5mM Ethylene Diamine Tetrameric Acid (EDTA), 10 mM Tris-HCl, PH 8.0, 0.05%SDS, was added into the homogenate and incubated at 65°C for 1.5 h. A 100 μ l of Protein Precipitation Solution (PPS) consisting of 1M EDTA and 8M ammonium acetate was then added, the mixture vortexed for 1 min and incubated on ice for 30 min. Afterwards, the mixture was centrifuged at 16400rpm x g at 4°C for 10 min. All the supernatant was transferred into 300 μ l isopropanol, in a new sterile 1.5ml micro centrifuge tube. The mixture was then mixed thoroughly by inverting 100 times, centrifuged for 30 min at 16400 rpm x g at 4°C. The supernatant was discarded and pellets suspended using 300 μ l of 70% ethanol (ice-cold). The mixture was inverted 50 times to mix thoroughly, centrifuged for 30 min at 4°C, 16400 rpm x g, and thereafter the supernatant was pipetted off and pellets were inverted and left to air-dry overnight. The DNA pellets were eluted using 50 μ l PCR grade water and using NanoDrop™ 2000 Spectrophotometer, the extracted DNA were quantified (Adams *et al.*, 2008)

For *Ae. aegypti* specimens, PCR targeting a 860 bp barcode region of mitochondrial Cytochrome C Oxidase subunit 1 gene (*COI* gene) was amplified using the COI-FOR and

CO1 –REV primer (Table 1); (Paupy *et al.*, 2012). The PCR was performed using Mytaq HS Mix kit (Bioline, Germany), the reaction volume comprised of 5µl of 2xMytaq HS mix polymerase, 10 M of each primer and a template DNA of 2 µl using the thermal cycling conditions of 95°C for 2 min followed by 40 cycles at 95°C for 30 sec, 60°C for 30 sec and 72°C for 35 sec and 72°C for 7 min.

However, for *Ae. simpsoni* sl specimens, PCR was conducted targeting a portion of the ITS2 region using the primers ITS2A-FOR and ITS2B-REV (Beebe & Saul, 1995) as shown in Table 1. The PCR was performed comprising of 20 µl reaction volume including 3µl 5x Hot Firepol Blend master mix kit (Estonia) 0.5 µl of 10 M of each forward and reverse primer, 5µl of 2xMytaq HS mix polymerase and 1 µl DNA template (~20ng) . The thermal cycling conditions were 95°C for 15 minutes followed by 40 cycles at 95°C for 30 seconds, 60°C for 30 seconds and 72°C for 45 seconds and 72°C for 7 minutes. The PCR products for both targets were run in 2% gel electrophoresis as described earlier. Finally, the PCR products were purified using Sure Clean Plus kit and outsourced to Microgen for sequencing however, in both the forward and reverse direction, as shown in Table 1.

Table 1: Summary of primers used in this study

Primer	Oligonucleotide sequence	Reference
CO1-22-F	5'-TGTAATTGTAACAGCTCATGCA-3'	Paupy <i>et al.</i> , 2012
CO1-22-R	5'-AATGATCATAGAAGGGCTGGAC-3'	
ITS2A-F	5'-TGTGAACTGCAGGACACAT-3'	Beebe & Saul, 1995
ITS2B- R	5'-TATGCTTAAATTCAGGGGGT-3'	

12S3F	5'-GGGATTAGATACCCCACTATGC-3'	Roca <i>et al.</i> , 2004
12S5R	5'-TGCTTACACATGTTACGACTT-3'	

3.5 Genetic and Phylogenetic Analysis

The nucleotide sequences were cleaned and aligned using Molecular Evolutionary Genetics Analysis Version 6.0 (Tamura *et al.*, 2013). The nucleotide sequences were searched against the GenBank database using the Basic Local Alignment Search Tool, BLAST (www.ncbi.nlm.nih.gov/blast). Evaluation of sequence matches closest on or near matches of threshold >98% was used, to conclude the species individuality of the blood meal source.

The *COI* nucleotide sequences were viewed, cleaned and aligned using MEGA v.6 preceding to the phylogenetic analysis. In the GenBank database through BLASTn searches, corresponding *COI* gene sequences of *Ae. aegypti formosus* (GenBank accession no: AY056597 and domestic *Ae. aegypti* (GenBank accession no: AF390098 and MF 194022) were identified and included in the alignments to infer a Maximum Likelihood (ML) phylogenetic tree. For *Ae. simpsoni* sl homologous *ITS2* sequence of *Ae. simpsoni* clone GenBank accession no: AF 439601, *Aedes bromeliae* GenBank accession no: KF135509 and *Aedes lillii* (MH277635, MH277623 and MH277625) were included in a Maximum Likelihood phylogenetic tree (Tamura *et al.*, 2013).

3.6 Data Analysis

The percentage of total number of parous mosquitoes scored of the total number mosquitoes dissected was calculated as the parity rate (PR). This was determined both for each species and for each trapping period. For each species, the PR (proxy for survival) was compared between the trapping periods, using Pearson Chi-square test (χ^2) at P=0.05. The human blood

index was calculated as the proportion of human blood meals of the total number of blood-fed mosquitoes examined. The proportions of human blood meal between the mitochondrial CO1 lineages of *Ae. aegypti* and established genetic forms of *Ae. simpsoni* were compared and tested for significant differences using Chi-square test (χ^2) at $P= 0.05$, using R programming software (R Core Team, 2013).

CHAPTER FOUR

4.0 RESULTS

4.1 Mosquitoes retrieved from Kerio valley and Rabai

Out of 1,578 adult female mosquitoes (morphologically identified *Ae. aegypti* and *Ae. simpsoni* sl) were retrieved from the -80 freezers at EID lab in ICIPE, a total of 1,520 mosquitoes were successfully dissected and scored for parity. However, 58 mosquitoes were not successfully identified for parity owing to physical damage. Out of 1,008 of *Ae. aegypti*, 981 (97.3%) and for *Ae. simpsoni* sl out of 570, 539 (94.2%) were successfully dissected and scored for parity both in Kerio valley and Rabai. For the blood fed cohorts, a total of 80 engorged mosquitoes (morphologically identified *Ae. aegypti* and *Ae. simpsoni* sl), were retrieved from Kerio Valley and Rabai, these comprised of 53 *Ae. aegypti* and 27 *Ae. simpsoni* sl. The blood-meal sources from *Ae. aegypti* 48 of 53 (90.6%) and *Ae. simpsoni* sl 24 of 27(88.9%) were successfully identified.

4.2 Parity rates of *Ae. aegypti* and *Ae. simpsoni* sl.

A total of 1,520 mosquitoes were successfully dissected and scored for parity from Kerio Valley and Rabai across the three sampling periods. This comprised 981 *Ae. aegypti* and 539 *Ae. simpsoni* sl. The parous/ parity rate (PR) were calculated as the percentage of total number of parous mosquitoes scored of the total number of mosquitoes dissected. Regardless of site or sampling period, parous rate for *Ae. aegypti* was 74.9% (735/981) and 85% (458/539) for *Ae. simpsoni* sl. For *Ae. simpsoni* sl parous rates were 82.5% (71/86) in August-September, 2019 in Rabai, 85.1% (189/222) in February 2020 in Rabai and 85.7% (198/231) in Kerio Valley in November 2019. There was no significant difference in the parous rates across the sampling periods $p > 0.05$ ($\chi^2 = 0.50$, $df = 2$, $P = 0.78$). *Aedes aegypti* parous rates were 67.7 % (212/313) in August-September, 2019 in Rabai, 77.8% % (277/356) in February

2020 in Rabai and 78.8% (246/312) in Kerio Valley in November 2019. Analysis showed that there was a significant difference in the parous rates across the sampling periods $p < 0.05$ ($\chi^2 = 12.75$, $df = 2$, $P = 0.002$) as shown in figure 5 below.

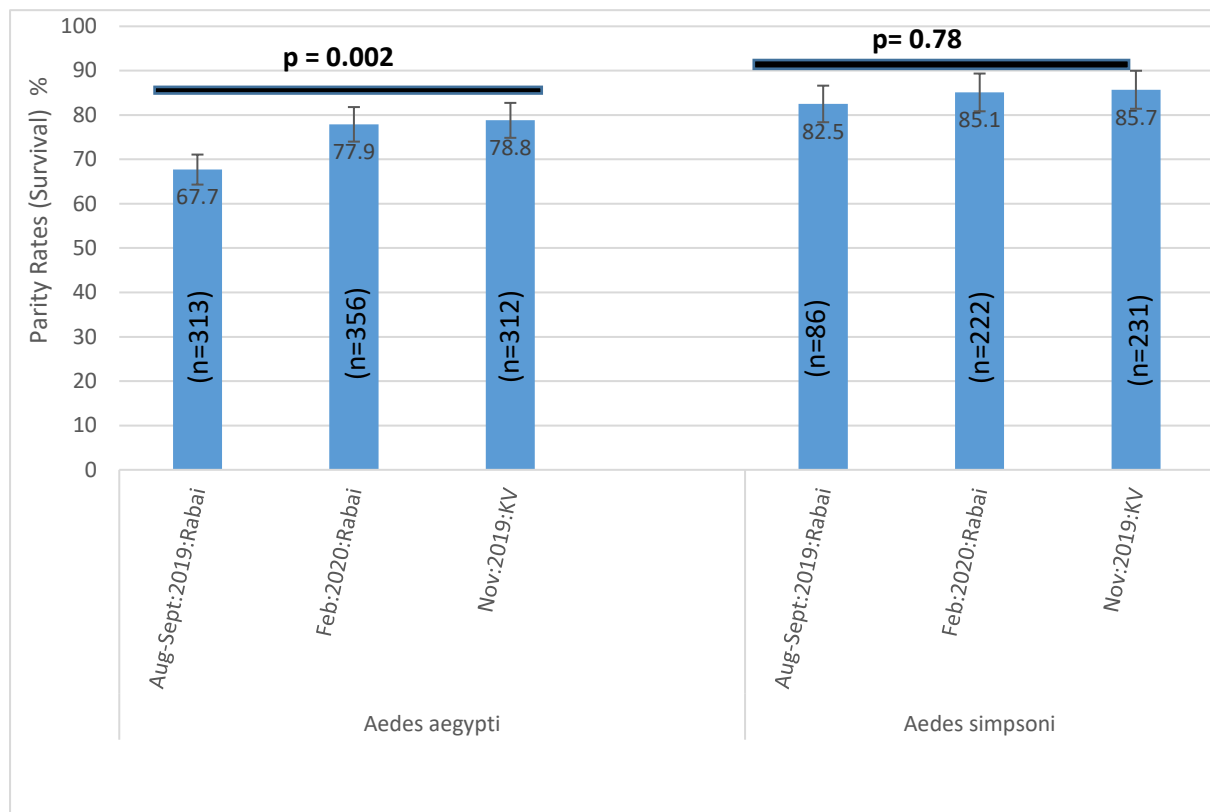


Figure 5: Variation in parity rates estimated for *Ae. aegypti* and *Ae. simpsoni* s.l. collected at different periods in Kerio Valley (KV) and Rabai. (n) Represents, total number of mosquitoes dissected and Aug-Sept: 2019, Feb: 2020 and Nov: 2019, represents August-September: 2019, February: 2020 and November: 2020 respectively.

4.3 Survival rates and longevity of *Ae. aegypti* and *Ae. simpsoni* sl mosquitoes

From the parous data estimated daily survival rates for *Ae. simpsoni* sl was 0.947 in February 2020 in Rabai, 0.94 in August –September 2019 in Rabai and 0.95 in November -2019 in Kerio Valley, translating to longevity of 18.6, 16.2 and 19.5 days, respectively. In Rabai, *Aedes aegypti* had estimated daily survival rates of 0.92 in February -2020 and 0.87 in August – September 2019 while value for Kerio Valley in November-2019 was 0.923 translating to longevity of 12,7.7 and 12.4 days, respectively (Table 2).

Table 2: Daily survival rates and life expectancy (longevity) of *Ae. aegypti* and *Ae. simpsoni* sl in Rabai and Kerio Valley.

Species	Trapping period	Parity Rate (PR)	Daily Survival Rates (P)	Longevity (Days)
<i>Aedes aegypti</i>	Rabai August-September :2019	0.677	0.878	7.7
	Rabai February :2020	0.779	0.92	12
<i>Aedes simpsoni</i>	Kerio Valley November :2019	0.788	0.923	12.4
	Rabai August- September:2019	0.825	0.94	16.2
<i>Aedes simpsoni</i>	Rabai February :2020	0.851	0.947	18.6
	Kerio Valley November :2019	0.857	0.95	19.5

4.4 Blood meal patterns of *Ae. aegypti* and *Ae. simpsoni* sl

A total of 80 blood fed mosquitoes were analysed from Kerio Valley and Rabai, these consisted 53 *Ae. aegypti* and 27 *Ae. simpsoni* sl. The blood meal sources were successfully identified from 48 of 53 (90.6%) and 24 of 27(88.9%) *Ae. aegypti* and *Ae. simpsoni* sl respectively, revealing a total of 13 different host species. Of the 48 blood fed *Ae. aegypti* successfully identified, three were from Kerio Valley and forty-five from Rabai. The 3 *Ae.*

aegypti from Kerio Valley had 3 different host species; rodent, goat and squirrel each at 33.3% (1/3). In Rabai majority were human blood fed at 53.3% (24/45), followed by domestic dog 17.7% (8/45) rodent 8.9% (4/45), lizard 6.7% (3/45), domestic cat at 4.4% (2/45) and finally bat, tortoise, goat and hedgehog had each at 2.2% (1/45) as shown in Figure.6 below.

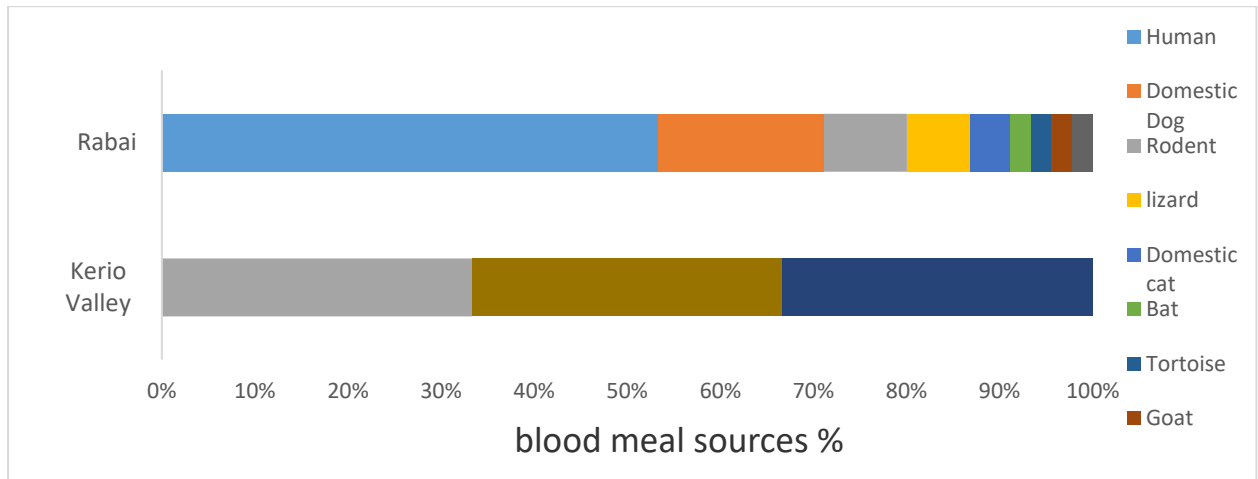


Figure 6: Blood meal patterns of *Aedes aegypti* in Kerio Valley and Rabai.

Out of 24 *Ae. simpsoni* sl successfully identified for blood meal sources, 15 were from Rabai and 9 in Kerio Valley, showed narrow specific blood meal host species. In Kerio Valley those found to feed on human was highest at 44.4% (4/9), then rodents at 33.3% (3/9), goat and domestic cat each at 11.1% (1/9). In Rabai, those feed on human were 26.6% (4/15), squirrel 20% (3/15), mongoose and rodent each at 13.3% (2/15), wild cat, lizard, cow and goat each were at 6.6% (1/15) as shown in figure 7 below.

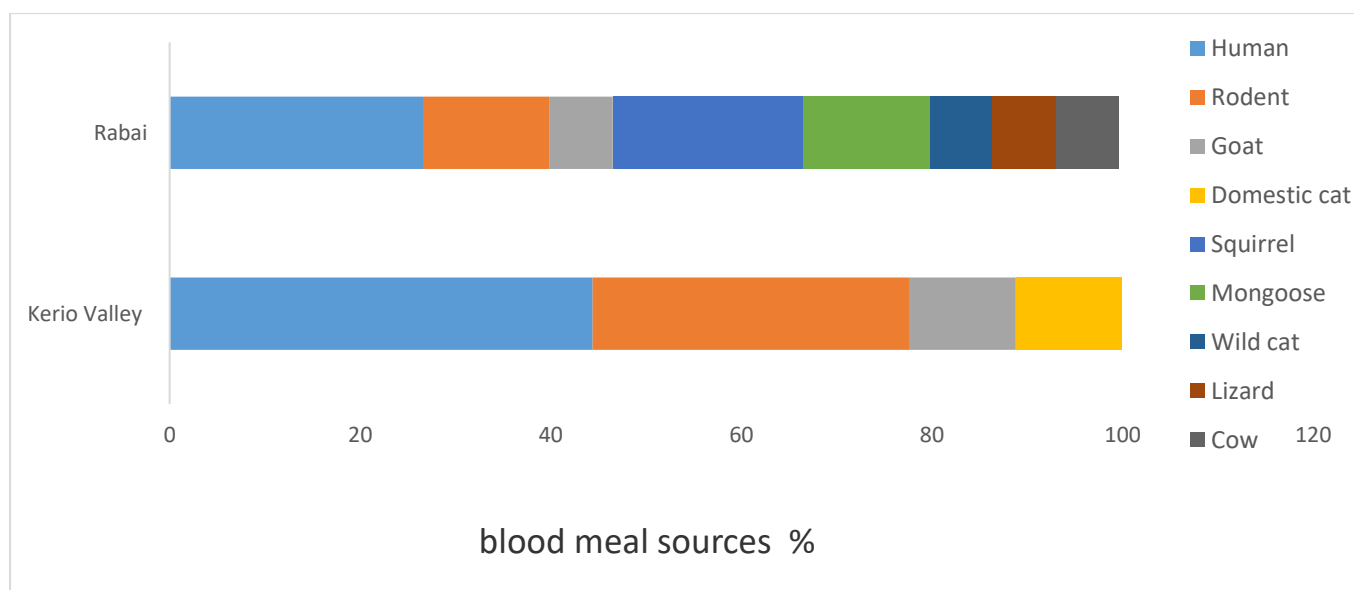


Figure 7: Blood meal patterns of *Aedes simpsoni sl* in Kerio Valley and Rabai.

4.5 Human blood feeding habits among the genetic forms of *Ae. aegypti* and subspecies of *Ae. simpsoni sl*.

Phylogenetic analysis of 38 blood-fed *Ae. aegypti* from the two study areas, showed three distinct lineages (Figure 8). One of these, clustered with the domestic form (*Ae. aegypti aegypti* GenBank No: Af390098 and MF 194022), and Kerio Valley (n=1 and majority of the samples from Rabai (n=25) and Lineage 2 had samples from Rabai (n=5) while lineage 3 contained samples from Rabai (n=7), and clustered with the forest form (*Ae. aegypti formosus* GenBank No: AY056597).

Data for human blood index for each of the mitochondrial lineages are presented in Figure 8. In Rabai, lineage 1 which clustered with the domestic form had a HBI of 0.59(13/22). HBI were 0.75(3/4) and 0.43(3/7) for lineage 2 and 3, respectively. The proportions of human feeds did not vary among the mitochondrial lineages $p > 0.05$ ($\chi^2 = 1.13$, $df = 2$, $p = 0.565$.) In Kerio Valley had one sample in lineage 3, which fed on a non-human host (rodent).

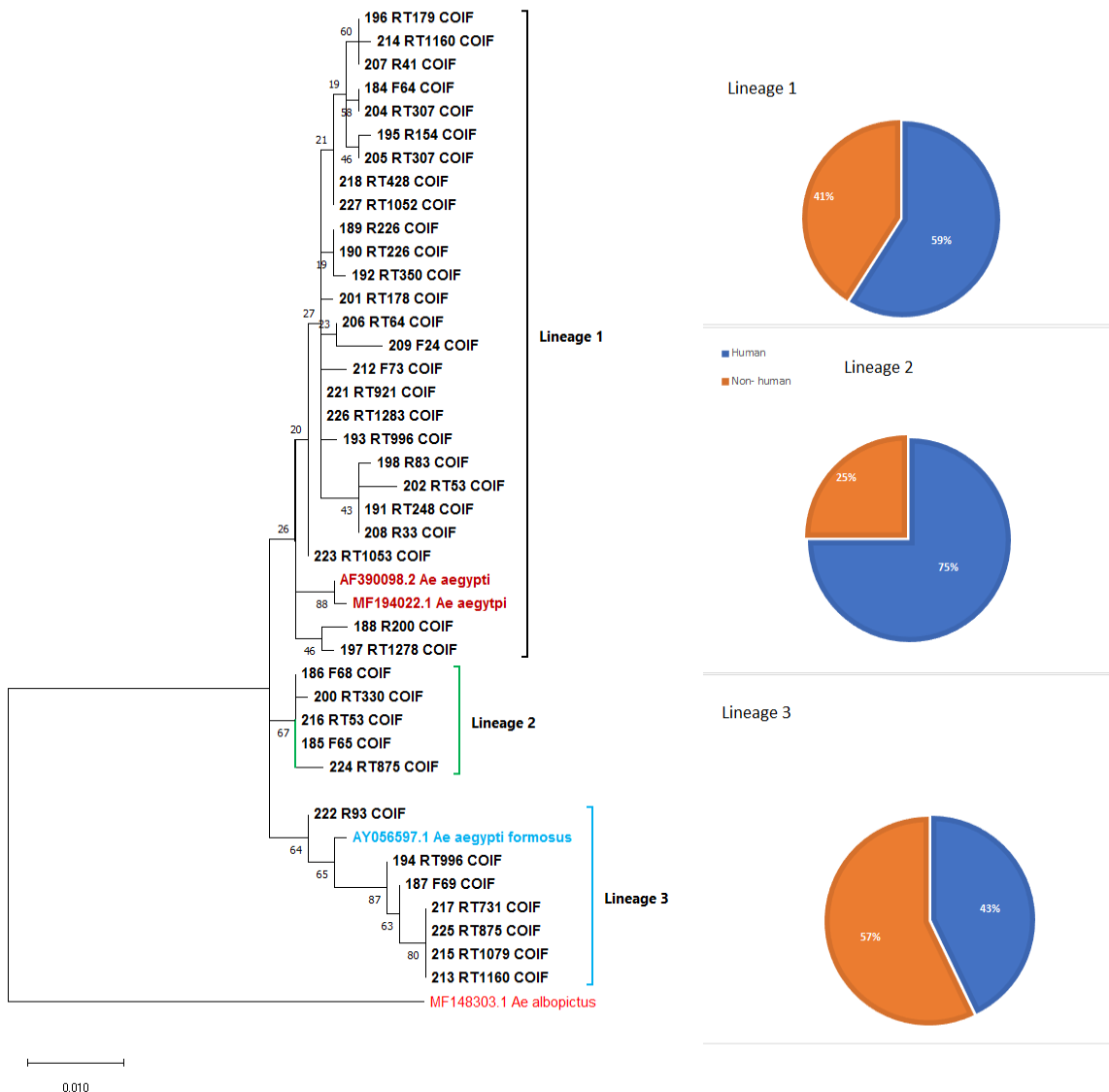


Figure 8: A Maximum likelihood phylogenetic tree of 41 nucleotide mosquitoes COI gene sequences using Tamura-Nei model. GenBank accessions and species are highlighted in blue and red. The sequences acquired in the study specified in black. *Ae. albopictus* was used as out group. Bootstrap values = 1000 replicates. Adjacent are the presentation of human blood feeding proportions, among the *Ae. aegypti* mitochondrial lineages in Rabai.

A total of $n=27$ *Ae. simpsoni* blood-fed samples were profiled for phylogenetic analysis Kerio Valley ($n=9$) and majority of samples from Rabai ($n=15$). The *Ae. simpsoni* samples resolved into 3 clades with well supported bootstrap values (Figure 9). One of these, clustered with *Aedes bromeliae* (*Aedes bromeliae* GenBank No: KF135509, *Ae. simpsoni* clone GenBank accession No: AF 43960) and majority of the samples from Rabai ($n=11$) and Kerio Valley

(n=6). Clade 2 contained samples entirely from Rabai (n=5) and designated *Ae. simpsoni* spp 1, while clade 3 had samples solely from Kerio Valley (n=3) (*Ae. simpsoni* spp 2).

Data for human blood index for each of the ITS clades are presented in Figure 9 and In Rabai, clade1 which clustered with *Ae. bromeliae* had a HBI of 0.18(2/11) and in Kerio Valley 0.33 (2/6). Similar data for the other clades were 0.67(2/3) in Rabai and 0.67(2/3) in Kerio Valley for clade 2 and 3, respectively.

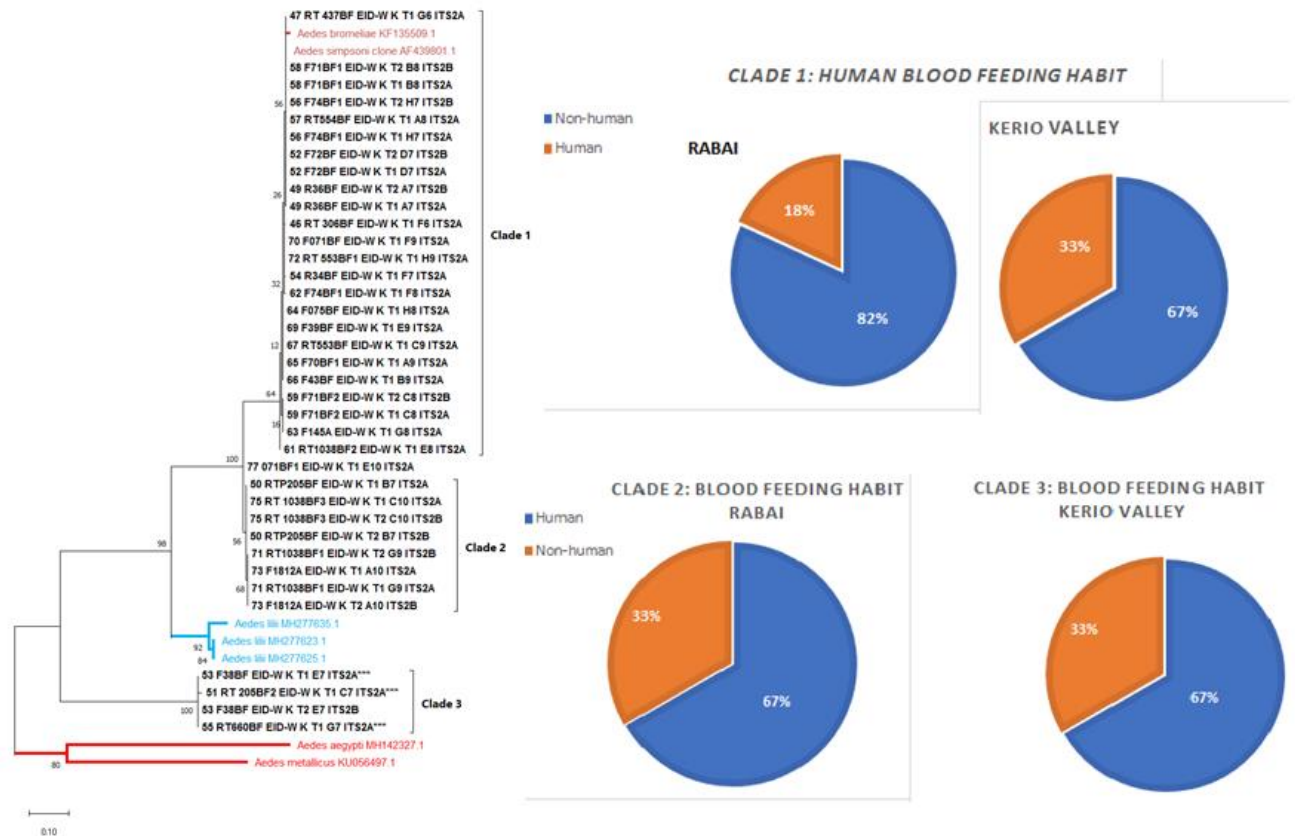


Figure 9: A Maximum likelihood phylogenetic tree of 46 nucleotide mosquitoes, ITS2 sequences using Tamura-Nei model. GenBank accessions and species are highlighted in blue and red. The sequences obtained in the study specified in black. *Ae. aegypti* and *Ae. metallicus* were used as out group. An asterisks indicated an additional sequence. Bootstrap values = 1000 replicates. Adjacent are the presentation of human blood feeding proportions, among the clades.

CHAPTER FIVE

5.0 DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

5.1 Discussion

5.1.1 Parity rates of *Ae. aegypti* and *Ae. simpsoni*

Pathogen transmission patterns are greatly influenced by ecological, biological and behavioral characteristics of vectors. Here, the estimates of survival and blood feeding of *Ae. aegypti* and *Ae. simpsoni* collected from the two areas that contrast in level of urbanization. High parity and the survival rates/longevity was found in both species although with a notable variation among populations of *Ae. aegypti* based on trapping periods. The mosquitoes which were scored parous, would have been involved in the circulation of pathogens in the selected study area (Rift Valley and Coastal regions). This is the reason that the parous mosquito would have obtained blood from infected hosts during their first fresh blood meal and perhaps transmit the pathogen to another host during the second blood meal, which would have kept the diseases to the circulation. The infectious mosquito mirrors the age structure of adult female mosquito (Smith *et al.*, 2004), and regardless of the modest human feeding rates observed in this study, the findings suggests an increased pathogen/disease transmission (vectorial capacity) by these two vectors.

Estimates of parity rates (PR) for natural populations of these species are few or non-existent (e.g *Ae. simpsoni*) especially in endemic countries of Africa. The values estimated for *Ae. aegypti* generally mirror those in recent published literature although the rates are dependent on the habitat and prevailing climatic conditions (e.g., temperature, rainfall and humidity) (Hugo *et al.*, 2014). For instance (Garcia-rejon *et al.* 2018) observed mean parity rates of 0.58 (range: 0-0.88) and 0.61 (range: 0.11-1.00) in the dry and rainy seasons, respectively, in a Mexico cemetery. Relatively lower *Ae. aegypti* parity rate (27%) was reported in the city of

São Paulo, in Brazil (Andrade *et al.*, 2018) . It is also worth noting that PR may be affected by mosquito sampling method (Maciel-de-Freitas *et al.*, 2006; David *et al.*, 2009). The analyzed samples were representative of captures over a number of days for each trapping period. Overall, the study provides new estimates for PR for *Ae. simpsoni* and the high values for both species indicate high contact rates with vertebrate hosts, creating opportunities for infection and pathogen transmission.

5.1.3 Survival rates and longevity of *Ae. aegypti* and *Ae. simpsoni* sl mosquitoes

Parity rate was used to estimate the vectors' daily survival rates assuming a gonotrophic cycle of 3 days. Estimates of the gonotrophic cycle likely vary between these mosquito species with published estimates for *Ae. aegypti* varying between 3-4 days (Garcia-rejon *et al.*, 2018). No such data exist for *Ae. simpsoni*. The derived survival rates for *Ae. aegypti* are consistent with data for this species in Rabai in the mid 1980's based on mark-release recapture method, with female survival of 0.8 (Trpis and Hausermann, 1986). A mean adult female age was 10.7 days (max. value 42 days) generally in agreement with our present data for this species. The model used to estimate survival from parity assumes that mortality is the same for all ages i.e., age-independent survival (Brie, 2002) which may not necessarily be accurate (Charlwood *et al.*, 1985). The variation in survival rates observed in this study could be modulated by other environmental factors e.g., quality of plant diet sources (Nyasembe *et al.*, 2021; Wanjiku *et al.*, 2021). The variation of these parameters could be a function of trapping period, indicating seasonal transmission risk as previously noted, (Agha *et al.*, 2017; Agha *et al.* 2019) especially for the coastal endemic area which has been prone to several DEN outbreaks with annual rises experienced after the short and long rains between February and June (Konongoi *et al.*, 2016; WHO, 2021).

5.1.4 Blood meal patterns of *Ae. aegypti* and *Ae. simpsoni* sl

The feeding behaviour is another bionomic trait and a significant principle of vector species explored in this study, which allows to understand the interaction between potential hosts and vector species in the risk posed in transmission of pathogen (Kilpatrick *et al.*, 2006). For instance *Ae. aegypti* has been involved in transmission of dengue virus especially in coastal Kenya (Kyungah *et al.*, 2020) and reported to prefer feeding on human (Scott *et al.*, 1993; Thomas W Scott & Takken, 2012; Musa *et al.*, 2020). However, according to this study on blood feeding patterns of *Ae. aegypti* in Rabai, it shows that, an anthropophilic mosquito, equally feeding on human and diversely feeding on other hosts such as domestic dog, rodents, domestic cat, lizard, goat, bat, hedgehog, squirrel and tortoise, and it's not exclusive as observed in Mombasa and contrary observed on other studies done in endemic areas such as Thailand (Harrington, 2005) and Australia (Stephenson *et al.*, 2019) where *Ae. aegypti* was found to exclusively blood feed on human at proportions rate of 99%.

Although the actual data on the densities of domestic animal is currently unavailable in the study areas, the moderate human feeding behaviour observed in this study may be associated by higher availability of non- human hosts or low human density in the study area, and this may indicate a high potential risk in transmission of enzootic or zoonotic diseases. Similar studies associated with high non-human feeding among *Ae. aegypti* had been reported where majority 50 % were found to blood feed on domestic dog (Olson *et al.*, 2020). This diverse blood meals sources of *Ae.aegypti* demonstrates the feeding preference is commonly based on their genetic forms occupying different habitats, *Ae. aegypti aegypti* reportedly more anthropophilic and *Ae. aegypti formosus* zoophilic (Joyce *et al.*, 2018).

In addition, the blood meal pattern displayed *Ae. simpsoni* feeding on various hosts. Although the overall engorged *Ae. simpsoni* mosquitoes analysed was fairly small (n=24), which was related with difficulty in trapping blood meal mosquito samples, the blood meal pattern showed a moderate human blood index. The human blood feeding and non-human blood meal sources including ; rodents, squirrel, mongoose, goat, cow, domestic cat, lizard and wild cat, could increase the risk to humans of diverse vector- borne pathogens such as dengue, zika and chikungunya viruses, which could include zoonotic ones circulating in livestock or rodents hosts.

5.1.5 Human blood feeding habits among the genetic forms of *Ae. aegypti* and subspecies of *Ae. simpsoni* sl.

Blood meal data showed feeding on diverse hosts for the two vectors with correspondingly low human blood index (*Ae. aegypti* 0.53 in Rabai, *Ae. simpsoni* 0.44 in Kerio Valley and 0.26 in Rabai). Similar values for *Ae. aegypti* was recorded in a recent study in Rabai in coastal Kenya (Agha *et al.*, 2019). However, the estimated HBI contrasts findings, elsewhere this species is known to be primarily anthropophilic with rates (>0.87-1) in Australia (Stephenson *et al.*, 2019) and Thailand (Harrington, 2005). This is consistent with most previous data on the urban populations of this species classified as anthropophilic (Diallo *et al.*, 2020). The difference could relate to where the samples were captured (indoor vs outdoor) and habitat (urban vs rural/sylvatic) (Joyce *et al.*, 2018). Surprisingly, recent data suggest outdoor rather than indoor resting habitats for populations of *Ae. aegypti* in urban and rural areas of Kenya (Ngugi *et al.*, 2020), in support of our trapping that focused on the outdoors. This may point to fundamental differences in the behavior of *Ae. aegypti* from those in west Africa and outside Africa requiring further elucidation. Nonetheless, few studies on the trophic behavior of *Ae. aegypti* in Africa found that this species fed mainly on

animals such as dog, wild and domestic animals in Nigeria (Davis and Philip 1931) and Senegal (Diallo *et al.*, 2013).

The degree of anthropophagy in *Ae. aegypti* has been posited to have a genetic basis with the domestic form generally considered to prefer human over the forest form with a more zoophilic habit (Joyce *et al.*, 2018). Phylogenetic analysis of blood-fed specimens resolved mainly as mitochondrial lineages mirroring these genetic forms, with no differences in the estimated HBI between the lineages. While the sample size was small to allow definitive conclusion on the trophic behavior between the species, the co-occurrence in domestic habitat (Agha *et al.*, 2019) poses enhance risk of pathogens to humans. Feeding on humans by both forms escalates the risk of transmission to humans of various vector-borne pathogens such as DENV, CHIKV, and ZIKV that are known to have animal reservoirs.

Blood-fed specimens of *Ae. simpsoni* were mostly *Ae. bromeliae* the principal YFV vector in eastern Africa and seems to be most abundant sub-species in *Ae. simpsoni* complex. This species has been described as solely biting sub-species in this complex, although low HBI was attributed to the species. Remarkably, based on the phylogenetic tree with well supported clades, there seems to have a presence of a yet-to-be described species exhibiting human feeding tendencies. Trapping of blood-fed cohorts are very difficult. Generally, the blood-fed mosquitoes samples analyzed was fairly small (n= 80) reflected by the trapping method that is biased towards host-seeking females. Further studies should include resting collections to increase freshly blood- fed mosquitoes (Diallo *et al.*, 2021). Despite the low number of engorged females tested, a better understanding of host selection by *Aedes* species was possible implicating these vectors including *Ae. aegypti* in these foci as principled blood-

feeding vector, blood feeding on a broad variety of host including mammals, rodents and reptiles.

Our feeding profiles were exclusively single feeds on the hosts described with no record of mixed blood meals. This may have been attributed to the molecular method of analysis using a single marker only and Sanger sequencing technique. Multiple feeds on different hosts have been unraveled using ELISA or multiple markers including next generation sequencing (Logue *et al.*, 2016). A high-throughput sequencing has been used for comprehensively evaluating the composition of insect blood meals (Muturi *et al.*, 2020). Molecular assays only test for the presence of a few pre-selected species (Logue *et al.*, 2016). A minor proportion of blood meals were unsuccessfully analyzed and could be related to the advanced digestion of some blood meals, or due to unknown causes.

5.2 Study Limitations

- i. The overall engorged *Ae. simpsoni* samples analyzed were relatively small (n=24), reflecting the hitches encountered in trapping blood-fed mosquitoes. This could be associated with the trapping methods used probably biased towards host-seeking females. However the study provided valuable baseline data indicating a diverse blood feeding habit.
- ii. Nested PCR-based method has also been developed to distinguish *Ae. simpsoni* *S I* sub-siblings; *Ae. bromeliae*, *Ae. lili* and *Ae. simpsoni spp* based on the ITS region (Bennett *et al.*, 2015b). The application of this protocol in this study resulted to mixed and inconclusive because some of the samples showed amplification, both in the specific primers used in targeting their sub-siblings.

5.3 Conclusions

- i. Overall, it was evident that in both species the parity rates which translated to survival rates /longevity, were generally high with variation based on the trapping period among *Ae. aegypti* populations.
- ii. High values of survival/parity rates indicated the possibility of high contact rates with vertebrate hosts, allowing tremendous chances for the transmission of pathogen and infections. These designate a high vectorial capacity for YFV and DENV transmissions by these vectors.
- iii. The analysis of blood fed cohorts showed a diverse host feeding range for *Ae. aegypti* with estimated low HBI, which did not vary between mitochondrial lineages indicative of domestic and forest genetic forms.
- iv. The contemporary results of both genetic forms of *Ae. aegypti* species feeding on human, indicated an enhanced risk of a diverse vector borne disease transmission in human. *Ae. simpsoni* complex similarly, had a broad host feeding range, with *Ae. bromeliae* being the utmost predominant sub-species although unveiling low HBI.
- v. The phylogenetic analysis suggested the presence of new sub-species which is not yet described within the *Ae. simpsoni* complex, affiliated with human blood-feeding.

5.4 Recommendation

- i. These findings demonstrate the applicability to incorporate other bionomic parameters such as vector competence, which describes vectorial capacity, for an effective understanding of spread and recurrence risk of these arboviruses.
- ii. To enhance disease prediction and interventions which are cost effectiveness, this study demonstrates the urgency to actuate surveillance information of vector population based on genotype analyses.

- iii. To fully understand the effect of high survival ability and human feeding found in *Ae aegypti* at different periods of the year, other environmental factors should be incorporated such as temperature, relative humidity, to intensively report on the intense dengue virus transmission in coastal regions.
- iv. Availability of yet to be identified sub-species of *Ae. simpsoni complex* found in this study, speculates that the species diversity may be greatly higher than earlier thought, which calls for more studies.

5.4.1 Recommendations for further research

- i. The relatively small engorged specimen used in this study could have been affected by the trapping methods. For other further studies, this study recommends to include resting mosquitos' collections to increase freshly engorged mosquitoes.

REFERENCES

- Adams, E. R., Hamilton, P. B., Malele, I. I., & Gibson, W. C. (2008). The identification , diversity and prevalence of trypanosomes in field caught tsetse in Tanzania using ITS-1 primers and fluorescent fragment length barcoding. *8*, 439–444.
- Agha, S. B., Tchouassi, D. P., Bastos, A. D. S., & Sang, R. (2017). Dengue and yellow fever virus vectors : seasonal abundance , diversity and resting preferences in three Kenyan cities. 1–10.
- Agha, S. B.; Tchouassi, D. P., Turell, M. J., Bastos, A. D. S., Sang, R., & Reiner, R. C. (2019). Entomological assessment of dengue virus transmission risk in three urban areas of Kenya. *PLOS Neglected Tropical Diseases*, *13*(8),
- Anderson, J. R., & Rico-hesse, R. (2006). *Aedes aegypti* vectorial capacity is determined by the infecting genotype of dengue virus. *75*(5), 886–892.
- Andrade, S., Urbinatti, P. R., Coelho, R., Marques, R. M., Almeida, D. S., Ribeiro, S. S., & Lima-camara, T. N. De. (2018). Parity and gonotrophic discordance of females of *Aedes aegypti* and *Aedes albopictus* (*Diptera* : *Culicidae*) in the city of São Paulo , SP , Brazil. *44*(2), 233–240.
- Arum, S. O., Weldon, C. W., Orindi, B., Tigoi, C., Musili, F., Landmann, T., Tchouassi, D. P., Affognon, H. D., & Sang, R. (2016). Plant resting site preferences and parity rates among the vectors of Rift Valley Fever in northeastern Kenya. *Parasites & Vectors*.
- Atieno, W. (2017, May). Dengue fever outbreak hits 153 in Mombasa. 7.
- Detinova. T. S (1962) Age-Grouping Methods in Diptera of Medical Importance: With Special Reference to Some Vectors of Malaria. *The Journal of Parasitology*, *48*(3), 456
- Babayan, S. A., Khazaeli, P., Reedy, E., Glew, T., Doyle, M., Walton, F., Okumu, F. O., Ranford-cartwright, L., Niang, A., Siria, D. J., Diabaté, A., Ferguson, H. M., Wynne, K., & Churcher, T. S. (2019). Prediction of mosquito species and population age structure using mid-infrared spectroscopy and supervised machine learning [*version 3* ; *peer review* : 2 approved] *Mario González Jiménez*.
- Beebe, N. W., & Saul, A. (1995). Discrimination of all members of the *Anopheles punctulatus* complex by polymerase chain reaction-restriction fragment length polymorphism analysis. *American Journal of Tropical Medicine and Hygiene*, *53*(5), 478–481.
- Beebe, Nigel W. (2021). DNA barcoding mosquitoes : advice for potential prospectors.
- Bennett, K. L., Linton, Y. M., Shija, F., Kaddumukasa, M., Djouaka, R., Misinzo, G., Lutwama, J., Huang, Y. M., Mitchell, L. B., Richards, M., Tossou, E., & Walton, C. (2015a). Molecular Differentiation of the African Yellow Fever Vector *Aedes bromeliae* (Diptera: Culicidae) from Its Sympatric Non-vector Sister Species, *Aedes lili*. *PLoS Neglected Tropical Diseases*, *9*(12), 1–19.

- Bennett, K. L., Linton, Y., Shija, F., Kaddumukasa, M., Djouaka, R., Misinzo, G., Lutwama, J., Huang, M., Mitchell, L. B., Richards, M., Tossou, E., & Walton, C. (2015b). Molecular Differentiation of the African Yellow Fever Vector *Aedes bromeliae* (Diptera : Culicidae) from Its Sympatric Non- vector Sister Species , *Aedes lili*. 1–19.
- Bhatt, S., Gething, P. W., Brady, O. J., Messina, J. P., Farlow, A. W., Moyes, C. L., Drake, J. M., Brownstein, J. S., Hoen, A. G., Sankoh, O., Myers, M. F., George, D. B., Jaenisch, T., William Wint, G. R., Simmons, C. P., Scott, T. W., Farrar, J. J., & Hay, S. I. (2013). The global distribution and burden of dengue. *Nature*, 496(7446), 504–507.
- Brie, O. J. T. (2002). A simple method for calculating mosquito mortality rates , correcting for seasonal variations in recruitment. 22–27.
- Blake, H.; Malik, S.; Mo, P. K. H.; Pisano, C. (2011). 'Do as I say, but not as I do': Are next generation nurses role models for health?. *Perspectives in Public Health*, 131(5), 231–239.
- Bellini, Romeo; Albieri, Alessandro; Balestrino, Fabrizio; Carrieri, Marco; Porretta, Daniele; Urbanelli, Sandra; Calvitti, Maurizio; Moretti, Riccardo; Maini, Stefano (2010). Dispersal and Survival of *Aedes albopictus* (Diptera: Culicidae) Males in Italian Urban Areas and Significance for Sterile Insect Technique Application. *Journal of Medical Entomology*, 47(6), 1082–1091
- Bosa, H. Kyobe; Montgomery, J.M.; Kimuli, I.; Lutwama, J.J. (2014). Dengue fever outbreak in Mogadishu, Somalia 2011: Co-circulation of three dengue virus serotypes. *International Journal of Infectious Diseases*, 21
- Cebri, S. (2020). A Literature Review of Host Feeding Patterns of Invasive *Aedes* Mosquitoes in Europe.
- Chepkorir, E., Venter, M., Lutomiah, J., Mulwa, F., Arum, S., Tchouassi, D. P., & Sang, R. (2018). The occurrence, diversity and blood feeding patterns of potential vectors of dengue and yellow fever in Kacheliba, West Pokot County, Kenya. *Acta Tropica*, 186(February), 50–57.
- Cook, J., Nuccitelli, D., Green, S. A., Richardson, M., Winkler, B., Painting, R., Way, R., Jacobs, P., & Skuce, A. (2013). Quantifying the consensus on anthropogenic global warming in the scientific literature. *Environmental Research Letters*, 8(2).
- Cook, P. E., Mcmeniman, C. J., & Neill, S. L. O. (2008). *Hapter 11*.
- Davis, G. E ; Philip, C. B. (1931). The Identification of the Blood-meal in West African Mosquitoes by means of the Precipitin Test. *American Journal of Hygiene* Vol.14 No
- David, M. R., Lourenço-de-oliveira, R., & Freitas, R. M. De. (2009). Container productivity , daily survival rates and dispersal of *Aedes aegypti* mosquitoes in a high income dengue epidemic neighbourhood of Rio de Janeiro : presumed influence of differential urban structure on mosquito biology. 104(September), 927–932.

- Davidson, G. (1954). Estimation of the Survival-Rate of *Anopheline* Mosquitoes in Nature. , 174(4434), 792–793
- Diallo, D., & Diallo, M. (2020). Resting behavior of *Aedes aegypti* in southeastern Senegal. *Parasites & Vectors*, 1–7.
- Diallo, Diawo; Faye Oumar; Ousmane; Diallo, Mawlouth; Weidmann, Manfred; Sall, Amadou (2013). Quantitative real-time PCR detection of Zika virus and evaluation with field-caught Mosquitoes. *Virology Journal*, 10(1)
- De Laval, Franck; Plumet, Sébastien; Simon, Fabrice; Deparis, Xavier; Leparç-Goffart, Isabelle (2012). Dengue Surveillance among French Military in Africa. *Emerging Infectious Diseases*, 18(2), 342–343.
- Edwards FW (1941). Mosquitoes of the Ethiopian Region. III.-*Culicine* Adults and Pupae, London, UK: British Museum (Nat. Hist.).499
- Eiras AE, Buhagiar TS, Ritchie SA. Development of the Gravid *Aedes* Trap for the capture of adult female container–exploiting mosquitoes (*Diptera: Culicidae*). *J Med Entomol*. 2014; 51(1):200–209.
- Faraji, A., Egizi, A., Fonseca, D. M., Unlu, I., Crepeau, T., Healy, S. P., & Gaugler, R. (2014). Comparative Host Feeding Patterns of the Asian Tiger Mosquito , *Aedes albopictus* , in Urban and Suburban Northeastern USA and Implications for Disease Transmission. 8(8).
- Garcia-rejon, J. E., Ulloa-garcia, A., Cigarroa-toledo, N., Talavera-aguilar, L. G., & Torreschable, O. M. (2018). *Original article. June*, 1–14.
- Garske, T., Van Kerkhove, M. D., Yactayo, S., Ronveaux, O., Lewis, R. F., Staples, J. E., Perea, W., Ferguson, N. M., Burke, D., De La Hoz, F., Grenfell, B., Hansen, P. M., & Hutubessy, R. (2014). Yellow Fever in Africa: Estimating the Burden of Disease and Impact of Mass Vaccination from Outbreak and Serological Data. *PLoS Medicine*, 11(5).
- Gaythorpe, K. A. M., Hamlet, A., Jean, K., Ramos, D. G., Cibrelus, L., Garske, T., & Ferguson, N. (2021). The global burden of yellow fever. *ELife*, 10, 1–22.
- Getachew, D., Tekie, H., Gebre-michael, T., Balkew, M., & Mesfin, A. (2015). Breeding Sites of *Aedes aegypti* : Potential Dengue Vectors in Dire Dawa , East Ethiopia. 2015.
- Gloria-Soria, A., Ayala, D., Bheecarry, A., Calderon-Arguedas, O., Chadee, D. D., Chiappero, M., Coetzee, M., Elahee, K. Bin, Fernandez-Salas, I., Kamal, H. A., Kamgang, B., Khater, E. I. M., Kramer, L. D., Kramer, V., Lopez-Solis, A., Lutomiah, J., Martins, A., Micieli, M. V., Paupy, C., ... Powell, J. R. (2016). Global genetic diversity of *Aedes aegypti*. *Molecular Ecology*, 25(21), 5377–5395.
- Gloria-soria, A., Ayala, D., Bheecarry, A., Calderon-arguedas, O., Chadee, D. D., Chiappero, M., Coetzee, M., Elahee, K. B. I. N., Fernandez-salas, I., Kamal, H. A., Kamgang, B., Khater, E. I. M., Kramer, L. D., Micieli, M. V., Paupy, C., Ponlawat, A.,

- Sanchez-casas, R. M., Seixas, A. L. O., Sousa, C. A., & Rica, C. (2016). *Global genetic diversity of Aedes aegypti*. 5377–5395.
- Gwee, S. X. W., St John, A. L., Gray, G. C., & Pang, J. (2021). Animals as potential reservoirs for dengue transmission: A systematic review. *One Health*, 12, 100216.
- Hadinegoro, S. R. S. (2012). The revised WHO dengue case classification: Does the system need to be modified *Paediatrics and International Child Health*, 32(SUPP1), 33–38.
- Harrington, L. C. (2005). Blood Feeding Patterns of *Aedes aegypti* and *Aedes albopictus* in Thailand. 844–849.
- Huber, K., Ba, Y., Dia, I., Mathiot, C., Sall, A. A., & Diallo, M. (2008). *Aedes aegypti* in Senegal : Genetic Diversity and Genetic Structure of Domestic and Sylvatic Populations. 79(January 2003), 218–229.
- Hugo, L. E., Jeffery, J. A. L., Trewin, B. J., Wockner, L. F., Thi Yen, N., Le, N. H., Nghia, L. T., Hine, E., Ryan, P. A., & Kay, B. H. (2014). Adult Survivorship of the Dengue Mosquito *Aedes aegypti* Varies Seasonally in Central Vietnam. *PLoS Neglected Tropical Diseases*, 8(2).
- Huhtamo, E., Korhonen, E. M., & Vapalahti, O. (2013). Imported dengue virus serotype 1 from Madeira to Finland 2012. 1–4.
- Sheillah. B. A., Tchouassi, D. P., Turell, M. J., Id, A. D. S. B., & Sang, R. (2019). Entomological assessment of dengue virus transmission risk in three urban areas of Kenya. i, 1–14.
- Joy, T. K., Jeffrey Gutierrez, E. H., Ernst, K., Walker, K. R., Carriere, Y., Torabi, M., & Riehle, M. A. (2012). Aging Field Collected *Aedes aegypti* to Determine Their Capacity for Dengue Transmission in the Southwestern United States. *PLoS ONE*, 7(10).
- Joyce, A. L., Torres, M. M., Torres, R., & Moreno, M. (2018). Genetic variability of the *Aedes aegypti* (*Diptera* : *Culicidae*) mosquito in El Salvador , vector of dengue , yellow fever , chikungunya and Zika. 1–14.
- Kent, R. J. (2009). Molecular methods for arthropod bloodmeal identification and applications to ecological and vector-borne disease studies.
- Kilpatrick, A. M., Kramer, L. D., Jones, M. J., Marra, P. P., & Daszak, P. (2006). West Nile Virus Epidemics in North America Are Driven by Shifts in Mosquito Feeding Behavior. 4(4), 4–8.
- Kramer, L. D., & Ebel, G. D. (2003). dynamics of flavivirus infection in mosquitoes. 60, 187–232.
- Konongoi, Limbaso; Ofula, Victor; Nyunja, Albert; Owaka, Samuel; Koka, Hellen; Makio, Albina; Koskei, Edith; Eyase, Fredrick; Langat, Daniel; Schoepp, Randal J.; Rossi, Cynthia Ann; Njeru, Ian; Coldren, Rodney; Sang, Rosemary (2016). Detection of dengue virus serotypes 1, 2 and 3 in selected regions of Kenya: 2011–2014. *Virology*

Journal, 13(1), 182

- Kyungah, J., Hadley, S., Alexander, N., Lee, J., Sung, K., Namkung, S., Andia, E., Oyembo, N., Lim, S., Kanyi, H., Hee, S., Seung, J., Ochola, M. A., Edwards, T., Yoon, I., & Njenga, S. M. (2020). International Journal of Infectious Diseases Clinical and epidemiologic characteristics associated with dengue fever in Mombasa , Kenya. *International Journal of Infectious Diseases, 100*, 207–215.
- Ladeau, S. L., Allan, B. F., Leisnham, P. T., & Levy, M. Z. (2015). *The ecological foundations of transmission potential and vector-borne disease in urban landscapes.* 889–901. Logue, K., Keven, J. B., Cannon, M. V, Reimer, L., Siba, P., Walker, E. D., Zimmerman, P. A., & Serre, D. (2016). *Unbiased Characterization of Anopheles Mosquito Blood Meals by Targeted High- Throughput Sequencing.* 1–18.
- Mukwaya L. G; Kayondo J. K.; Crabtree M. B; Savage H. M.; B. J. Biggerstaff; B. R. Miller (2000). *Genetic differentiation in the yellow fever virus vector, Aedes simpsoni complex, in Africa: Sequence variation in the ribosomal DNA internal transcribed spacers of anthropophilic and non-anthropophilic populations. , 9(1), 85–91.*
- Londono-Renteria, B., Troupin, A., & Colpitts, T. M. (2016). Arbovirosis and potential transmission blocking vaccines. *Parasites & Vectors, 9(1)*, 516.
- Maciel-de-freitas, R., Eiras, Á. E., & Lourenço-de-oliveira, R. (2006). Field evaluation of effectiveness of the BG-Sentinel , a new trap for capturing adult *Aedes aegypti* (Diptera : Culicidae). 101(April), 321–325.
- Maciel-De-Freitas, R., Eiras, Á. E., & Lourenço-De-Oliveira, R. (2008). Calculating the survival rate and estimated population density of gravid *Aedes aegypti* (Diptera, Culicidae) in Rio de Janeiro, Brazil. *Cadernos de Saude Publica, 24(12)*, 2747–2754.
- Malik, Afshan N.; Czajka, Anna; Cunningham, Phil (2016). Accurate quantification of mouse mitochondrial DNA without co-amplification of nuclear mitochondrial insertion sequences. *Mitochondrion.*
- McAvin, J. C., Bowles, D. E., Swaby, J. A., Blount, K. W., Blow, J. A., Quintana, M., Hickman, J. R., Atchley, D. H., & Niemeyer, D. M. (2005). Identification of *Aedes aegypti* and its respective life stages by real-time polymerase chain reaction. *Military Medicine, 170(12)*, 1060–1065.
- Moritz U G Kraemer, Nuno R Faria, Robert C Reiner Jr, Nick Golding, Birgit Nikolay (2017). Spread of yellow fever virus outbreak in Angola and the Democratic Republic of the Congo 2015–16
- Muir, L. E., & Kay, B. H. (1998). *Aedes aegypti* survival and dispersal estimated by mark-release-recapture in Northern Australia. *58(3)*, 277–282.
- Musa, A. A., Muturi, M. W., Musyoki, A. M., Ouso, D. O., Oundo, J. W., Makhulu, E. E., Wambua, L., Villinger, J., & Jeneby, M. M. (2020). Arboviruses and blood meal sources

- in zoophilic mosquitoes at human-wildlife interfaces in Kenya. *Vector-Borne and Zoonotic Diseases*, 20(6), 444–453.
- Muturi, E. J., Dunlap, C., Tchouassi, D. P., & Swanson, J. (2020). Scientific Note Next generation sequencing approach for simultaneous identification of mosquitoes and their blood-meal hosts. 46(1), 1–6.
- Mwanyika, G. O., Mboera, L. E. G., Rugarabamu, S., Ngingo, B., Sindato, C., Lutwama, J. J., Misinzo, G. (2021). Dengue Virus Infection and Associated Risk Factors in Africa: A Systematic Review and Meta-Analysis. *Viruses*, 13(4), 536
- Ndoen, E., Wild, C., Dale, P., Sipe, N., & Dale, M. (2012). Mosquito Longevity , Vector Capacity , and Malaria Incidence in West Timor and Central Java , Indonesia. 2012.
- Neira, M., Lacroix, R., Cáceres, L., Kaiser, P. E., Young, J., Pineda, L., Black, I., Sosa, N., Nimmo, D., Alphey, L., & McKemey, A. (2014). Estimation of aedes aegypti (Diptera: Culicidae) population size and adult male survival in an urban area in Panama. *Memorias Do Instituto Oswaldo Cruz*, 109(7), 879–886.
- Neiderud Carl-Johan (2015) How urbanization affects the epidemiology of emerging infectious diseases, *Infection Ecology & Epidemiology*, 5:1
- Ngoi, C. N., Price, M. A., Fields, B., Bonventure, J., Ochieng, C., Mwashigadi, G., Hassan, A. S., Thiong’O, A. N., Micheni, M., Mugo, P., Graham, S., & Sanders, E. J. (2016). Dengue and chikungunya virus infections among young febrile adults evaluated for acute HIV-1 infection in coastal Kenya. *PLoS ONE*, 11(12).
- Ngugi, H. N., Nyathi, S., Krystosik, A., Ndenga, B., Mbakaya, J. O., Aswani, P., Musunzaji, P. S., Irungu, L. W., Bisanzio, D., Kitron, U., Labeaud, A. D., & Mutuku, F. (2020). Risk factors for Aedes aegypti household pupal persistence in longitudinal entomological household surveys in urban and rural Kenya. *Parasites & Vectors*, 1–14.
- Nyasembe, V. O., Tchouassi, D. P., Muturi, M. N., Pirk, C. W. W., Sole, C. L., & Torto, B. (2021). Plant nutrient quality impacts survival and reproductive fitness of the dengue vector Aedes aegypti. *Parasites and Vectors*, 14(1), 1–10.
- Ogola, E. O., Chepkorir, E., Sang, R., & Tchouassi, D. P. (2019). A previously unreported potential malaria vector in a dry ecology of Kenya. 1–6.
- Olson, M. F., Nde, M. L., Juarez, J. G., Garcia-luna, S., Martin, E., Borucki, M. K., Frank, M., Estrada-franco, J. G., Rodr, M. A., Fern, N. A., Jes, G. De, Javier, L., Garc, A., Huidobro-guevara, R. E., Badillo-vargas, I. E., & Hamer, G. L. (2020). High Rate of Non-Human Feeding by Aedes aegypti Reduces Zika Virus Transmission in South Texas. 1–20.
- Omondi D, Masiga DK, Ajamma YU, Fielding BC, Njoroge L, et al. (2015) Unraveling Host-Vector-Arbovirus Interactions by Two-Gene High Resolution Melting Mosquito Bloodmeal Analysis in a Kenyan Wildlife-Livestock Interface. *PLOS ONE* 10(7):
- Paupy, C., Le, G., Brengues, C., Guerra, M., Revollo, J., Barja, Z., Hervé, J., & Fontenille, D.

- (2012). Infection , Genetics and Evolution Genetic structure and phylogeography of *Aedes* *Evolution*, 12(6), 1260–1269.
- Powell, J. R., & Tabachnick, W. J. (2013). History of domestication and spread of *Aedes aegypti* - A Review. *108*(October), 11–17.
- Reinhold, J. M., & Lazzari, C. R. (2018). Effects of the Environmental Temperature on *Aedes aegypti* and *Aedes albopictus* Mosquitoes : A Review.
- Reiter P, Cordellier R, Ouma JO, Cropp CB, Savage HM, Sanders EJ, Marfin AA, Tukei PM, Agata NN, Gitao LG, Rapuoda BA and Gubler DJ. First recorded outbreak of Yellow fever in Kenya, 1992-93 II. Entomologic Investigations. *American Journal of Tropical Medicine and Hygiene*. 1998;59(4): 650-656.
- Russell J. Reiter; Juan M. Guerrero; Joaquin J. Garcia; Dario Acuña-Castroviejo (1998). Reactive Oxygen Intermediates, Molecular Damage, and Aging: Relation to Melatonin. , *854*(none), 410–424.
- R Core Team (2013). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.
- Roca AL, Bar-Gal GK, Eizirik E, Helgen KM, Maria R, Springer MS, O'Brien SJ, Murphy WJ. Mesozoic origin for West Indian insectivores. *Nature*. 2004;429(6992):649-51.
- Scott, Thomas W, & Takken, W. (2012). Feeding strategies of anthropophilic mosquitoes result in increased risk of pathogen transmission. *Trends in Parasitology*, 28(3), 114–121.
- Sentinel Surveillance for Yellow Fever in Kenya , 1993 to 1995*. (1996). 2(3), 1995–1997.
- Simo, F. B. N., Bigna, J. J., Kenmoe, S., Ndongang, M. S., Temfack, E., Moundipa, P. F., & Demanou, M. (2019). Dengue virus infection in people residing in Africa: a systematic review and meta-analysis of prevalence studies. *Scientific Reports*, 9(1), 1–9.
- Sivan, A., Shriram, A., Vidhya, P. T., & Organisation, D. (2015). Host-feeding pattern of *Aedes aegypti* and *Aedes albopictus* (Diptera: Culicidae) in heterogeneous landscapes of South Andaman, Andaman and Nicobar Islands, India. August.
- Smith, D. L., Dushoff, J., & McKenzie, F. E. (2004). The risk of a mosquito-borne infection in a heterogeneous environment. *PLoS Biology*, 2(11).
- San Martin, J. L.; Brathwaite, O.; Zambrano, B.; Solorzano, J. O.; Bouckennooghe, A.; Dayan, G. H.; Guzman, M. G. (2010). The Epidemiology of Dengue in the Americas Over the Last Three Decades: A Worrisome Reality. *American Journal of Tropical Medicine and Hygiene*, 82(1), 128–135.
- Charlwood, J.D., Birley, M.H., Dagoro, H., Paru, R., & Holmes, P.R. (1985). Assessing

- survival rates of *Anopheles farauti* (Diptera: Culicidae) from Papua New Guinea. *Journal of Animal Ecology*, 54, 1003-1016.
- Stephenson, E. B., Murphy, A. K., Jansen, C. C., Peel, A. J., & Mccallum, H. (2019). Interpreting mosquito feeding patterns in Australia through an ecological lens: an analysis of blood meal studies. *Parasites & Vectors*, 1–11.
- Sylla, M., Bosio, C., Urdaneta-Marquez, L., Ndiaye, M., & Black, W. C., 4th (2009). Gene flow, subspecies composition, and dengue virus-2 susceptibility among *Aedes aegypti* collections in Senegal. *PLoS neglected tropical diseases*, 3(4), e408.
- Tamura, K., Stecher, G., Peterson, D., Filipinski, A., & Kumar, S. (2013). MEGA6 : Molecular Evolutionary Genetics Analysis Version 6 . 0. *30*(12), 2725–2729.
- Tchouassi, D. P., Bastos, A. D. S., Sole, C. L., Diallo, M., Lutomiah, J., Mutisya, J., Mulwa, F., Borgemeister, C., Sang, R., & Torto, B. (2014). Population Genetics of Two Key Mosquito Vectors of Rift Valley Fever Virus Reveals New Insights into the Changing Disease Outbreak Patterns in Kenya. *8*(12).
- Tchouassi D.P., Torto B., Sang R., Riginos C., & Ezenwa V.O (2020). Large herbivore loss has complex effects on mosquito ecology and vector-borne disease risk. *Transboundary and Emerging Diseases*. 00:1–11.
- Trpis, M., Hartberg, W. K., Teesdale, C., & McClelland, G. A. (1971). *Aedes aegypti* and *Aedes simpsoni* breeding in coral rock holes on the coast of Tanzania. *Bulletin of the World Health Organization*, 45(4), 529–531.
- Trpis, M., & Hausermann, W. (1986). Dispersal and other population parameters of *Aedes aegypti* in an African village and their possible significance in epidemiology of vector-borne diseases. *The American journal of tropical medicine and hygiene*, 35(6), 1263–1279.
- Walter, K. S., Brown, J. E., & Powell, J. R. (2014). Microhabitat Partitioning of *Aedes simpsoni* (Diptera: Culicidae) . *Journal of Medical Entomology*, 51(3), 596–604.
- McGinnis Ye, J., & Madden, T. L. (2006). BLAST: improvements for better sequence analysis. *Nucleic Acids Research*, 34(Web Server), W6–W
- WHO Disease Outbreak News (DONs). <https://www.who.int/emergencies/disease-outbreak-news/4>.
- WHO Regional Office for Africa. Yellow fever. <https://www.afro.who.int/health-topics/yellow-fever>.
- Weetman, D., Kamgang, B., Badolo, A., Moyes, C. L., Shearer, F. M., Coulibaly, M., Pinto, J., Lambrechts, L., & McCall, P. J. (2018). *Aedes* mosquitoes and *Aedes*-borne arboviruses in Africa: Current and future threats. *International Journal of Environmental Research and Public Health*, 15(2), 1–20.

APPENDICES

Appendix. I: Graduate School Research Approval



**KENYATTA UNIVERSITY
GRADUATE SCHOOL**

E-mail: dean-graduate@ku.ac.ke

P.O. Box 43844, 00100
NAIROBI, KENYA
Tel. 020-8704150

Website: www.ku.ac.ke

Internal Memo

FROM: Dean, Graduate School**DATE:** 25th February, 2021

TO: Ms. Winnie W. Kamau
C/o Department of Medical
Laboratory Science

REF: P150/38587/2017**SUBJECT: APPROVAL OF RESEARCH PROPOSAL**

=====

We acknowledge receipt of your Research Proposal after fulfilling recommendations raised by the Graduate School Board of 27th January, 2021.

You may now proceed with your Data collection, subject to clearance with the Director General, National Commission for Science, Technology & Innovation and Ethics Review Committee, Kenyatta University.

As you embark on your data collection, please note that you will be required to submit to Graduate School completed Supervision Tracking and Progress Report Forms per semester. The forms are available at the University's Website under Graduate School webpage downloads.

Thank you.

JULIA GITU**FOR: DEAN, GRADUATE SCHOOL**

CC. Chairman, Department of Medical Laboratory Science

Supervisors:

1. Dr. Nelson C. Menza
C/o Department of Medical Laboratory Science
Kenyatta University
2. Prof. Rosemary Sang
International Centre of Insect Physiology & Ecology (ICIPE)
C/o Department of Medical Laboratory Science
Kenyatta University
3. Dr. David P. Tchouassi
International Centre of Insect Physiology & Ecology (ICIPE)
C/o Department of Medical Laboratory Science
Kenyatta University

Appendix. II: Publication

PLOS NEGLECTED TROPICAL DISEASES

RESEARCH ARTICLE

Survival rate, blood feeding habits and sibling species composition of *Aedes simpsoni* complex: Implications for arbovirus transmission risk in East Africa

Winnie W. Kamau^{1,2}, Rosemary Sang^{1,3}, Edwin O. Ogola¹, Gilbert Rotich¹, Caroline Getugi¹, Sheila B. Agha¹, Nelson Menza³, Baldwin Torto¹, David P. Tchouassi^{1*}

1 International Centre of Insect Physiology and Ecology, Nairobi, Kenya, **2** Kenyatta University, Nairobi, Kenya, **3** Center for Virus Research, Kenya Medical Research Institute, Nairobi, Kenya

* dptchouassi@icipe.org



OPEN ACCESS

Citation: Kamau WW, Sang R, Ogola EO, Rotich G, Getugi C, Agha SB, et al. (2022) Survival rate, blood feeding habits and sibling species composition of *Aedes simpsoni* complex: Implications for arbovirus transmission risk in East Africa. *PLoS Negl Trop Dis* 16(1): e0010171. <https://doi.org/10.1371/journal.pntd.0010171>

Editor: Felix Hol, Institut Pasteur, FRANCE

Received: October 2, 2021

Accepted: January 14, 2022

Published: January 24, 2022

Peer Review History: PLOS recognizes the benefits of transparency in the peer review process; therefore, we enable the publication of all of the content of peer review and author responses alongside final, published articles. The editorial history of this article is available here: <https://doi.org/10.1371/journal.pntd.0010171>

Copyright: © 2022 Kamau et al. This is an open access article distributed under the terms of the [Creative Commons Attribution License](https://creativecommons.org/licenses/by/4.0/), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

Data Availability Statement: Sequences generated in this work have been deposited in the GenBank under accession numbers DK339453 – DK339472 and OJ339480 – OJ339521 (*Aedes bromeliae*).

Abstract

Aedes simpsoni complex has a wide distribution in Africa and comprises at least three described sub-species including the yellow fever virus (YFV) vector *Ae. bromeliae*. To date, the distribution and relative contributions of the sub-species and/or subpopulations including biometric characteristics in relation to YF transmission dynamics remain poorly studied. In this study conducted in two areas with divergent ecosystems: peri-urban (coastal Rabai) and rural (Rift Valley Kerio Valley) in Kenya, survival rate was estimated by parity in *Ae. simpsoni s.l.* mosquitoes sampled using CO₂-baited BG Sentinel traps. We then applied PCR targeting the nuclear internal transcribed spacer 2 (ITS2), region followed by sequencing and phylogenetic analytics to identify the sibling species in the *Ae. simpsoni* complex among perous and blood fed cohorts. Our results show that *Ae. bromeliae* was the most dominant sub-species in both areas, exhibiting high survival rates, human blood-feeding, and potentially, high vectorial capacity for pathogen transmission. We document for the first time the presence of *Ae. bi* in Kenya and potentially yet-to-be described species in the complex displaying human feeding tendencies. We also infer a wide host feeding range on rodents, reptiles, and domestic livestock besides humans especially for *Ae. bromeliae*. This feeding trend could likely expose humans to various zoonotic pathogens. Taken together, we highlight the utility of genotype-based analyses to generate precision surveillance data of vector populations for enhanced disease risk prediction and to guide cost-effective interventions (e.g. YF vaccinations).

Author summary

Yellow fever (YF) remains a significant public health risk in East Africa, however, with gaps in the transmission ecology. Important YF virus vectors include *Aedes simpsoni* mosquitoes that comprise subspecies with varying vectoring abilities and, poorly described