UNIVERSITY OF GHANA

COLLEGE OF HEALTH SCIENCES

SCHOOL OF BIOMEDICAL AND ALLIED HEALTH SCIENCES

SPATIO-TEMPORAL DISTRIBUTION AND INSECTICIDE RESISTANCE STATUS OF *AEDES* MOSQUITOES IN GHANA

BY

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THIS THESIS IS SUBMITTED TO THE UNIVERSITY OF GHANA, LEGON IN PARTIAL FULFILMENT OF THE REQUIREMENT FOR THE AWARD OF MPHIL MEDICAL MICROBIOLOGY DEGREE

JULY, 2018

DECLARATION

I, Christopher Mfum Owusu-Asenso, declare that the work presented in this thesis is the result of my own research work carried out in the Department of Medical Microbiology and the Parasitology Department of the Noguchi Memorial Institute for Medical Research (NMIMR) - University of Ghana under the supervision of Professor Yaw Asare Afrane and Professor Julius Abraham Addo Mingle (Department of Medical Microbiology) and that all references cited in this work have been duly acknowledged.

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DEDICATION

This work is dedicated to my loving and supportive family, friends, participants, supervisors, collaborators and the Medical Microbiology Department of the School of Biomedical and Allied Health Sciences (SBAHS). This momentous milestone would not have been achieved without your commitment, support and love.

ACKNOWLEDGEMENTS

My utmost appreciation goes to the Almighty God for His grace, guidance and favor. I wish to express my heartfelt gratitude to my supervisors, Professor Yaw Asare Afrane and Professor Julius Abraham Addo Mingle of the Medical Microbiology Department, School of Biomedical and Allied Health Sciences (SBAHS), for their valuable contributions, script editing and proofreading, constructive criticism and relentless supervision and training. Special thanks and appreciation to Dr. Simon Kwaku Attah, Department of Medical Microbiology, SBAHS for his critical proofreading of my thesis, also to Mr. Isaac Boamah, Isaac K. Sraku and Prince Ashong-Pappoe of the Virology laboratory – Medical Microbiology Department, Fredrick Afful, Elvis Baseriyi Batuu, colleagues of the insecticide resistance and malaria transmission (IMAT) Research team, interns (especially Prince Boateng and Sebastian Kow Egyin Mensah) and service persons (2017/2018) for their immense support. My sincerest gratitude to Opanin Kwabena Mensah, Mr. George Owusu Mfum and Mr. Benjamin Adu-Bobbie for their advice and financial assistance, God richly bless you all. An immense thanks to my lovely course mates (2016/2017) (especially Gabriel Akosah Brempong), lecturers and staff of the Medical Microbiology Department, SBAHS, Parasitology Department of the Noguchi Memorial Institute for Medical Research (NMIMR), staff of the President Malaria Initiative (PMI) – Tamale (especially Mr. Sylvester Coleman, Mr. Ernest Yaw Nsefo, Mr. Eddie Grand-Agyeman, Mr. Imoro Nurudeen and Mr. Abdullai Abdul-Rahman) and staff of the Entomology Department of the Navrongo Health Research Centre (NHRC) (especially Dr. Victor Asoala, Mr. Stephen Kantum and Ms. Bernice Ama Baako) for enabling this work to come to fruition. I also thank all the local people who volunteered and offered their assistance during the data collection activities. Ultimately, I thank everyone who in diverse ways helped me in my research work, I say GOD richly bless you.

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LIST OF ABBREVIATIONS

Ae.	*Aedes*
BF	Blood-fed
bp	Base pair
BG	Biogent
ANOVA	Analysis of variance
CFR	Case fatality ratio
CHIKV	Chikungunya virus
CTAB	Cetyl trimethylammonium bromide
DDT	Dichlorodiphenyltrichloroethane
DENV	Dengue virus
DENV-1	Dengue virus serotype 1
DENV-2	Dengue virus serotype 2
DENV-3	Dengue virus serotype 3
DNA	Deoxyribonucleic acid
E	East
EPRC	Ethics and protocol review committee
G	Gravid
GPS	Global positioning system

HBI	Human blood index
HG	Half gravid
HLC	Human landing catch
in	inches
IRS	Indoor residual spraying
kdr	Knockdown resistance gene
L	NEB 100bp LADDER
M	Molar
NC	Negative control
N	North
NIS	Number identified successfully
PCR	Polymerase chain reaction
PPK	Prokopack
RDT	Rapid diagnostic test
rmp	Rotation per minute
RNA	Ribonucleic acid
RVFV	Rift valley fever virus
ST	Samples tested

UF	Unfed
µl	microlitre
W	West
WHO	World Health Organisation
YF	Yellow fever
YFV	Yellow fever virus

ABSTRACT

Background: The *Aedes* mosquito is an important vector of arboviruses, which include the Yellow fever virus (YFV), Dengue virus (DENV), and Chikungunya virus (CHIKV). Spatial distribution by their shared vectors is grounded on environmental and demographic factors. Currently, vector control is still the main instrument to reduce or eradicate incidence of arboviruses since there are no effective vaccines for a number of public health important arboviruses. With an upsurge in insecticide resistance, surveillance of the target population for ideal choice of insecticides is of great significance. The feeding habits of *Aedes* mosquitoes is more likely anthropophilic, exophagic and endophilic. For such reason, they can serve as vectors for numerous pathogens responsible for both human and animal diseases.

Aim of Study: This study investigated the spatio-temporal distribution and insecticide resistance status of *Aedes* mosquitoes in Ghana.

Method: The study was a cross-sectional study. Mosquito samples were collected from seven study sites in three ecological zones of Ghana. Indoor and outdoor sampling was done with Biogent sentinel-2 mosquito traps (BG traps), human landing catch (HLC) and prokopack aspirator (PPK). The mosquito vectors were sorted into species morphologically. Their spatial and temporal distribution was determined seasonally, indoor and outdoor for all site. Phenotypic resistance status of *Aedes* mosquitoes to insecticides was determined using the WHO susceptibility bioassay. Host blood meal sources was determined by PCR.

Results: A total of 2193 adult *Aedes* mosquitos were collected in this study; *Aedes aegypti* (97.3%), *Aedes africanus* (2.2%) and *Aedes luteocephalus* (0.05%). The human landing catch method recorded the highest densities of 210.9 (77%) followed by PPK 74 (17.8%) and BG trap 15.5 (5.2%). The dry and wet seasons recoded 73.5 (42.1%) and 103.95 (57.9%) respectively.

A total of 390 resting female adult *Aedes* mosquito were collected using the PPK method: The gonotrophic status of the *Aedes* collected using the Prokopack were 68.9 % (269/390) unfed, 11.3% (44/390) blood-fed, 17.9% (70/390) half-gravid and 1.8% (7/390) gravid. The test results showed that *Aedes* mosquito populations from all study sites were resistant to DDT (0 - 84%) mortality. Vectors showed resistance to deltamethrin in Tema (68%) and patchy resistance in the other sites. Vectors showed resistance to permethrin in Accra (40.0%) and Larabanga (88.8%), patchy resistance in Dwease (90%), Navrongo (90%) and Paga (96%). *Aedes* mosquitoes showed resistance to bendiocarb in Larabanga, susceptibility in Ada foah and Accra, patchy resistance in Tema (95.0%), Accra (97.5%), Dwease (96.3%), Navrongo (96%) and Paga (97%). *Aedes* mosquitoes were susceptible to organophosphates in all the sites. Blood meal analysis showed that the *Aedes* mosquitoes were mostly anthropophilic with HBI of 0.9 i.e. [(human = 90%), (human and dog = 5%), (dog and cow = 5%)].

Conclusion: *Aedes* mosquitoes are found in all the ecological zones of Ghana and have the potential to contribute actively to arboviral transmission. The development of resistance to pyrethroids and carbamates by these mosquitoes may have an operational impact on the efficacy of insecticide on vector control interventions.

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background

The *Aedes* mosquito is an important vector of arboviruses, which include the Yellow fever virus (YFV), the Dengue virus (DENV), the Chikungunya virus (CHIKV) (Ferreira-de-Brito *et al*., 2016; Farraudière *et al*., 2017). Arboviruses have a well-documented account of emergence through numerous mechanisms, including geographic expansion such as urbanisation and travel, often facilitated through human travel. Most arboviruses are located in tropical areas and transmitted among animals by the sylvatic cycle (Weaver and Reisen, 2010).

Many cause disease after spill over transmission by the vector from the reservoir to humans and domesticated animals. These devices threaten to intensify in the future due to increased deforestation, travel, human population growth in the tropics and deforestation. Global warming also has the possibility to surge the distribution of vectors and to heighten transmission in temperate regions by increasing host-vector contact by shortening vector gonotrophic cycles, prolong transmission seasons, and limiting extrinsic incubation times (Weaver and Reisen, 2010).

Female *Aedes* mosquitoes need a blood meal from a vertebrate host to reproduce (Bhatt *et al*., 2013). The nutrients taken up with the blood goes into production of large amounts of yolk proteins that are deposited in the eggs. The need for blood in order to reproduce makes mosquitoes effective disease vectors because they require, at least, one insect host contact for every set of eggs they develop. During feeding, adult female *Aedes* mosquitoes can take up more than their own body weight of blood (Clements, 1992).

The mosquitoes exhibit different feeding habits; anthropophilic, endophagic and endophilic which means that some, prefer to feed on humans (anthropophilic). Some feed indoors (endophagic) and rest indoors after blood feeding (endophilic) while the meal is digested and the eggs mature, respectively (Scott and Morrison, 2010). For such reason, they can serve as vectors for numerous pathogens responsible for both human and animal diseases (Bhatt *et al*., 2013). Some of the most important arthropod-borne viruses include the Dengue virus (DENV) with epidemics recently reported in Burkina Faso and Côte d'Ivoire (WHO, 2017a) which are neighboring countries to Ghana, the Yellow fever virus (YFV) which is endemic in Ghana (Appawu *et al*., 2006) and the Chikungunya virus (CHIKV). All of the arboviruses are mainly transmitted by *Aedes* species including the highly efficient and anthropophilic *Ae. aegypti* and the highly invasive *Ae. albopictus* (Weaver and Reisen, 2010). The warm climatic conditions within tropical areas allows for year-round transmission by the arthropod vectors. Other important factors determining geographic distribution of arthropod vectors include insecticide resistance, rainfall, humidity, and vegetation (Weaver and Reisen, 2010).

Currently, vector control is still the main tool to control or eliminate incidence of arboviruses since there are no effective vaccines for a number of public health important arboviruses (WHO, 2009). Insecticides play a fundamental role in the regulation of mosquito-borne diseases. With an upsurge of insecticide resistance, surveillance of the target population for ideal choice of insecticides is of great significance (Owusu *et al*., 2015). Comparisons of test data from a sentinel site over time are useful for assessing temporal trends in resistance. Contrasting of test data from multiple sites provide helpful information about the geographical distribution of resistance. The resistance frequencies, the prevalence of resistance mechanisms in a single locality vary markedly with time (Owusu *et al*., 2015).

1.2 Problem Statement

Epidemics of arboviral diseases including Yellow fever and Dengue fever transmitted mainly by mosquitoes pose significant public health problems in the tropics (Monath, 2001). Yellow fever is endemic in Ghana (Appawu *et al.*, 2006) ever since it was documented in 1901 (Scott, 1965). A major epidemic of yellow fever, involving 319 cases and 79 deaths, occurred in 1969-70 and was the heaviest outbreak recorded during any 2-year period since 1901 (Agadzi *et al.*, 1984). On 20 December 2011, the Ministry of Health of Ghana notified WHO of a yellow fever (YF) outbreak occurring in 3 districts; Builsa and Kassena-Nankana-West in the Upper East Region and Kintampo-South in the Brong Ahafo Region located in the mid-western part of the country (WHO, 2018b). Recently, some African countries including Ghana reported yellow fever outbreaks (WHO, 2018a).

As of November 3, 2017 Burkina Faso recorded a total of 5773 (64%) cases of Dengue fever (Rapid diagnostic test (RDT) positive) out of 9029 suspected cases reported, with a cumulative total of 18 deaths (CFR 0.2%) (WHO, 2017a). In Côte d'Ivoire, a total of 192 cases out of 623 suspected cases of Dengue fever including two deaths (case fatality rate: 0.3%) have been confirmed by PCR of which 66% were dengue virus serotype 2 (DENV-2), 29% were dengue virus serotype 3 (DENV-3) and 5% were dengue virus serotype 1 (DENV-1) as of 11th July 2017 (WHO, 2017a). It is therefore possible that there could be an outbreak of Dengue fever in Ghana, since Ghana borders these two West African countries and there is constant movement of people and vehicles between these countries which could also facilitate the movement of the vector and transmission of the viruses.

Currently, there is no evidence of Dengue fever cases in Ghana.

However, the risk is high due to Ghana's proximity to the endemic countries, increased travel to Ghana from arboviral disease endemic West and East African countries where recent epidemics have been recorded, and the absence of an organized mosquito control programme in Ghana for *Aedes* mosquitoes. It is possible that the status of arbovirus transmission in the country is changing, however, there is no comprehensive data on insecticide resistance status of the vectors, preferred blood type and adult/larval densities of the mosquito vectors in Ghana. The purpose of this study is to determine the spatial and temporal distribution of *Aedes* mosquitoes, blood meal type and determine the phenotypic resistance status of the vectors in Ghana to be able to develop a surveillance system to arboviruses in the country.

1.3 Justification

According to the World Health Organisation (WHO), 2.5 billion people are at risk of DENV globally (Bhatt *et al*., 2013). *Aedes aegypti* is also the primary vector of yellow fever which is a disease prevalent in tropical South America and Africa (Tabachnick, 1991).

Changes in climate and other anthropogenic factors, epidemiology, insecticide resistance and viral genetics have stimulated the need to update and extend information on the role of these changes in the emergence of arboviruses in Ghana. Expanding urbanization and deforestation has resulted in the abundance of susceptible human hosts and favourable conditions for vector population expansion that facilitate arbovirus transmission. However, in addition to female adult feeding pattern, larval habitat and wide transient distribution makes the control of these mosquitos difficult (Smith *et al*., 2012). Mapping the distribution of these vectors and the geographical determinants of their ranges is essential for public health planning (Kraemer *et al*., 2015a). It is important to work out where these mosquito species are found in Ghana to identify the areas at risk.

It is also important to predict where these species could become established if they were introduced, in order to map out areas that could become at risk in the future.

Recent studies in Ghana have also recorded presence and high densities of vectors (Appawu *et al*., 2006) and insecticide resistance within the vectors (Suzuki *et al*., 2016). The reported dengue epidemics in neighbouring countries, Cote d'Ivoire and Burkina Faso (WHO, 2017a) is also of public health concern. Insecticide susceptibility bioassay data are an indicator if targeted mosquito population respond positively to the insecticides in use (Thomsen *et al*., 2014).

Hence, the purpose of this research study is to determine the spatio-temporal distribution and insecticide resistance status of *Aedes* mosquitoes in Ghana. This will aid the understanding of significant features of emerging arboviral risks in Ghana and how it can be mitigated.

1.4 Aim of Study

The aim of this study is to investigate the spatio-temporal distribution and insecticide resistance status of *Aedes* mosquitoes in Ghana.

1.5 Specific Objectives

The specific objectives are to determine:

i. the spatio-temporal distribution of *Aedes* mosquitoes.
ii. phenotypic resistance of the vectors to insecticides.
iii. the source of blood meal taken by the vectors.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 An Overview of Arboviruses

Arthropod-borne viruses (arboviruses) are transmitted biologically among vertebrate hosts by blood feeding arthropod vectors such as mosquitoes, other biting flies, and ticks. When transmitted biologically, arboviruses must replicate in the gut of arthropod vectors prior to transmission in contrast to being mechanically transmitted through contaminated mouthparts (Weaver, 1997). Vertical transmission, an example of biological transmission, involves the passage of the virus from an infected female vector to both male and female offspring (Weaver and Reisen, 2010). Horizontal transmission can be venereal, from a vertically infected male directly to a female vector, as well as oral from a female vector to a vertebrate host via the saliva during blood feeding. The latter horizontal mode of transmission is most common for the majority of arboviruses (Weaver and Reisen, 2010). The arboviruses include a wide variety of RNA virus family including the Alphaviruses, the Flaviviruses, the Bunyaviruses, the Orbiviruses, the Vesiculoviruses and the Thogotoviruses. These groups of RNA viruses have a variety of RNA genomes and replication processes. The only known DNA arbovirus is the African swine fever virus, an Asfarvirus of family Asfarviridae (van Regenmortel *et al*., 2000). The competence posed by these viruses to cause human disease is determined by factors ranging from epidemiology (urbanisation) to viral genetics. Examples of arboviral emergence associated with urbanisation, travel and trade are; the Dengue virus (DENV), Chikungunya virus (CHIKV), Yellow fever virus (YFV) and Rift valley fever virus (RVFV) (Weaver and Reisen, 2010).

In conjoint CHIKV, YF, and perhaps also DENV (Holmes and Twiddy, 2003), *Aedes aegypti* also certainly originated from Africa (Moore *et al*., 2013). Globally *Ae. aegypti* is the primary vector of most arboviruses, but a collection of other *Aedes* species are proficient and epidemiologically-important vectors. An important member of the *Aedes* genus is the *Ae. albopictus* (WHO, 2014). The Control of *Aedes*-transmitted arboviruses is a major 21st century challenge for global public health, heightened by extensive insecticide resistance in the vectors (Moyes *et al*., 2017) and a persistent growth in urban environments (Alirol *et al*., 2011). A systematic study of the burden, transmission biology and control of *Aedes*-transmitted arboviruses is critical to define, appreciate and prepare for possible future threats in Africa and worldwide (Weaver, 2017; Wetsman, 2017).

2.2 *Aedes* as Vectors of Arboviruses

Aedes is a genus of mosquitoes originating from the tropical and subtropical zones (Womack, 1993), but now it is found on all the continents except the Antarctica. *Aedes* mosquitoes are visually distinctive because they have visible black and white markings on their body and legs. They are diurnal, thus active and bite only during the daytime. The peak biting periods are early in the mornings and in the evenings before dusk (Womack, 1993).

Members of the *Aedes* genus are well-known for various arboviral infections, but the two most prominent species that transmit arboviruses are *Aedes aegypti* and *Aedes albopictus* (Leparc-Goffart *et al*., 2014; CDC, 2016).

2.2.1 *Aedes aegypti*

The *Ae. aegypti* can be identified by white markings on its legs and a marking in the form of a lyre on the superior surface of its thorax. This mosquito originated in Africa (Mousson *et al*., 2005; Brown *et al*., 2014).

Only the female bites for blood, which is essential for maturing and nourishing her eggs (Muktar *et al*., 2016). To find a host, these mosquitoes are attracted to cues emitted by vertebrates (birds and mammals) such as body temperature (heat) and chemical compounds, including ammonia, carbon dioxide from sweat and breathing, lactic acid from certain bacteria, octenol from sweat, cholesterol, folic acid, skin lotions, and perfume (O'Brien, 2010).

Adults of the *Ae. aegypti* are highly domesticated mosquitoes, highly anthropophilic and typically endophilic (Scott and Takken, 2012). Although *Aedes aegypti* mosquitoes most commonly feed at dusk and dawn, in shady areas, or when the weather is cloudy, they can bite and spread infection all year long and at any time of the day (Scott and Takken, 2012; CDC, 2016). The *Aedes aegypti* is more closely associated with human habitation. The larvae develop preferentially in artificial containers (Lounibos, 2002; Brown *et al*., 2011, 2014; Powell and Tabachnick, 2013), including discarded car tires, toilet tanks and water storage vessels often in urban settings (Morrison *et al*., 2004; Newswise, Inc., 2008). Although the lifespan of an adult *Ae. aegypti* is two to four weeks depending on environmental conditions (Catherine and Phillip, 2010), the eggs can be viable for over a year in a dry state, which allows the mosquito to re-emerge after hibernation or aestivation (Mortimer, 2010). The anthropophagic behaviour of the *Ae. aegypti* is dependent on expression of the odorant receptor AeegOr4 (McBride *et al*., 2014).

Breeding of the jungle *Ae. aegypti* (*Ae. aegypti formosus*) occurs in both sylvatic and domestic environments. *Ae. aegypti* larvae within domestic environments develop in artificial containers within or in proximity to human habitation whereas larvae of the sylvatic ecotype breed in natural habitats such as rock pools, tree holes, plant axils, fruit husks in forested areas (Powell and Tabachnick, 2013).

Larvae of the two *Ae. aegypti* ecotypes are exposed to different bacterial groups in their respective breeding sites, possibly resulting in variances in vectorial capacity (Dickson *et al*., 2017). Naturally, two morphological subspecies have been acknowledged that generally relate with these ecotypes: *Ae. aegypti aegypti* and *Ae. aegypti formosus*. Evidence however shows that *Ae. aegypti formosus* is increasingly found in urban environments (Powell, 2016), and the indicative morphological characters i.e. presence/absence of white abdominal scaling patterns (Mattingly, 1967) often differentiates the ecotypes. On the contrary, clear genetic boundaries are absent, probably as a result of widespread of recent historical gene flow (Huber *et al*., 2008; Paupy *et al*., 2010).

2.2.2 *Aedes albopictus*

Aedes albopictus, referred to as the Asian tiger mosquito is the most invasive species; which occurs even in temperate regions. In recent times the distribution of *Ae. albopictus* from Asia to areas of Africa, Europe and the Americas by the used tire trade has escalated (Delatte *et al*., 2009; Medlock *et al*., 2012; Carvalho *et al*., 2014).

Aedes albopictus in contrast to Ae. *aegypti* is usually exophagic and bites humans and animals opportunistically (Paupy *et al*., 2009), but it has also been shown to exhibit anthropophilic behaviour similar to *Ae. aegypti* (Ponlawat and Harrington, 2005; Delatte *et al*., 2010). *Aedes albopictus* thrives in a varied range of habitats than *Ae. aegypti*; this includes coconut husks, cocoa pods, bamboo stumps, tree holes and rock pools. In addition to artificial containers such as vehicle tyres and flower pot saucers. They also show similar larval development behaviour in artificial containers as *Ae. aegypti*. This diversity of habitats explains the abundance of *Ae. albopictus* in rural as well as peri-urban areas and shady city parks, feeding readily on a diversity of mammalian and avian species (Gratz, 2004).

2.2.3 Other *Aedes* Species

The possibility of arboviruses to adapt to new vectors rapidly occur, and this can have great significant consequences (Weetman *et al*., 2018). Other *Aedes* species play a vital role in the transmission of arboviruses in Africa because they link sylvatic and human transmission cycles and/or they are involved in sylvatic arbovirus transmission cycles. For example, *Aedes africanus* is considered as the main vector of yellow fever virus in Africa within the sylvatic environment (Haddow *et al*., 1948) and can also act as a bridge vector to humans, together with *Ae. luteocephalus, Ae. taylori, Ae. bromeliae*, *Ae. furcifer, Ae. metallicus, Ae. opok, Ae. vittatus*, and species of the *Ae. simpsoni* complex (Hanley *et al*., 2013). Sylvatic dengue viruses in Africa are transmitted among non-human primates by *Ae. furcifer* and *Ae. luteocephalus* within the sylvatic habitat, and usually cross over to humans through biting by *Ae. furcifer* (Hanley *et al*., 2013). Bridge vectors may initiate a human outbreak, but large epidemics typically occur only when virus transmission involves urban populations of *Ae. aegypti* or *Ae. albopictus*, though there can be exclusions (Weetman *et al*., 2018). The main stream of these *Aedes* vector species are established in rural or forest areas, and so, are less likely to present a threat in the urban environments where *Ae. aegypti* populations thrive (Weetman *et al*., 2018). Nevertheless, increasing erosion of their natural habitats could lead to contact with humans, and/or previously obligate sylvatic species might adapt to new urban environments and hosts, potentially with a greater role as vectors (Weaver and Reisen, 2010). Many readily feed on animals (both domestic and wild (primates)), as well as humans, hence their potential importance as bridging vectors and zoonotic transmissions (Diallo *et al*., 2005 and 2013).

2.3 Spatial Distribution of *Aedes* Mosquitoes

The extent of arbovirus distributions in Africa is largely unknown, and knowledge of the variation in transmission risk within these zone is hampered by a lack of disease data (Weetman *et al*., 2018). Knowledge of the distribution of their shared vectors, the *Aedes mosquito* is incomplete and is complicated by travel and trade (Kraemer *et al*., 2015a). Kraemer *et al*. (2015a), in their study, showed that these *Aedes* mosquitoes are now located across all continents, including North America and Europe.

Given the public health impact of these arboviral diseases and their rapid global spread, understanding the current and future distribution, and determining the geographic limits of transmission and transmission intensity, will enable more efficient planning for vector and disease control (Carrington and Simmons, 2014; Semenza *et al*., 2014). Because these diseases can only persist where the vectors are present (Brown *et al*., 2014).

Year-round transmission of arboviruses by the arthropod vectors can be favoured by the warm climatic condition. Spatial distribution of both species is grounded on environmental factors, often with a select focus on rainfall, humidity, vegetation, temperature and demographic factors such as urbanisation (Kobayashi *et al*., 2002; Lounibos, 2002; Brown *et al*., 2014). Survival of *Aedes* mosquitoes is highly dependent on temperature and water availability (Luz *et al*., 2008). *Aedes albopictus* has higher survival rates than *Ae. aegypti*, though adults of the latter can tolerate a wider range of temperature. Adult mosquitoes and their eggs require moisture to survive, with low moisture levels affecting adult mortality (Russell *et al*., 2001). Vegetation canopy cover decreases evaporation and wind speed, thereby protecting mosquito breeding and development sites (Fuller *et al*., 2009; Hahn *et al*., 2014).

Ecological models have been used to estimate the ranges and spatial distributions of arboviruses (Bhatt *et al*., 2013; Messina *et al*., 2016). African ranges are unknown, even for the two major *Aedes* vector species, and there are insufficient data on their distribution (Kraemer *et al*., 2015a), and though the tropical region of Africa indicate extensive areas of suitability and a large potential for sympatric occurrence, these estimated distributions need to be treated with caution (Weetman *et al*., 2018). This geographical location indicates where the species could potentially occur but not necessarily where they have been found. For example, much of sub-Saharan Africa is predicted to be suitable for *Aedes* vectors, but records remain patchy (Kraemer *et al*., 2015b). Since the worldwide records of each species were complied, new studies in Africa have reported *Ae. aegypti* in Ghana (Kawada *et al*., 2016), Mozambique (Massangaie *et al*., 2016) and Namibia (Noden et al., 2014), and *Ae. albopictus* in Mali (Müller *et al*., 2016), Morocco (Bennouna *et al*., 2017), Mozambique (Kampango and Abilio, 2016) and São Tomé and Príncipe (Reis *et al*., 2017).

2.4 Vector Control Strategies for *Aedes* mosquitoes

Preventing or reducing arbovirus transmission depends exclusively on controlling the mosquito vectors or interruption of human–vector contact. The WHO endorses the strategic approach known as Integrated Vector Management (IVM) for control mosquito vectors (WHO, 2017b). The ultimate goal is to prevent the transmission of vector-borne diseases. Transmission control activities should target the egg, larva, pupa and adult stages in the household and immediate vicinity. This includes other settings where human–vector contact occurs, such as schools, hospitals and workplaces.

Aedes mosquitoes use a wide range of confined larval habitats, both man-made and natural. Control efforts should target the habitats that are most productive. Vector transmission is reduced through the use of IVM.

Management of breeding habitats can be effective for Dengue (Boyce *et al*., 2013). Chemical and biological larviciding formulations, such as pyriproxyfen and *Bti* respectively, represent vital implements in the arsenal for control of *Aedes* immatures (Abad-Franch *et al*., 2017). Attractive toxic sugar baits (ATSB) might eventually prove to be effective against *Aedes* species, whilst evading deleterious effects of traps on economically-beneficial insects (Fiorenzano *et al*., 2017).

2.5 Insecticide Resistance in *Aedes* Mosquitoes

The use of safe and efficacious insecticides against the adult and larval populations of mosquito vectors is one of the most effective ways to rapidly interject transmission of arboviruses (WHO, 2016b). However, with time, resistance to these insecticides develop.

Insecticide resistance is a change in susceptibility of mosquito population to an insecticide, which is generally reflected in the product's failure to achieve the expected level of control or efficacy. Control of arboviral vectors (*Aedes* mosquito) is the key approach to prevent arboviruses. The use of pyrethroids such as deltamethrin and permethrin as a fraction of all the total insecticides use for dengue control has increased over the past decade (WHO, 2011). Organophosphates, such as pirimiphos methyl, malathion and fenitrothion are also widely used, predominantly in response to disease outbreaks or, at times, when densities of mosquitoes are high. The organochloride, dichlorodiphenyltrichloroethane (DDT) played an essential role in the control of *Aedes* mosquitoes for several years; however, it is now infrequently used.

While insecticide-based interventions have efficiently controlled *Aedes* mosquito populations for several decades, resistance has now developed (Ranson *et al*., 2010) as a result of the dependence on a few active ingredients registered and used in public health. Resistance to all four classes of insecticides has developed in *Ae. aegypti* (Ranson *et al*., 2010).

There is paucity of data on insecticide resistance in *Aedes* mosquitoes worldwide, the majority of data originating from South-East Asia and Latin America (Moyes *et al*., 2017). Resistance to DDT is pervasive and there have been reports from every country where it has been tested, and in both *Ae. aegypti* and *Ae. albopictus* (Weetman *et al*., 2018). Resistance to pyrethroids (primarily permethrin and deltamethrin) is patchy but there are confirmed reports in *Ae. aegypti* from mainland West, Central and East Africa (Moyes *et al*., 2017). It should also be noted that doses of pyrethroids used for *An. gambiae* are almost always used for assessment of pyrethroids in *Aedes* mosquitoes; this probably underestimates resistance because *Ae. aegypti* discriminant doses are lower (WHO, 2016a). Pyrethroid resistance may be less critical presently in Latin America and South East Asia (Moyes *et al*., 2017), but appears to be developing in Africa. This is not restricted to particular regions. There has been infrequent use of organophosphates (malathion or fenitrothion), but resistance to this insecticide by *Ae. aegypti* has only been reported in Portugal, Madeira and Sudan. In West Africa, all studies testing carbamates (usually propoxur) have reported resistance. Susceptibility in other studies has also been reported elsewhere, but very recent testing in Yaoundé, Cameroon, reported bendiocarb resistance in both *Ae. aegypti* and *Ae. albopictus* (Kamgang *et al*., 2017). Fortunately, first-line biological (*Bti*) and chemical (temephos) larvicides, have not recorded any challenging level of resistance in West Africa. Susceptibility to *Bti* is expected based on its multifaceted mode of toxicity and absence of any prior reports of resistance in *Aedes* populations in the field (Moyes *et al*., 2017). In contrast, Latin America and Asia have recorded high levels of temephos resistance (Moyes *et al*., 2017), yet due to apparently full susceptibility in Africa, temephos is regarded as a possible option for water treatment.

Resistance in *Ae. albopictus* appears relatively low, compared to *Ae. aegypti* (Ranson *et al*., 2010).

This is possibly so because *Ae. aegypti* mosquitoes have been pre-exposed to household insecticides and organized indoor spray treatments more often than *Ae. albopictus*. This can be attributed to the endophilic and exophilic behaviours demonstrated by the *Ae. aegypti* and *Ae. albopictus* respectively. However, due to the increase of *Ae. albopictus* populations into areas where insecticide use is intensive; adulticides, or selection pressure by agriculture in its new breeding sites (Gratz, 2004), it is highly probable that insecticide resistance will finally, negatively influence our ability to control this vector as well in the near future. There are a number of possible adaptations that permit a mosquito to endure harmful doses of an insecticide, usually classified based on their biochemical/physiological properties as either mechanisms of decreased response to the insecticides (alterations in the target site), or mechanisms of decreased exposure (increased excretion/reduced uptake, distribution, detoxification) (Taylor and Feyereisen, 1996). Most cases consist of alterations in the sensitivity of the insecticide target due to mutations, which decreases the affinity of the insecticide for its target, or detoxification/sequestration of the insecticide before it reaches its target site due to variations in detoxification enzymes (Li *et al*., 2007; Stevenson *et al*., 2012).

Insecticide resistance monitoring in field populations of *Aedes* is required to determine the levels, mechanisms and geographical distribution of resistance in order to select appropriate insecticides for vector control. Evidence-based decisions will ensure that effective insecticides are selected and used. Changes in insecticide susceptibility status should also direct policy and operational decisions (WHO, 2016b). Insecticide resistance monitoring is an essential part of entomological surveillance. Together with information on adult mosquito density, larval and pupal densities, ecology and habitats, and efficacy of vector control interventions, suitable responses to prevent and control arboviruses can be developed (WHO, 2016b).

2.5.1 Mechanism of Resistance in *Aedes* Vectors

Presently there is limited information on the mechanisms of insecticide resistance in African populations of key *Aedes* vector species. Knockdown resistance (kdr) mutations in the voltage-gated sodium channel, which can confer high levels of resistance when present in combinations (Hirata *et al*., 2014), have in recent times been detected in West Africa. However, limited investigations have been carried out elsewhere (Weetman *et al*., 2018). High frequencies of F1534C, the only kdr mutation known to show a worldwide distribution (Moyes *et al*., 2017), has been found in samples from Ghana (Kawada *et al*., 2016), while the Latin American V1016I variant have been detected in a single Ghanaian sample (Weetman *et al*., 2018).

In the absence of target site mutations, indication for metabolic resistance through efficiency of synergists in bioassays or high activity of mixed-function esterases and glutathione-S-transferases and oxidases in biochemical assays has been recommended as, at least, a partial explanation for some resistant phenotypes (Weetman *et al*., 2018). The bases of resistance in African *Aedes* populations is less obvious compared to areas of Asia and Latin America which have been subjected to targeted control programmes. Extensive use of DDT and pyrethroids for indoor residual spraying (IRS) against malaria vectors might be one reason; even so, introductions, rather than simply in situ selection from local genetic variation, might be important. For example, the F1534C kdr mutation detected in Africa is associated with a non-African, presumably immigrant, haplotype (Kraemer *et al*., 2015). In Madeira, Portugal, a multiple-insecticide resistant *Ae. aegypti* population already equipped with two kdr mutations and over expression of several pyrethroid metabolising genes (Kasai *et al*., 2014) may have been introduced in recent times, possibly from Latin America (Seixas *et al*., 2017). In general, insecticide resistance by vectors suggests that viable insecticidal options remain accessible to target African *Aedes* populations.

However, the possibility for insecticide resistant *Ae. aegypti* and *Ae. albopictus* to be introduced through human-aided movement of mosquitoes over long distances (Grigoraki *et al*., 2017), rather than through slow natural migration, is a major concern, and a challenge to prevent the spread of resistance.

2.6 Blood Meal Sources

The identification of the source of blood meal in mosquito vectors is important in understanding the transmission dynamics of vector-borne pathogens and vectorial capacity of species and populations (Niare *et al*., 2016). To obtain an overview of human risk exposure to *Aedes* bites, the identification of blood meal sources in recently blood-fed mosquito vectors is an alternative for assessment of hosts-vector contacts (Niare *et al*., 2016). This is because their feeding behaviour differs, with some species feeding exclusively on either humans (anthropophily) or animals (zoophily), or both. *Aedes* species that take human blood are potential vectors of arboviruses of public health importance and those that feed on both human and animals are potential vectors to zoonoses. Host-feeding patterns and preferences vary according to environmental factors, including host availability and abundance, flight behaviour and feeding periodicity of mosquitoes (Molaei *et al*., 2008).

Blood meal Polymerase chain reaction (PCR) yields species-specific fragments of varying sizes of amplified products from the Cytochrome b gene encoded in the mitochondrial genome of vertebrates. Successful identification of hosts by PCR-based methods are directly linked to the quality and quantity of the vertebrate's DNA contained in the mosquito abdomen (Ngo and Kramer, 2003).

Therefore, identification of the blood meal source(s) of species-identified female mosquitoes provides relevant information about possible zoonotic transmission and arbovirus vector incrimination.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Study Site

This study was carried out in both rural and urban sites in three ecological zones of Ghana from May 2017 to May 2018. The sahel savannah ecological zone consisted of Larabanga in the West-Gonja District (rural), Navrongo in the Kassena-Nankana District (urban) and Paga in the Kassena-Nankana West District (rural). The site within the forest zones consisted of Dwease in the Konongo district. The coastal sites consisted of Ada Foah in the Ada East District (rural), Tema in the Tema Metropolitan District (urban), and Accra in the Accra Metropolitan District (urban).

Urban defines geographic areas that are situated within towns and cities while rural defines geographic areas that are situated outside towns and cities, typically less developed with significant land cover under agriculture and/or natural vegetation.

Larabanga is a small town but it is the capital of the West-Gonja District in the Northern Region of Ghana. It is about 5 km from Mole, the biggest game reserve in the country. It is located on the Fufulso-Damongo road at coordinates 9°5′0″ N and 1°49′0″ W. The game reserve is about 300 km^2 and harbours diverse species of animals including chimpanzees, elephants and antelopes. The settlements are dispersed and nucleated with mud houses and a few cement block houses. Majority of the inhabitants are farmers and keep poultry and livestock as well. The vegetation is typical sahel savannah with short trees including baobab and shea butter and long grass.

Navrongo is the capital of the Kassena-Nankana District which is within the Upper East Region of Northern Ghana. It is sited within the North-Eastern part of Northern Ghana and lies beside the Burkina Faso border at coordinates 10°53'5"N and 01°05'25"W.

The landscape is flat and the ecology is typical of Sahel savannah with arid grassland with occasional shrubs. Navrongo has a variation of dispersed and nucleated settlements with houses built from cement blocks. The inhabitants are into different occupations which includes livestock farmers. Some of these animals are kept semi-intensively, hence found in and around the town

Paga, a town within the Upper East Region is the capital of the Kassena Nankana West District. It is situated on the border of Burkina Faso and it is about 166 km south of Ouagadougou with coordinates 10°59'32"N and 01°06'48"W. Paga is composed of scattered settlement with mud houses and a few cement block houses. Majority of the inhabitants are farmers and keep poultry and livestock as well.

Dwease is a farming/mining community situated in the Konongo District of the Ashanti Region of Ghana. It is about 53 kilometres (33 miles) from Kumasi with coordinates 06°37′00″N and 001°13′00″W. The town has a nucleated settlement with variation of housing types made of mud and cement blocks. The climate is the wet semi equatorial type with monthly average rainfall between 120 and 150 mm. Dwease has a tropical savannah climate with monthly mean temperatures of 26°C or above.

Ada Foah is a town in the Greater Accra Region of Ghana with coordinates 5°47' N and 0°38' E. The town is situated along the Volta River just off the Accra-Aflao motorway and it is also the capital of the Dangme East District and the seat of the District Assembly. Ada Foah has a mixture of nucleated and linear settlements, the housing is made from cement blocks with some inhabitants keeping poultry and some livestock in an intensive and semi-intensive farming system.

Ada Foah has a tropical savannah climate with monthly mean temperatures above 18°C (64°F) in every month of the year and typically a pronounced dry season, with the driest month having precipitation of less than 60 mm

Tema is a city on the Bight of Benin and the Atlantic coast of Ghana. It is located 25 kilometres (16 miles) east of the capital city, Accra with coordinates 5°40′0″ N and 0°0′0″ E; in the region of Greater Accra, and it is the capital of the Tema Metropolitan District. Tema is made up of nucleated and linear settlements, the housing is made from cement blocks. Some of the inhabitants keep poultry and some livestock in an intensive and semi-intensive farming system. Tema features a dry equatorial climate. It lies in the driest part of southern Ghana, experiencing an average annual rainfall of about 750 millimetres (30 in). Average temperatures are high, often exceeding 30°C all year-round.

Accra is the capital and the most densely inhabited city of the Greater Accra Region and the Republic of Ghana. It stretches along the Ghanaian Atlantic coast and extends north inland with coordinates 5°33′0″ N and 0°12′0″ W. Accra lies in the coastal savannah and features a tropical savannah climate that consists of a hot semi-arid climate. Accra has a mixture of clustered and linear settlements with cement block housing type. Some inhabitants keep poultry and some livestock in an intensive and semi-intensive farming system. The average annual rainfall is about 730 mm, which falls primarily during Ghana's two rainy seasons. The chief rainy season begins in April and ends in mid-July, whilst a weaker second rainy season occurs in October. The mean monthly temperature ranges from 25.9 °C (78.6 °F) in August (the coolest) to 29.6 °C (85.3 °F) in March (the hottest), with an annual average of 27.6 °C (81.7 °F).

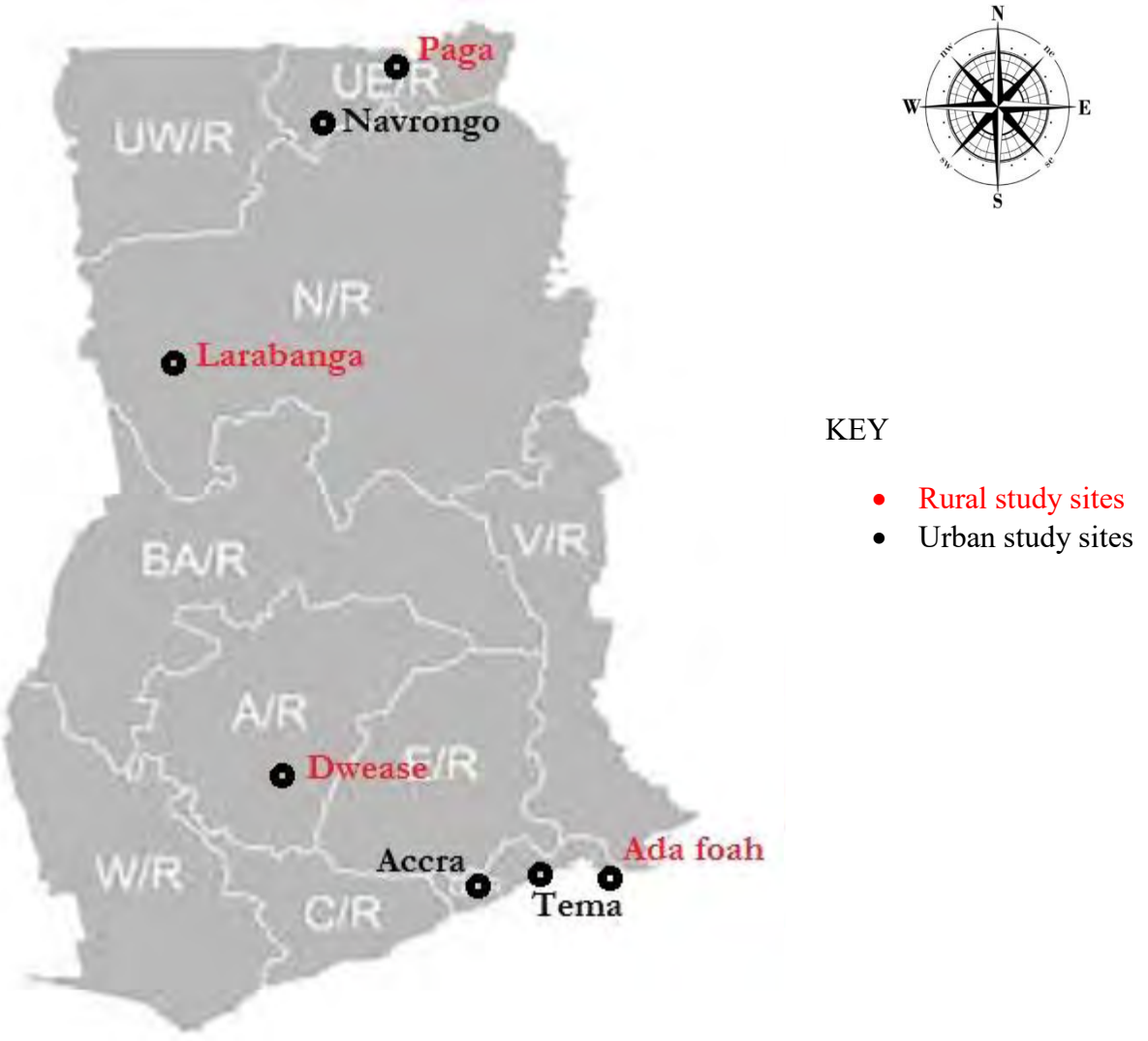


Figure 3.1: Map of Ghana showing the study sites. Urban sites (black) and rural sites (red).

Source: (Self)

3.2 Spatio-temporal Distribution of *Aedes* Mosquitoes

The spatial and temporal distribution of adult *Aedes* mosquitoes were determined by sampling both indoors and outdoors during the wet and dry seasons. Three (3) sampling methods were employed to collect the vectors indoors and outdoors, namely, Biogent sentinel-2 mosquito traps (BG traps) (Biogents AG Weissenburgstr 22 93055 Regensburg, Germany), human landing catch (HLC) and prokopack (PPK) (Vazquez-Prokopec *et al*., 2009).

Human dwellings and outdoor situations (parks/garden) from all sites were sampled to determine the presence and densities of *Aedes* mosquitoes. Ovitraps and larval sampling was employed to sample *Aedes* immatures from all sites. The GPS coordinates of all collection points were taken.

3.2.1 Adult Mosquito Collection Using Biogent Sentinel Traps (BG traps)

BG traps were set both indoors (sitting room) and outdoors (open, but secure, verandas or under a shed/tree about 2 meters from the house) during the hours of 5:00 am-8:00 am and 3:00 pm-7:00 pm. The BG traps were baited with carbon dioxide (CO_2) which was produced by either BG-lures or from a mixture of 17.5 g yeast (Angel Yeast (Egypt) Co. Ltd.), 250 g sugar in 1 litre of water (Ndenga *et al*., 2017). At 1-hour interval the mosquito collection bag of the BG-trap was changed. Mosquitoes trapped within the collection net were placed in a cooler box containing ice and then transported to the insectary at the Noguchi Memorial Institute for Medical Research. Twenty houses were randomly selected in each site, with 4 houses sampled each day. Sampling using the BG trap was done for four consecutive days.

3.2.2 Adult Mosquito Collection Using Human Landing Catch (HLC)

Four houses were randomly selected at each site. On each day, two people were positioned to catch *Aedes* mosquitoes indoors by human landing catches and another two sat outdoors. This was done in 4 days at each site. Collection was done hourly from 5:00 am-8:00 am and then from 3:00 pm-7:00 pm. Collected *Aedes* were placed in paper-cups, placed in cool box with ice packs and transported to the laboratory for identification.

Sampling was done during the dry season from January 2018 - March 2018 in the coastal savannah and forested savannah, and from November 2017 – December 2017 in the sahel savannah, and during the wet season from May 2018 - June 2018 in the sahel savannah, and from August 2017 - October 2017 in the coastal savannah for Adult Mosquito.

3.2.3 Adult Mosquito Collection Using Prokopack Automated Aspirators (PPK)

The Prokopack was only used in three (3) study sites namely, Larabanga, Navrongo and Paga. Twenty (20) houses were randomly selected for *Aedes* collection. Sampling for *Aedes* mosquitoes was done indoors (bedroom and sitting rooms) and outdoors (veranda and roofing crevices) for 4 days (five houses per day). Both indoor and outdoor sampling was done simultaneously. Collected *Aedes* were placed inside paper cups, labelled, placed in a cooler box containing ice pack and transported to the insectary for identification.

3.3 Distribution of Larval *Aedes* mosquitoes

Larval surveys were conducted by searching for water holding containers and recording their types and site locations. The presence of immature stages of mosquitoes was visually evaluated in all water-holding containers present within the sampling area.

The number and type of containers inspected were recorded, including information on which had or did not have immature stages of mosquitoes. Larvae and pupae presumed to be *Aedes* mosquitoes were into a plastic bowl collected using a pipette and ladle. They were placed immediately in labelled specimen bowls with water filled from a water container after collection and transported to the insectary at NMIMR. The entire contents of the various containers were emptied into a large larval tray and the immature *Aedes* specimens were picked out using a dropper. Oviposition traps were also set up at sentinel breeding sites. All collected larvae and pupae were reared in the laboratory until adults emerged. The GPS coordinates of all collection points were taken.

3.4 Laboratory Analysis

3.4.1 Morphological Species Identification

At the insectaries, all mosquitoes were killed by holding them at -20°C in a freezer or chloroform for 1 minute. Sorting and identification was done to determine the genus (*Aedes*, *Anopheles* or *Culex)* and sex. Females *Aedes* mosquitoes were further grouped according to their gonotrophic status as unfed, blood-fed, half-gravid or gravid. Further morphological identification of *Aedes* mosquitoes was done using identification keys by Huang *et al*., (2004), to differentiate the *Ae. aegypti* and the other *Aedes* species.

3.4.2 Phenotypic Resistance in *Aedes* Mosquitoes

3.4.2.1 Larval collections: To determine phenotypic resistance status of *Aedes* mosquitoes, larvae collected from the study sites (Ada Foah, Tema, Accra, Dwease, Larabanga, Navrongo and Paga) were raised to adults in the insectary and 3-5 days old females which were fed sugar but not blood-fed before were used to undertake the WHO susceptibility bioassay test.

3.4.2.2 WHO susceptibility bioassay Test: To undertake the susceptibility test, WHO standard test strips of two Pyrethroids (0.05% deltamethrin and 0.75% permethrin); one Organochloride (4% DDT); one Organophosphate (5% malathion) and one carbamate (0.1% bendiocarb) were used. Four replicates and 2 controls were used for each insecticide (WHO, 2016a).

The procedures for the WHO Susceptibility test is as spelt below (WHO, 2016a)

Six sheets of clean white paper (12 × 15 cm) rolled into a cylinder shape, were inserted into six holding tubes (with the green dot), one per tube, and fastened into position against the wall of the tube with a steel spring wire clip. The slide unit is attached to the tubes at the other end. One hundred and twenty (120) to 150 active female mosquitoes were aspirated (in batches) from a mosquito cage into the six green-dotted holding tubes through the filling hole in the slide, to give six replicate samples of 20–25 mosquitoes per tube.

Once the mosquitoes were transferred, the slide unit was then closed and the holding tubes set in an upright position for 1 hour. At the end of this time, any moribund mosquitoes (i.e. those unable to fly) and dead mosquitoes were removed.

Oil-treated paper (the control) was inserted into each of two yellow-dotted tubes, ensuring that the label of the paper is visible on the outside of the tube. The paper was fastened with a copper clip and the tube closed with a screw cap.

Four (4) exposure tubes with red dots were prepared in much the same way as the yellow-dotted tubes. Each of the four red-dotted exposure tubes were lined with a sheet of insecticide-impregnated paper such that the print label was visible on the outside.

Each paper was then fastened into its position against the wall with a copper spring-wire clip and the tube was closed with a screw cap.

The empty exposure tubes were then attached to the vacant position on the slides and, with the slide unit open, the mosquitoes were blown gently into the exposure tubes. Once all the mosquitoes were in the exposure tubes, the slide unit was closed and the holding tubes were detached and set aside.

Mosquitoes were kept in the exposure tubes, which were set in a vertical position with the mesh-screen end uppermost for a period of 1 hour. The tubes were placed in an area of reduced lighting or covered with cardboard discs to reduce light intensity and to discourage test mosquitoes from resting on the mesh screen lid.

At the end of the 1-hour exposure period, the number of knocked down mosquitoes were counted and recorded. An adult mosquito was considered to be alive if it was able to fly, regardless of the number of legs remaining. A mosquito was classified as knocked down if it was immobile or unable to stand or take off. The mosquitoes were then transferred back to the holding tubes. The exposure tubes were detached from the slide units. A pad of a cotton wool soaked in 10% sugar solution was placed on the mesh-screen end of the holding tubes.

The mosquitoes were then maintained in the holding tubes for 24 hours in a chamber maintained at 27°C ± 2°C temperature and 75% ± 10% relative humidity. Temperature and humidity was recorded with a hygrometer and a thermometer during the recovery period.

At the end of the recovery period (i.e. 24 hours post-exposure), the number of dead mosquitoes were counted and recorded.

An adult mosquito was considered to be alive if it was able to fly, regardless of the number of legs remaining. A mosquito was classified as dead if it was immobile or unable to stand or take off.

On completion of the susceptibility test, mosquitoes were transferred into individual, clearly labelled eppendorf tubes (separating dead and live mosquitoes into separate tubes) for preservation until such time as they can be transferred to suitable facilities for species identification and supplementary testing.

3.5 Blood Meal Analysis of Engorged Female Mosquitoes

Bloodfed *Aedes* mosquitoes were preserved in absolute ethanol delivered into 1.5ml eppendorf tubes or the blood from the abdomen was squeezed and stained on pre-labelled filter paper and stored and preserved with silica gel in plastic zip lock bags. This was carried to the laboratory for further molecular analysis by PCR.

3.5.1 DNA Extraction for Blood Meal Analysis

DNA was extracted from the blood engorged *Aedes* using QIAamp DNA extraction kit (QIAGEN, Hilden, Germany). Blood stain of individual mosquitoes on filter papers or abdomen of bloodfed mosquitoes were grounded in 100µl grind buffer in microfuge tubes. The tubes were immediately placed in 65°C water-bath for 20-40 minutes. Fourteen (14) microlitres of 8M potassium acetate was added and mixed well. The mixture was then cooled on crushed ice for 30 min and then centrifuged at top speed for 10 min. The supernatant was saved in a new sterile microfuge tube. Two hundred microlitres (200µl) of 95% cold ethanol was added, placed in a freezer for 10 min and inverted (10x) after refrigeration. Samples were then spinned at 15000 rpm for 20 min and the ethanol poured off, 200 ul of 70% ethanol was then added and spinned at 15000 rpm for 5 min. The ethanol was poured of and 200µl of 95% ethanol was added and then poured off again.

The tubes were then arranged on its side on a clean tissue paper and allowed to dry completely (usually for about an hour). Pellets were resuspended in 100µl of distilled water for at least 15 min.

3.5.2 Polymerase Chain Reaction (PCR) Amplification for Blood Meal Analysis

Polymerase chain reaction was performed using a thermocycler (Applied Biosystems™ SimpliAmp™) with reaction conditions consisting of a single step of 95ºC for 15 min, followed by 54ºC for 5 min. After these, the reaction was subjected to 45 cycles of 72ºC for 30s, 94ºC for 20s, and 54ºC for 30s. The final step is a 5 min extension at 72ºC. Size-fraction of the PCR products was performed on 2% agarose gel stained with Ethidium bromide (Biotium, Hayward, California, USA). The gel was ran at 70V for 1h and visualized under ultraviolet light. For each reaction positive controls containing DNA of five different vertebrate (Pig, Human, Goat, Dog and Cow) blood and a negative control of DNA from chicken blood was used, this was because no primer was selected for chicken DNA. Species specific primers that will be used for amplification process are listed below.

Table 3.1: Primer Sequences for the Cytochrome B-Based PCR Blood Meal Identification Assay.

Primer	5'-3' Sequence	Product Size with UNREV1025
Cow121F	CATCGGCACAAATTTAGTCG	561
Dog368F	GGAATTGTACTATTATTCGCAACCAT	680
Goat894F	CCTAATCTTAGTACTTGTACCCTTCCTC	132
Human741F	GGCTTACTTCTCTTCATTCTCTCCT	334
Pig573F	CCTCGCAGCCGTACATCTC	453
UnRev1025	GGTTG(T/G)CCTCCAATTCATGTTA	-

3.6 Ethical Consideration

Ethical approval was sought from the Ethics and Protocol Review Committee (EPRC) of the College of Health Sciences (CHS) of the University of Ghana with protocol identification number: CHS-Et/M.9 – P1.5/2017-2018

3.7 Data Management and Statistical Analysis

Daily densities of *Aedes* mosquitoes were used in all analyses for the mosquitoes collected by BG traps, HLC and PPK.

Adult densities were calculated by the total number of mosquitoes caught per the sum of the number of traps multiplied by trap days.

$$\text{Adult mosquito density} = \frac{Total\ number\ of\ mosquitoes\ caught}{Number\ of\ traps\ used \times Trap\ days}$$

While larval densities were calculated by the number of larvae collected per positive breeding sites

$$\text{Larval density} = \frac{Total\ number\ of\ larvae}{Number\ of\ positive\ breeding\ sites}$$

Data for adult (dry and wet seasons, indoor and outdoor and sampling method) and larval density were compared using ANOVA and independent T- test. Human blood index (HBI) was calculated as number of positive human blood specimens divided by the total number of specimen tested.

$$\text{HBI} = \frac{Total\ number\ of\ positive\ \ human\ blood\ specimens}{Total\ number\ tested}.$$

Statistical Package for Social Sciences (SPSS version 22) SPSS Inc., Chicago, IL and Prism V%.0 (GraphPad Software) were used to analyse the data and the results were presented in the form of tables and graphs. The Frequency distribution was used to determine the most prevalent *Aedes* species in Ghana.

WHO insecticide susceptibility levels were estimated using the WHO criteria (WHO, 2016). With 98% mortality, a test population was considered susceptible; mortalities between 90% and 97% suggested a possible resistance in the mosquito population; and below 90% of the test population was resistant.

CHAPTER FOUR

4.0 RESULTS

4.1 Spatio-temporal Distribution of *Aedes* Mosquitoes in Ghana

4.1.1 Densities of Adult *Aedes* Mosquitoes

A total of 2193 *Aedes* mosquitoes were collected from the study sites. Indoor sampling yielded a total of 473 (22.3%) and outdoor a total of 1720 (77.7%). HLC recorded the highest densities of 210.9 (77%) followed by PPK 74 (17.8%) and BG trap 15.5 (5.2%).

A total of 1689 *Aedes* mosquitoes were collected by HLC: 284 (16.8%) indoors and 1405 (83.2%) outdoors. The highest mosquito density was recorded in Accra, 9.5 followed by Navrongo, 7.0 with the lowest density recorded in Ada Foah 0.9. Out of the total *Aedes* mosquitoes sampled by HLC, 119.43 (19.8%) were collected during the morning hours and 274.1 (80.2%) were sampled during the evening hours.

A total of 114 (5.2%) *Aedes* mosquitoes were sampled using BG traps. 40 (35%) indoors and 74 (65%) outdoors. The highest mosquito density (1.3) were recorded in Accra as compared to the other sites, and the lowest density (0.2) recorded in Larabanga.

A total of 390 (17.8%) *Aedes* mosquitoes were sampled by prokopack (PPK) aspirators only within Larabanga, Navrongo and Paga. A total of 150 (38.5%) mosquitoes from indoor and 240 (61.5%) from outdoor sampling. The highest mosquito density of (30) was recorded in Paga followed by Navrongo, (18.8) and Larabanga, (15). The gonotrophic status of the *Aedes* collected with the Prokopack were 269 (68.9 %) for the unfed, 44 (11.3%) for the blood-fed, 70 (17.9%) for the half-gravid and 7 (1.8%) for the gravid.

4.1.2 Seasonal Densities

The wet season had the highest density 1270 (57.9%) of mosquitoes with Navrongo, 11.4 (32.2%) recording the highest density among all the sites. The lowest density of 0.1 (0.24%) was recorded in Dwease. During the dry season, 923 (42.1%) mosquitoes were sampled. The highest density of 12 (43.3%) mosquitoes was collected in Accra and the lowest of 0.3 (0.1%) was recorded in Paga. These are shown in Figure 4.1 below.

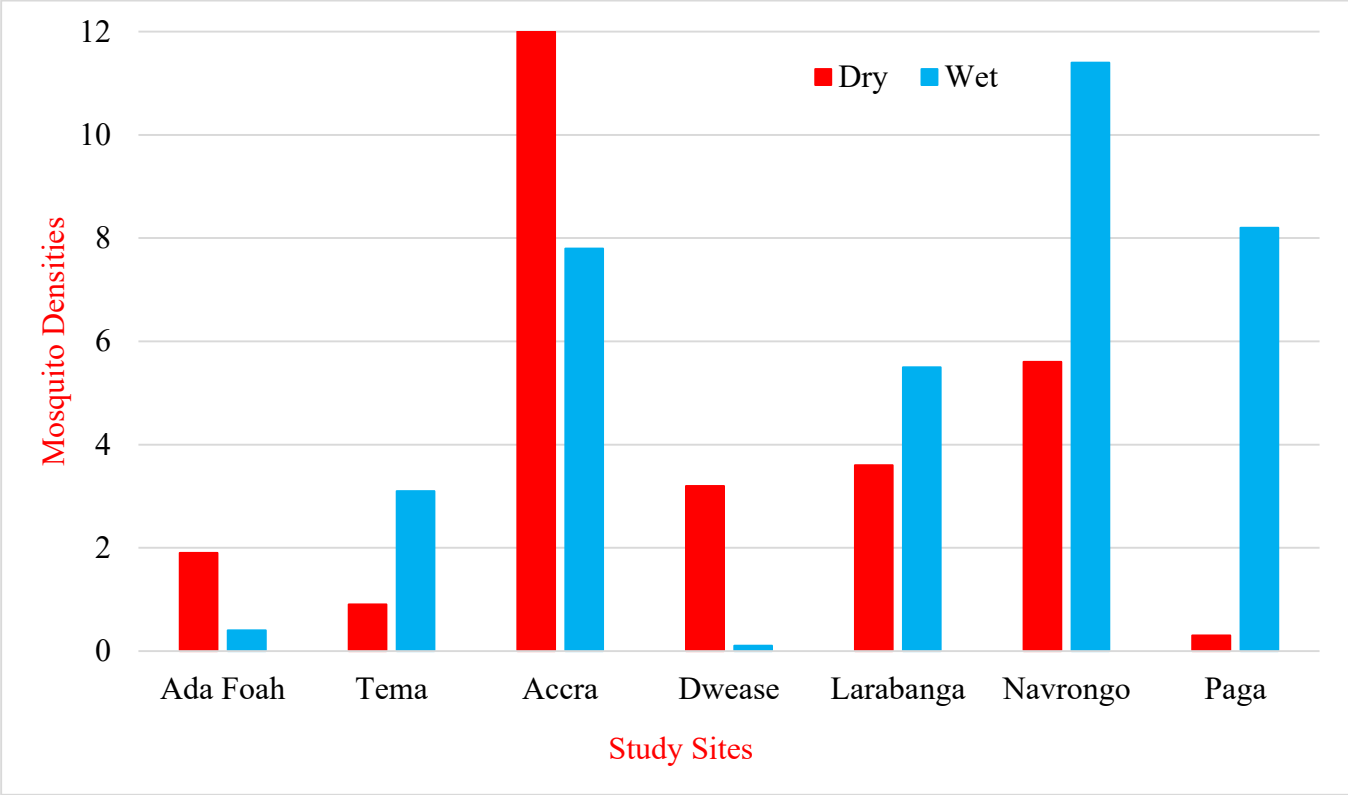


Figure 4.1: Seasonal distribution of *Aedes* mosquitoes at the study sites.

4.1.3 Indoor and Outdoor Densities

Mosquito abundance was highest outdoors compared to indoors except in Larabanga where indoor density 8.2 (34.5%) was equal to outdoor density 8.2 (34.5%). Larabanga had the highest indoor density of 8.2 (34.5%), and the lowest density was found in Tema 0.6 (1.9%). Accra had the highest outdoor density of 19.4 (36.1%)) and the lowest was found in Ada Foah 3.75 (3.49%). These are shown in Figure 4.2 below.

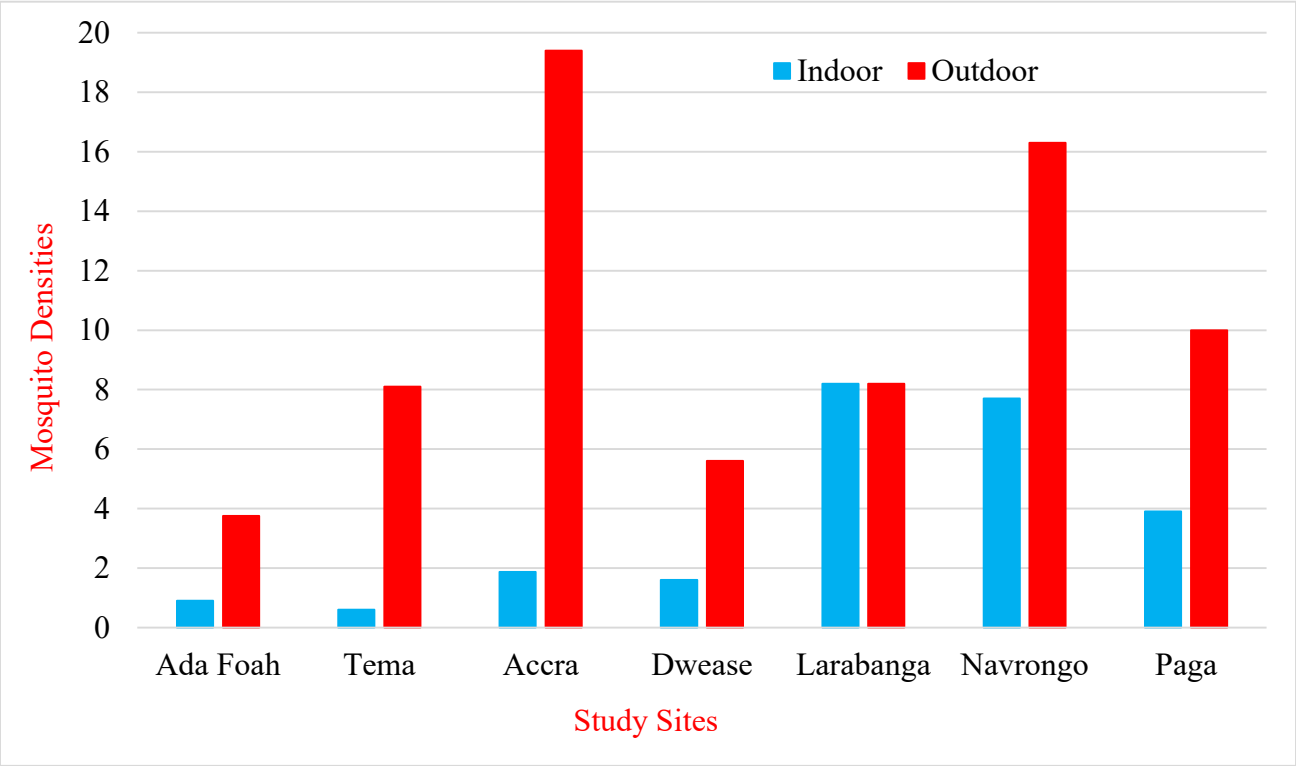


Figure 4.2: Indoor and outdoor densities of *Aedes* mosquitoes at the study sites.

4.1.4 Biting Time

Aedes mosquitoes were observed by HLC to be biting early at dawn between the hours of 5 am to 6 pm and at dusk during the hours of 4 pm to 6 pm. The evening hours recorded a higher biting activity shown in Figure 4.3a below. Host vector contact was high during the wet season as compared to the dry season (Figure 4.3a and 4.3b). The highest peak biting hours were from 5 pm – 6 pm as shown in Figure 4.3a and 4.3b.

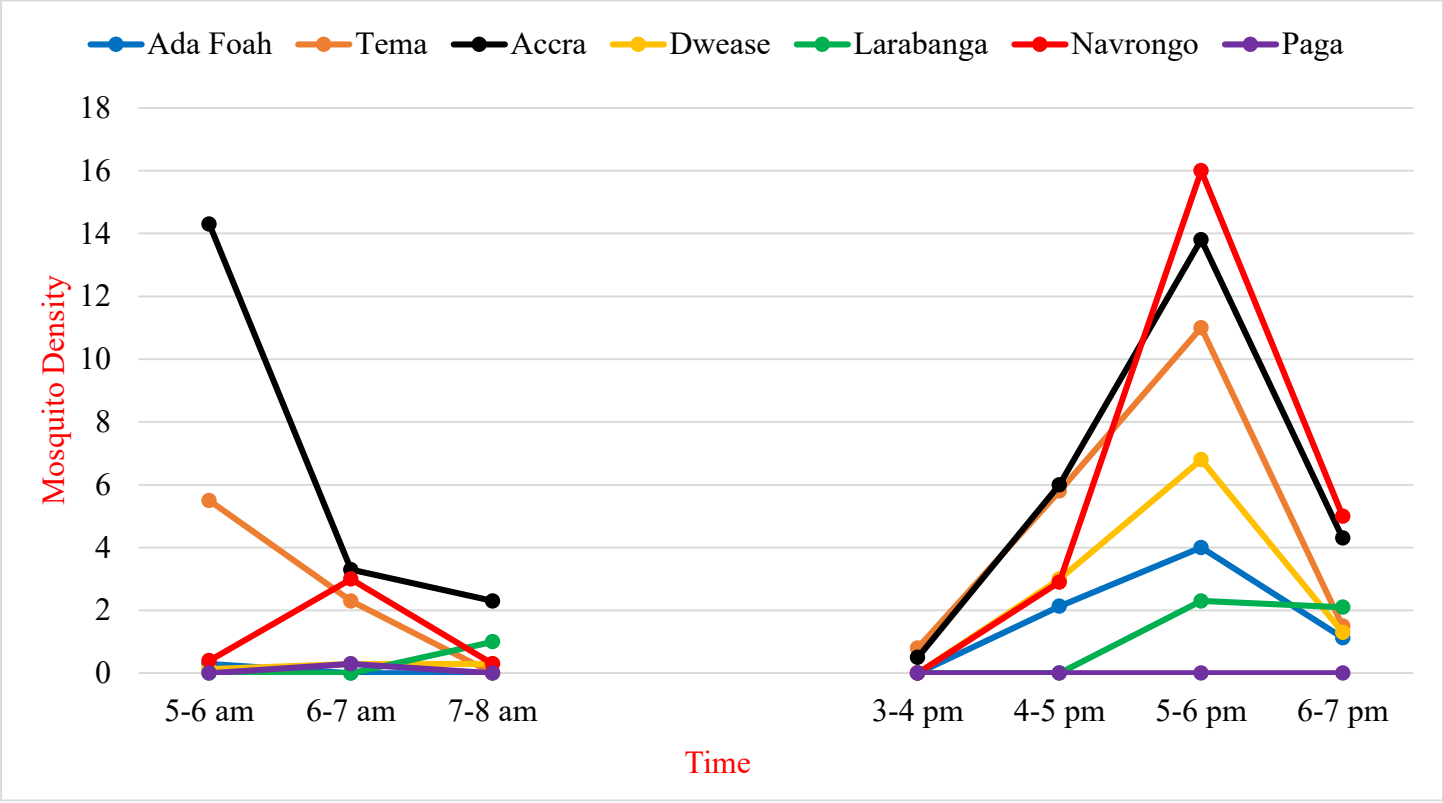


Figure 4.3a: Biting time of *Aedes* mosquitoes at the study sites for dry season.

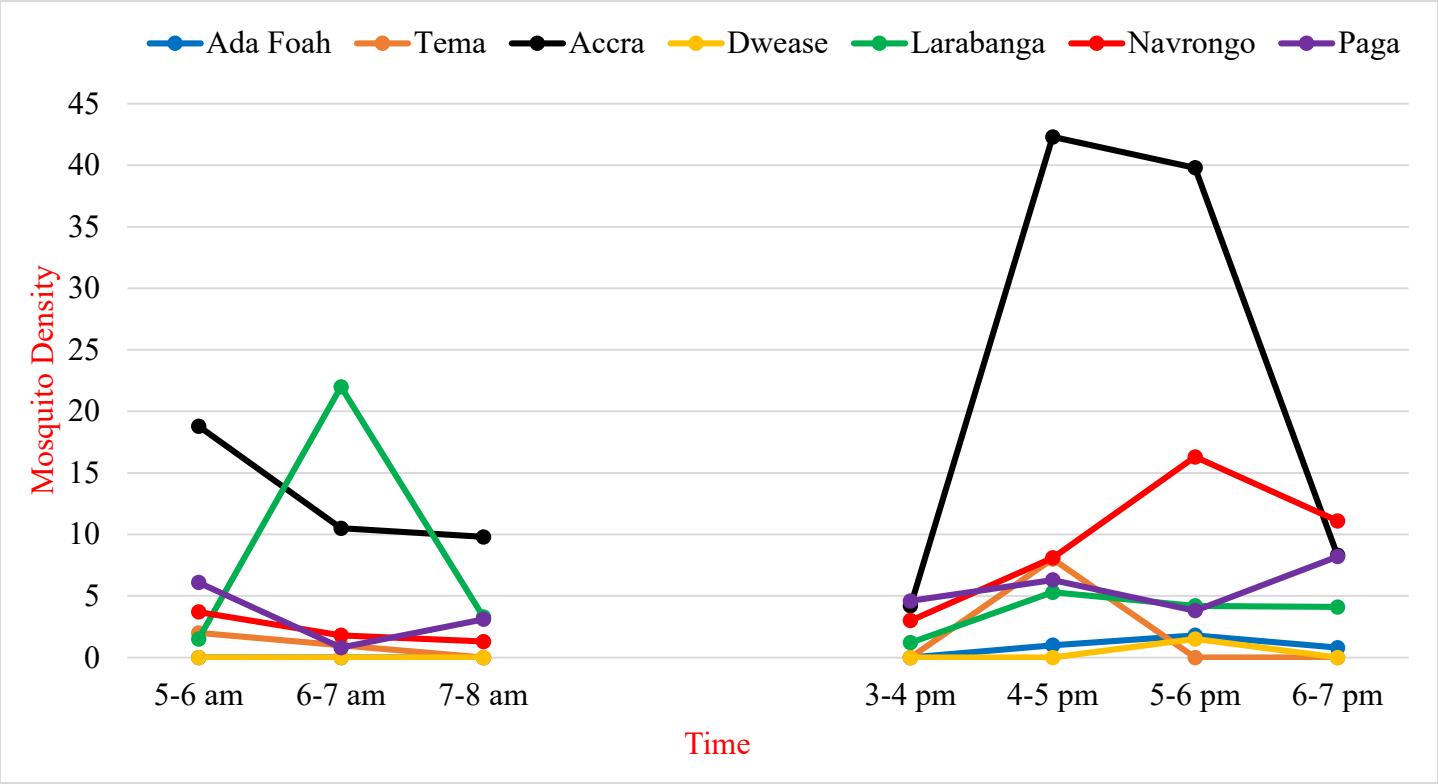


Figure 4.3b: Biting time of *Aedes* mosquitoes at the study sites for wet season.

4.1.5 *Aedes* Species Present in the Study Sites

Morphologically, *Aedes aegypti* 97.3% (2134/2193) were the abundant species present at all sites followed by *Aedes africanus* 2.2 % (49/2193) and *Aedes luteocephalus* 0.05% (1/2193). All the other sites had *Aedes africanus* except Accra, Tema and Paga. *Aedes luteocephalus* was only present in Larabanga. These are shown in Table 4.1 below.

Table 4.1: Morphological species identification of *Aedes* mosquitoes at the study sites.

Study Site	**Total from each site *No. (%)***	***Aedes aegypti* No. (%)**	***Aedes africanus* No. (%)**	***Aedes luteocephalus* No. (%)**
Ada foah	74 (3.4)	62 (83.8)	12 (16.2)	0 (0.0)
Tema	129 (5.9)	129 (100)	0 (0.0)	0 (0.0)
Accra	650 (29.3)	650 (100)	0 (0.0)	0 (0.0)
Dwease	106 (4.9)	86 (81.1)	20 (18.9)	0 (0.0)
Larabanga	327 (14.9)	311(94.5)	15 (4.6)	1 (0.3)
Navrongo	610 (27.9)	608 (99.7)	2 (0.3)	0 (0.0)
Paga	297 (13.6)	297 (100.0)	0 (0.0)	0 (0.0)
Total	**2193 (100)**	**2143 (97.7)**	**49 (2.2)**	**1 (0.05)**

4.2 Larval Densities

From the overall positive breeding sites, 17,657 immature *Aedes mosquitoes were* collected over the entire sampling period, of which 67% (11813/17657) were from tyres; 21% (3693/17657), ovitraps; 7% (1222/17657), discarded containers; 2.5% (434/17657), air-condition saucers; 1.3% (230/17657), buckets; 1.2% (210/17657), tanks; and 0.3% (55/17657), drums. Larval densities of 1306.8 (45.4%) was recorded during the dry season while 1574.7 (54.6%) was recorded during the wet season. A high larval density of 562 (43%) was recorded in Tema in the dry season while the lowest density of 122.2 (9.4%) was recorded in Dwease. No larvae were found during the dry season in Navrongo and Paga. The highest larval density during the wet season was recorded in Paga with a density of 346 (22%). These are shown in Figure 4.4 below.

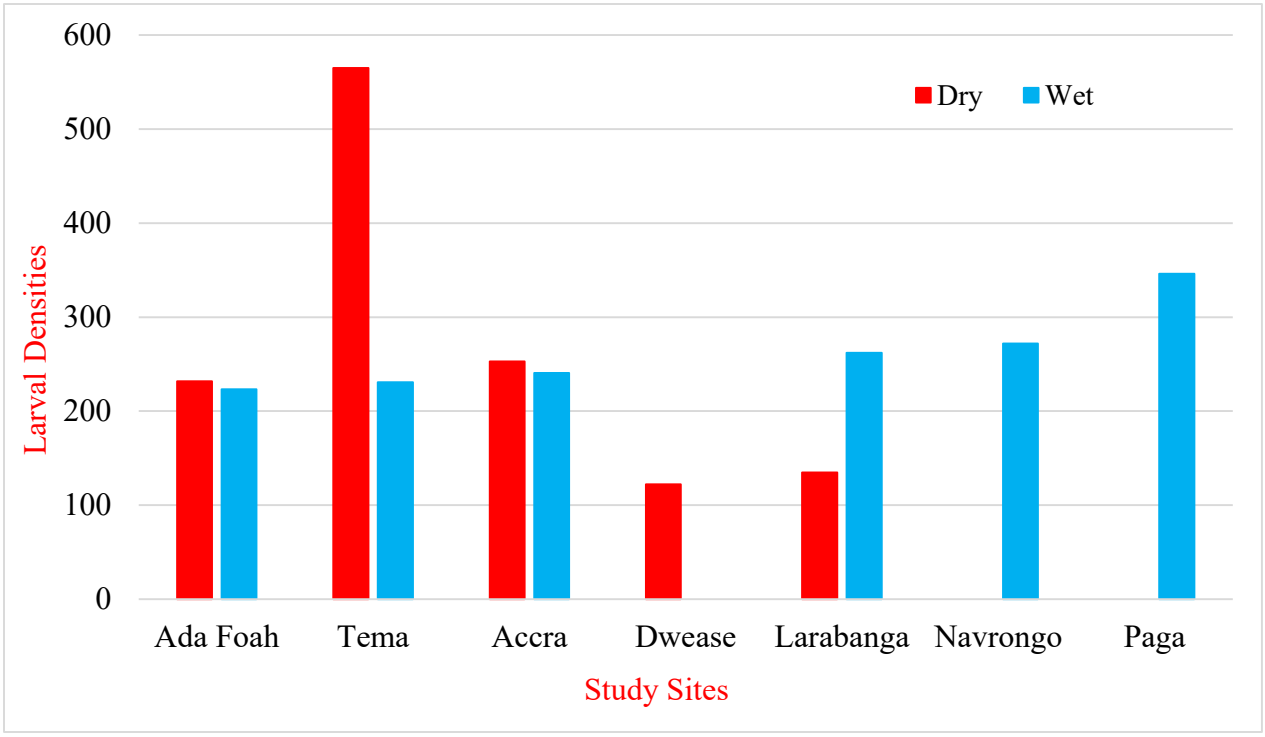


Figure 4.4: Densities of *Aedes* immatures per site.

4.3 Phenotypic Resistance of *Aedes* to Insecticides

The test results showed that *Aedes* mosquito populations from all study sites were resistant to DDT (0% - 88%). The highest resistant site was in Tema, where none of the mosquitoes died on exposure to DDT. Vectors showed resistance to permethrin in Tema (21%), Accra (40.0%), Larabanga (89%) and Navrongo (90%) and suspected resistance in Paga (96%) and Dwease (90%). Vectors showed resistance to deltamethrin in Tema (68%) and suspected resistance in Accra (91.3%), Ada foah (94%). Dwease (94%), Larabanga (93%), Navrongo (96%) and Paga (93%). *Aedes* mosquitoes were resistant to bendiocarb in Larabanga (81%), suspected resistance in Tema (95.0%), Dwease (96%), Navrongo (96%) and Paga (97%) and susceptible in Accra (98%) and Ada foah (99%). *Aedes* mosquitoes were susceptible to organophosphates (malathion) in all sites. These are shown in Figure 4.5 below.

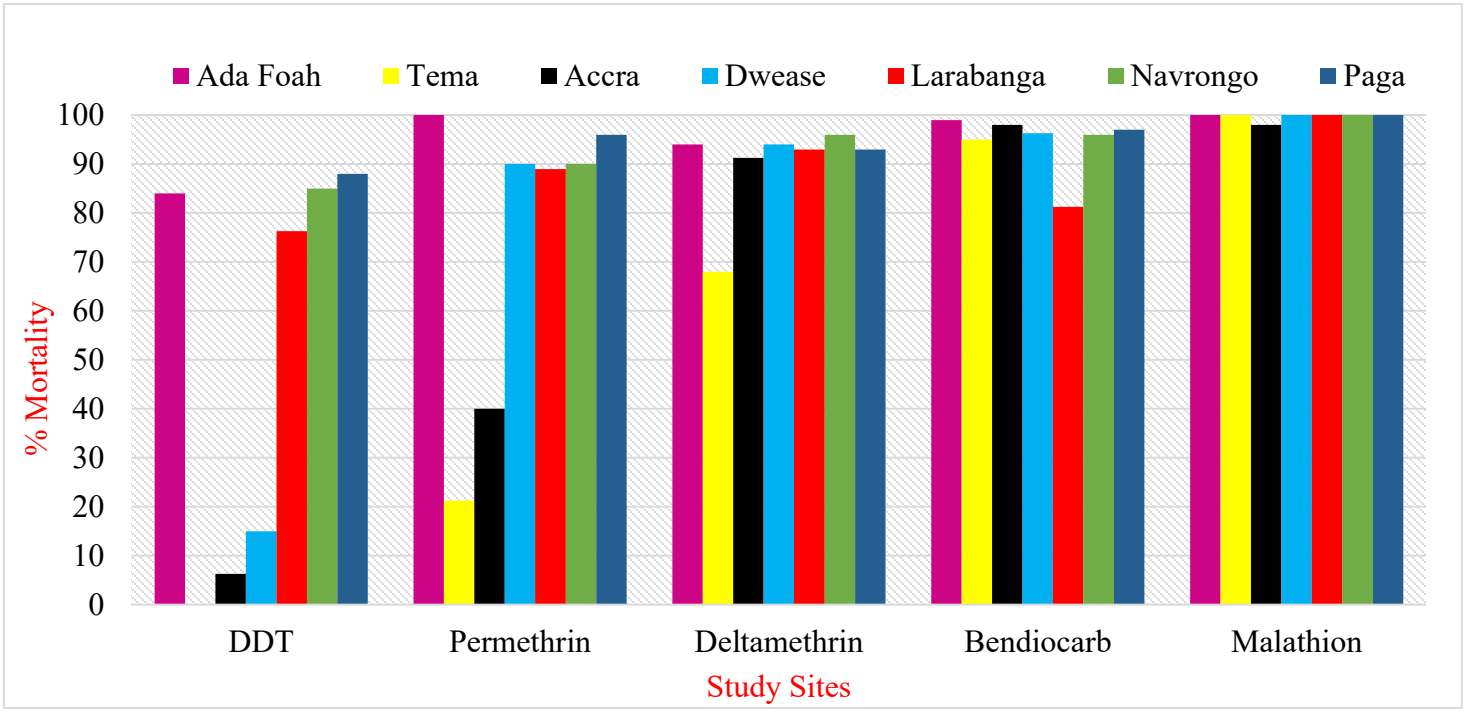


Figure 4.5: Phenotypic resistance status of *Aedes* mosquitoes to insecticides at all sites.

4.4 Blood Meal Analysis of the Vectors

Out of 44 blood-fed mosquitoes analysed by PCR, 20 amplified. Out of the 20 that amplified, 18 (90%) had taken human blood meal, 1 (5%) had fed on human and cow blood and 1 (5%) had taken dog and goat blood meal. These are shown in Table 4.2 below. The picture of the agarose gel showing the amplified samples can be found in Appendix IV.

Table 4.2: Blood meal analysis

Species	ST (%)	NIS (%)	HBI	Blood meal source (%)		
				Human	Dog + Goat	Human + Cow
Ae. aegypti	44 (100)	20 (45.5)	0.9	18 (40.9)	1 (2.3)	1 (2.3)

ST = Samples tested, NIS = Number identified successfully, HBI = Human blood index

CHAPTER FIVE

5.0 DISCUSSION

Aedes mosquitoes are vectors to several arboviruses. In Ghana they transmit yellow fever. With increasing travel and proximity to dengue endemic countries, these mosquitoes can serve as vectors to aid in its transmission.

Seasonal distribution and variation in population density is common for *Aedes* mosquitoes due to their sensitivity to change in temperature and moisture. This study found high densities of *Aedes* mosquitoes during the wet season compared to the dry season. The rains may have resulted in increased aquatic habitats for *Aedes* breeding (Li *et al*., 1985), thus increasing the vector population density and the risk of arbovirus transmission. This was similar to what was observed by Ndenga et al. (2017) in Kenya. However, a study by Appawu *et al*., (2006) in Ghana observed otherwise, where *Aedes* populations tended to increase during the dry season than the wet season. The difference observed between this present study and that of Appawu *et al*. may be due to the sampling methods used and number of sites surveyed. Drought however, also promotes vector abundance through increased storage of water in which *Aedes* mosquitoes breed (Pontes *et al*., 2000).

There was higher adult density of *Aedes* mosquitoes recorded for outdoor collection compared to indoor collection in this study. This could mean that *Aedes* mosquitoes are more exophagic (feed outdoors) and this was consistent with other studies from Kenya (Lutomiah *et al*., 2016); Malaysia (Wan-Norafikah *et al*., 2012) and Brazil (Favaro *et al*., 2008). This high outdoor density recorded is possibly because *Aedes* mosquitoes are diurnal (day biting).

The fact that people spend more time outdoors than indoors during the day influences the exophagic behaviours of these mosquitoes (Ndenga *et al*., 2017).

The bimodal blood seeking behaviour was observed through the use of the HLC method and the modal value is highest in the evening hours than in the morning hours. This bimodal blood meal seeking behaviour observed in this study agrees with findings in Trinidad and Tobago by Chadee *et al.* (1998) and in Kenya by Ndenga *et al.* (2017). This makes *Aedes* an efficient vector to transmit and rapidly spread arboviral diseases within a community. These *Aedes* adaptations have major implications for the possible transmission of diseases and for the planning of surveillance and control programmes

The *Aedes aegypti* found in this study tends to be more exophagic, anthropophilic and anthropophagic (BMI = 0.9); this agrees with findings in a study conducted in Thailand by Siriyasatien *et al* (2010) and in Kenya by McBride *et al* (2014), which found that female *Ae. aegypti* mosquitoes preferred human blood over that of animals. This study found *Aedes* to feed on more than one (multiple feeding) vertebrate host (human, dog, goat and cow) which was consistence with a study in Thailand by Khaklang and Kittayapong (2014). The ability of *Aedes* mosquitoes to feed on humans and different vertebrates has major implications of starting and sustaining a zoonotic transmission within a community.

In this study, *Aedes* mosquito larvae were found breeding more in car tyres than in other natural and artificial containers. The reason could be attributed to the fact that vulcanizers keep used car tyres for a longer period and these tyres hold water for that period providing a conducive environment for larval breeding in terms of optimal temperature, relative humidity and especially amount of light intensity. This observation is similar to that made by Bhat and Krishnamoorthy (2014) in India.

It was also observed that water collected in air conditioner saucers in Accra encourages breeding of *Aedes* mosquitoes. It will be prudent to investigate the chemical constituent of this particular type of water. Absence of pipe-borne water in Larabanga, where households tend to store a lot of water in tanks, drums, buckets and pots for longer periods, more especially during the dry season, allowed the breeding of *Aedes* mosquitoes in such artificial containers. This water storage practice has major implication of increasing vector densities.

This study found *Ae. aegypti* to be the predominant species in the study areas. This species has been incriminated as a yellow fever vector in Ghana and it is known to be the primary vector for the dengue fever virus (Suzuki *et al*., 2016). The high number of *Ae. aegypti* caught implies that the dengue vector is well established in Ghana and the risk of dengue fever and yellow fever transmission and outbreak is high in the absence of effective vector control. This calls for constant monitoring to prevent further outbreak of yellow fever and to prevent dengue fever outbreak. The zoonotic species: *Ae. africanus* and *Ae. luteocephalus* which transmit arboviruses between monkeys were found in this study. This could pose a great risk to humans as they have been incriminated to be potential vectors of yellow fever in Ghana and could have major implications of starting and sustaining a zoonotic transmission within these communities.

Aedes mosquito populations from all the study sites in this study were resistant to DDT (0 - 88%) which is similar to the observation made in Ghana by Suzuki and others (2016) on the University of Ghana campus in Legon. Deltamethrin, permethrin and bendiocarb resistance was also recorded. This could have serious implication, since there might be failure in vector control by these insecticides. Resistance to permethrin was recorded in this study and this was not in agreement with studies conducted in Central Africa by Kamgang *et al*. (2011) and in Ghana by Suzuki *et al*. (2016).

This study found *Aedes* mosquitoes to be susceptible to malathion across all sites which was different from the observation made in Sudan where resistance to organophosphate has been confirmed (Weetman *et al*., 2018). This finding therefore suggests the use of malathion in the control of *Aedes* mosquitoes in times of an outbreak. This implies that there will a readily available insecticide to control vectors in case there is an outbreak. This could also have major implications since the vectors are susceptible to only one insecticide and in the absence of that will be of great public health concern.

CHAPTER SIX

6.0 CONCLUSION AND RECOMMENDATION

6.1 Conclusion

High densities of *Aedes* mosquitoes were recorded during the wet season. Outdoor mosquito densities were high in all sites. Human landing catch recorded the highest densities among the three sampling methods used. High mosquito densities were recorded in Navrongo. A higher density of adult *Aedes* mosquitos was recorded during the wet season. The most predominant species was *Aedes aegypti*, other species also collected were *Ae. africanus* and *Ae. luteocephalus.* The highest larval density was recorded in Tema during the dry season. Dwease had the lowest larval density during this season.

Phenotypic resistance was recorded with DDT at all sites and deltamethrin, permethrin and bendiocarb in some sites, whilst there were suspected resistance recorded in deltamethrin, permethrin and bendiocarb in some sites, *Aedes* were susceptible to malathion at all sites.

Aedes mosquitoes were found to have fed on human blood by PCR (HBI=0.9), showing that the vectors were anthropophilic and exophagic.

6.2 Recommendation

1. This study determined the phenotypic resistance status of *Aedes* mosquitoes. However, it will be more important to determine the genetic and biochemical cause of the resistance for effective future interventions. It is therefore imperative that future studies should further look into resistance mutations in Ghana.

2. Since some blood meals taken by the *Aedes* were not determined because it could not be amplified, it is recommended that future studies should take a census of all vertebrates in the sites and use species specific primers to determine blood meals taken.

3. It is recommended that future studies should take into consideration, the determination of the carriage of arboviruses in *Aedes* mosquitoes to ascertain the possibility of an ongoing transmission.

4. Furthermore, a wider study that would include areas with agricultural sites where insecticides are frequently used must be carried out to determine the impact these insecticides have on the mosquitoes.

REFERENCES

1. Abad-Franch F., Zamora-Perea E. and Luz S.L. (2017). Mosquito-disseminated insecticide for citywide vector control and its potential to block arbovirus epidemics: Entomological observations and modeling results from Amazonian Brazil. *PLoS Med*. **14**, e1002213.
2. Agadzi V.K., Boatin B.A., Appawu M.A., Mingle J.A.A., Addy P.A. (1984). Yellow fever in Ghana, 1977-80. *Bull World Health Organ*.**62**(4):577-83.
3. Alirol E., Getaz L., Stoll B., Chappuis F. and Loutan L. (2011). Urbanisation and infectious diseases in a globalised world. *Lancet Infect. Dis*., **11**:131–141.
4. Appawu M., Dadzie S., Abdul H., Asmah H., Boakye D., Wilson M. and Ofori-Adjei D. (2006). Surveillance of Viral Haemorrhagic Fevers in Ghana: Entomological Assessment of the Risk of Transmission in the Northern Regions. *Ghana Medical Journal*, **40**(4):137–141.
5. Bennouna A., Balenghien T., El Rhaffouli H., Schaffner F., Garros C., Gardes L., Lhor Y., Hammoumi S., Chlyeh, G. and Fassi F.O.(2017). First record of Stegomyia albopicta (*Aedes albopictus*) in Morocco: A major threat to public health in North Africa? *Med. Vet. Entomol*., **31**, 102–106.
6. Bhat M. and Krishnamoorthy K. (2014). Entomological investigation and distribution of *Aedes* mosquitoes in Tirunelveli, Tamil Nadu, India. *Int J Curr Microbiol App Sci*., **3**(10):253-260.
7. 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., Wint G.R., Simmons C.P., Scott T.W., Farrar J.J. and Hay S.I. (2013). The global distribution and burden of dengue. *Nature* **496**:504-507.

8. Boyce R., Lenhart A., Kroeger A., Velayudhan R., Roberts B. and Horstick O. (2013). *Bacillus thuringiensis israelensis* (*Bti*) for the control of dengue vectors: Systematic literature review. *Trop. Med. Int. Hlth*, **18**:564–577.
9. Brown J.E., Evans B.R., Zheng W., Obas V., Barrera-Martinez L., Egizi A., Zhao H., Caccone A. and Powell J.R. (2014). Human impacts have shaped historical and recent evolution in *Aedes aegypti*, the dengue and yellow fever mosquito. *Evolution* **68**:514–525.
10. Brown J.E., McBride C.S., Johnson P., Ritchie S., Paupy C., Bossin H., Lutomiah J., Fernandez-Salas I., Ponlawat A., Cornel A.J., Black W.C., Gorrochotegui-Escalante N., Urdaneta-Marquez L., Sylla M., Slotman M., Murray K.O., Walker C. and Powell J.R.. (2011). Worldwide patterns of genetic differentiation imply multiple 'domestications' of *Aedes aegypti*, a major vector of human diseases. *Proc Biol Sci*. **278**(1717):2446-54.
11. Carrington L.B. and Simmons C.P. (2014). Human to mosquito transmission of dengue viruses. *Frontiers in Immunology* **5**: 1–8.
12. Carvalho R.G., Lourenc¸o-de-Oliveira R. and Braga I.A. (2014). Updating the geographical distribution and frequency of *Aedes* albopictus in Brazil with remarks regarding its range in the Americas. *Mem Inst Oswaldo Cruz*. **109**(6):787-96.
13. CDC (2016a). "Zika virus" Fact sheet Updated 6 September 2016.
14. CDC (2016b). Evaluating mosquitoes for insecticide resistance: Web-based instruction. Available from: http://www.cdc.gov/malaria.
15. Chadee D.D. (1988). Landing periodicity of the mosquito *Aedes* aegypti in Trinidad in relation to the timing of insecticidal space-spraying. *Med Vet Entomol*.; **2**(2):189-192. PMID: 2980173.
16. Clements A.N. (1992). The Biology of Mosquitoes.1st ed. London.

17. Delatte H., Desvars A., Boue´ tard A., Bord S., Gimonneau G., Vourc'h G. and Fontenille D. (2010). Blood-feeding behavior of *Aedes albopictus*, a vector of chikungunya on La Reunion. *Vector Borne Zoonotic Dis*. **10**(3):249-58

18. Delatte H., Gimonneau G., Triboire A. and Fontenille D. (2009). Influence of temperature on immature development, survival, longevity, fecundity, and gonotrophic cycles of *Aedes albopictus*, vector of Chikungunya and dengue in the Indian Ocean. *J Med Entomol*. **46**(1):33-41.

19. Diallo D., Chen R., Diagne C.T., Ba Y., Dia I., Sall A.A., Weaver S.C. and Diallo M. (2013). Bloodfeeding patterns of sylvatic arbovirus vectors in southeastern Senegal. *Trans. R. Soc. Trop. Med. Hyg*., **107**:200–203.

20. Diallo M., Sall A.A., Moncayo A.C., Ba Y., Fernandez Z., Ortiz D., Coffey L.L., Mathiot C., Tesh R.B. and Weaver S.C. (2005). Potential role of sylvatic and domestic African mosquito species in dengue emergence. *Am. J. Trop. Med. Hyg*., **73**: 445–449.

21. Dickson L.B., Jiolle D., Minard G., Moltini-Conclois I., Volant S., Ghozlane A., Bouchier C., Ayala D., Paupy C., Moro C.V. and Lambrechts L. (2017). Carryover effects of larval exposure to different environmental bacteria drive adult trait variation in a mosquito vector. *Science Advances*, **3**(8): e1700585.

22. Farraudière L., Sonor F., Crico S., Étienne M., Mousson L., Hamel R., Missé D., Failloux A.B., Simard F., Yébakima A. (2017). First detection of dengue and chikungunya viruses in natural populations of *Aedes aegypti* in Martinique during the 2013-2015 concomitant outbreak. *Rev Panam Salud Publica*. **41**(e63)

23. Favaro E.A., Dibo M.R., Mondini A, Ferreira A, Barbosa A.A.C., Eiras A.E., Neto F.C. (2008). Physiological state of *Aedes* (Stegomyia) *aegypti* mosquitoes captured with MosquiTRAPs^{TM} in Mirassol, São Paulo, Brazil. *Journal of Vector Ecology*. **33** (1): 8-16.

24. Ferreira-de-Brito A., Ribeiro I.P., Miranda R.M., Fernandes R.S., Campos S.S., Silva K.A., Castro M.G., Bonaldo M.C., Brasil P. and Lourenço-de-Oliveira R. (2016). First detection of natural infection of *Aedes aegypti* with Zika virus in Brazil and throughout South America. *Mem Inst Oswaldo Cruz*, Rio de Janeiro.; **111**(10):655-658.

25. Fiorenzano J.M., Koehler P.G. and Xue R.D. (2017). Attractive toxic sugar bait (ATSB) for control of mosquitoes and its impact on non-target organisms: A review. *Int. J. Environ. Res. Public Health*, **14**:398.

26. Fuller D.O., Troyo A. and Beier J.C. (2009). El Nin˜ o Southern Oscillation and vegetation dynamics as predictors of dengue fecer cases in Costa Rica. *Environmental Research Letters* **4**:140111–140118.

27. Gratz N.G. (2004).“Critical review of the vector status of *Aedes albopictus*”. *Med. Vet. Entomol*., **18**. pp. 215–227.

28. Grigoraki L., Pipini D., Labbe P., Chaskopoulou A., Weill M. and Vontas J. (2017). Carboxylesterase gene amplifications associated with insecticide resistance in *Aedes* albopictus: Geographical distribution and evolutionary origin. *PLoS Negl. Trop. Dis*., **11**, e0005533.

29. Haddow A.J., Smithburn K.C., Dick G.W.A., Kitchen S.F. and Lumsden W.H.R. (1948). Implication of the mosquito *Aedes* (Stegomyia) *africanus* Theobald in the forest cycle of yellow fever in Uganda. Ann. *Trop. Med. Parasitol*., **42**:218–223.

30. Hahn M.B., Gangnon R.E., Barcellos C., Asner G.P. and Patz J.A. (2014). Influence of deforestation, logging, and fire on malaria in the Brazilian Amazon. *PLOS ONE* **9**:e85725.

31. Hanley K.A., Monath T.P., Weaver S.C., Rossi S.L., Richman R.L., Vasilakis N. (2013). Fever versus fever: The role of host and vector susceptibility and interspecific competition in shaping the current and future distributions of the sylvatic cycles of dengue virus and yellow fever virus. *Infect. Genet. Evol*., **19**:292–311.

32. Hirata K., Komagata O., Itokawa K., Yamamoto A., Tomita T., Kasai, S. (2014). A single crossing-over event in voltage-sensitive Na+ channel genes may cause critical failure of dengue mosquito control by insecticides. *PLoS Negl. Trop. Dis*., **8**: e3085.

33. Holmes E.C. and Twiddy S.S. (2003). The origin, emergence and evolutionary genetics of dengue virus. *Infect. Genet Evol*., **3**:19–28.

34. Huang Y.M. (2004). The subgenus Stegomyia of *Aedes* in the Afrotropical Region with keys to the species (Diptera: Culicidae). Zootaxa 700 [Internet]. Online edi. Auckland: *Mongolia Press*. p. 1–120. Available from: http://www.mapress.com/zootaxa/(Accessed 10/12/2014)

35. Huber, K., Ba, Y., Dia, I., Mathiot, C., Sall, A.A. and Diallo, M. (2008). *Aedes aegypti* in Senegal: Genetic diversity and genetic structure of domestic and sylvatic populations. Am. *J. Trop. Med. Hyg*. **79**:218–229.

36. Kamgang B, Marcombe S, Chandre F, Nchoutpouen E, Nwane P, Etang J, Corbel V., Paupy C. (2011). Insecticide susceptibility of *Aedes aegypti* and *Aedes albopictus* in Central Africa. *Parasit Vectors*. **4**:79.

37. Kamgang B., Yougang A.P., Tchoupo M., Riveron J.M. and Wondji, C. (2017). Temporal distribution and insecticide resistance profile of two major arbovirus vectors *Aedes aegypti* and *Aedes albopictus* in Yaoundé, the capital city of Cameroon. *Parasites Vectors*, **10**: 469.

38. Kampango A. and Abilio A.P. (2016). The Asian tiger hunts in Maputo city-the first confirmed report of *Aedes* (Stegomyia) *albopictus* (Skuse, 1895) in Mozambique. *Parasites Vectors*. **9**,76.

39. Kasai S., Komagata O., Itokawa K., Shono T., Ng L.C., Kobayashi M. and Tomita, T. (2014). Mechanisms of pyrethroid resistance in the dengue mosquito vector, *Aedes aegypti*: Target site insensitivity, penetration, and metabolism. *PLoS Negl. Trop. Dis*., **8**: e2948.

40. Kawada H., Higa Y., Futami K., Muranami Y., Kawashima E., Osei J.H., Sakyi K.Y., Dadzie S., de Souza D.K., Appawu M., Ohta N., Suzuki T. and Minakawa N. (2016). Discovery of point mutations in the voltage-gated sodium channel from African *Aedes aegypti* populations: Potential phylogenetic reasons for gene introgression. *PLoS Negl. Trop. Dis*., **10**: e0004780.

41. Khaklang S. and Kittayapong P. (2014). Species composition and blood meal analysis of mosquitoes collected from a tourist island, Koh Chang, Thailand. *J Vector Ecol*., **39**(2):448-52.

42. Kobayashi A.M., Nihei N. and Kurihara T. (2002). Analysis of northern distribution of *Aedes albopictus* (Diptera: Culicidae) in Japan by geographical information system. *J* **39**:4–11.

43. Kraemer, M.U.G., Sinka, M. E., Duda, K. A., Mylne, A. Q.N., Shearer, F. M., Barker, C. M., Moore, G.M., Carvalho, R.G., Coelho, G.E., Van Bortel, W., Hendrickx, G., Schaffner, F., Elyazar I.R.F., Teng H., Brady, O.J., Messina, J.P., Pigott, D.M., Scott, T.W.,

Smith, D.L., Wint, G.R.W., Golding, N. and Hay, S. I. (2015a). The global distribution of the arbovirus vectors *Aedes aegypti* and *Ae. albopictus*. *eLife*, **4**: e08347.

44. Kraemer, M.U., Sinka, M.E., Duda, K.A., Mylne, A., Shearer, F.M., Brady, O.J., Messina, J.P., Barker, C.M., Moore, C.G., Carvalho, R.G., Coelho G.E., Van Bortel W., Hendrickx G., Schaffner F., Wint G.R., Elyazar I.R., Teng H.J., Hay S.I. (2015b) The global compendium of *Aedes aegypti* and *Ae. albopictus* occurrence. *Sci. Data* **7**;2:150035
45. Leparc-Goffart I., Nougairede A., Cassadou S., Prat C. and de Lamballerie X. (2014). Chikungunya in the Americas. *Lancet* **383**:514.
46. Li CF, Lim TW, Han LL, Fang R. (1985). Rainfall, abundance of *Aedes* aegypti and dengue infection in Selangor, Malaysia. *Southeast Asian J Trop Med Public Health*. **16**(4): 560-568. PMID:3835698
47. Li X., Schuler M.A., Berenbaum M.R. (2007). "Molecular mechanisms of metabolic resistance to synthetic and natural xenobiotics Annu. Rev. Entomol., 52, pp. 231–253.
48. Lounibos L.P. (2002). Invasion by insect vectors of human disease. *Annual Review of Entomology* **47**:233–266.
49. Lutomiah J., Barrera R., Makio A., Mutisya J., Koka H., Owaka S., Koskei E., Nyunja, A., Eyase, F., Coldren, R. and Sang R. (2016). Dengue outbreak in Mombasa City, Kenya, 2013-2014: Entomologic Investigations. *PLoS Negl Trop Dis*.; **10**(10): e0004981
50. Luz C, Tai M.H.H., Santos A.H. and Silva H.H.G. (2008). Impact of moisture on survival of *Aedes aegypti* eggs and ovicidal activity of Metarhizium anisopliae under laboratory conditions. *Memorias do Instituto Oswaldo Cruz* **103**:214–215.
51. Massangaie M., Pinto G., Padama F., Chambe G., da Silva M., Mate I., Chirindza C., Ali S., Agostinho S., Chilaule D., Weyer J., le Roux C., Abilio A.P., Baltazar C., Doyle T.J.,

Cliff J., Paweska J. and Gudo E.S. (2016). Clinical and epidemiological characterization of the first recognized outbreak of dengue virus-type 2 in Mozambique, 2014. *Am. J. Trop. Med. Hyg*., **94**,413–416.

52. Mattingly, P.F. (1967). Taxonomy of *Aedes aegypti* and related species. Bull. *Wld Hlth Organ*., **36**:552–554.
53. McBride C.S., Baier F., Omondi A.B., Spitzer S.A., Lutomiah J., Sang R., Ignell R. and Vosshall L.B. (2014). Evolution of mosquito preference for humans linked to an odorant receptor. *Nature*.; **13**(515,7526):222-227.
54. Medlock J.M., Hansford K.M., Schaffner F., Versteirt V., Hendrickx G., Zeller H. and Van Bortel W. (2012). A review of the invasive mosquitoes in Europe: ecology, public health risks, and control options. *Vector Borne Zoonotic Dis*.;**12**(6):435-47.
55. Messina, J.P., Kraemer, M.U., Brady, O.J., Pigott, D.M., Shearer, F.M., Weiss, D.J., Golding, N., Ruktanonchai, C.W., Gething, P.W., Cohn, E.,s Brownstein J.S., Khan K., Tatem A.J., Jaenisch T., Murray C.J., Marinho F., Scott T.W. and Hay S.I. (2016).Mapping global environmental suitability for Zika virus. *eLife* , **5**, e15272.
56. Molaei G., Andreadis T.G., Armstrong P.M. and Diuk-Wasser M. (2008). Host-feeding patterns of potential mosquito vectors in Connecticut, U.S.A. Molecular analysis of bloodmeals from 23 species of *Aedes, Anopheles, Culex, Coquillettidia, Psorophora*, and *Uranotaenia*. *J Med Entomol* **45**(6):1143-1151.
57. Monath T.P. (2001). Yellow fever. In: Service, M.W. (Ed.), The Encyclopedia of Arthropod-transmitted Infections. *CAB International*, Wallingford, UK, pp. 571–577.
58. Moore M., Sylla M., Goss L., Burugu M.W., Sang R., Kamau L.W., Kenya E.U., Bosio C., Munoz Mde L., Sharakova M. and Black W.C. (2013). Dual African origins of global

Aedes aegypti s.l. populations revealed by mitochondrial DNA. *PLoS Negl. Trop. Dis*. **7**, e2175.

59. Morrison A.C., Gray K., Getis A., Astete H., Sihuincha M., Focks D., Watts D., Stancil J.D., Olson J.G., Blair P. and Scott T.W. (2004). Temporal and geographic patterns of *Aedes aegypti* (Diptera: Culicidae) production in Iquitos, Peru. *J Med Entomol* **41**:1123–1142.
60. Mortimer R. (2010). "*Aedes aegypti* and dengue fever". *Onview.net Ltd*, Microscopy-UK. Retrieved -08- 27.
61. Mousson L., Dauga C., Garrigues T., Schaffner F., Vazeille M. and Failloux A.B. (2005). "Phylogeography of *Aedes (Stegomyia) aegypti* (L.) and *Aedes (Stegomyia) albopictus* (Skuse) (Diptera: Culicidae) based on mitochondrial DNA variations". *Genetics Research*. **86** (1):1–11.
62. Moyes C.L., Vontas J., Martins A.J., Ng L.C., Koou S.Y., Dusfour I., Raghavendra K., Pinto J., Corbel V., David J.P. and Weetman D. (2017). Contemporary status of insecticide resistance in the major *Aedes* vectors of arboviruses infecting humans. *PLoS Negl. Trop. Dis*., **11**: e0005625.
63. Muktar Y., Tamerat N. and Shewafera A. (2016). *Aedes aegypti* as a Vector of Flavivirus. *J Trop Dis* **4**: 223.
64. Müller G.C., Tsabari O., Traore M.M., Traore S.F., Kravchenko V.D., Junnila A. and Beier J.C. (2016). First record of *Aedes* albopictus in inland Africa along the River Niger in Bamako and Mopti, Mali. *Acta Trop*., **162**,245–247.
65. Ndenga B.A., Mutuku F.M., Ngugi H.N., Mbakaya J.O., Aswani P., Musunzaji P.S.,Vulule J., Mukoto D., Kitron U. and LaBeaud A.D. (2017). Characteristics of *Aedes aegypti* adult

mosquitoes in rural and urban areas of western and coastal Kenya. *PLoS ONE*, **12**(12): e0189971.

66. Newswise, Inc. (2008). "Lay Your Eggs Here". *Academy of Sciences* of USA **103**:6242–6247.
67. Ngo K.A. and Kramer L.D. (2003). Identification of mosquito bloodmeals using polymerase chain reaction (PCR) with order-specific primers. *J Med Entomol*. **40**(2):215-22.).
68. Niare S., Berenger J.M., Dieme C., Doumbo O., Raoult D., Parola P. and Almeras L. (2016). Identification of blood meal sources in the main African malaria mosquito vector by MALDI-TOF MS. *Malaria Journal*, **15**: 87.
69. Noden B.H., Musuuo M., Aku-Akai L., van der Colf B., Chipare I. and Wilkinson R. (2014). Risk assessment of flavivirus transmission in Namibia. *Acta Trop*. , **137**, 123–129.
70. O'Brien D. (2010). "ARS Study Provides a Better Understanding of How Mosquitoes Find a Host". U.S. Department of Agriculture. Retrieved 2010-08-27.
71. Owusu, H.F., Jan, D., Malone, D. and Müller, P. (2015). Comparability between insecticide resistance bioassays for mosquito vectors : *time to review current methodology*. 1–11.
72. Paupy C., Brengues C., Ndiath O., Toty C., Herve J.P. and Simard F. (2010). Morphological and genetic variability within *Aedes aegypti* in Niakhar, Senegal. *Infect. Genet. Evol.*, **10**:473–480.
73. Paupy C., Delatte H., Bagny L., Corbel V. and Fontenille D. (2009). *Aedes albopictus*, an arbovirus vector: from the darkness to the light. *Microbes Infect.*;**11**(14-15):1177-85.. Epub 2009 May 18.

74. Ponlawat A. and Harrington L.C. (2005). Blood feeding patterns of *Aedes aegypti* and *Aedes albopictus* in Thailand. *J Med Entomol* **42**:844–849.

75. Pontes J.S., Freeman J., Oliveira-Lima J.W., Hodgson J.C., Spielman A. (2000). Vector densities that potentiate dengue outbreaks in a Brazilian city Ricardo. *Am. J. Trop. Med. Hyg*. **62**(3): 378-383. PMID:11037781

76. Powell, J.R. (2016). Mosquitoes on the move. *Science*, **354**:971–972.

77. Powell J.R. and Tabachnick W.J. (2013). History of domestication and spread of *Aedes aegypti*. Review. *Mem Inst Oswaldo Cruz*, Rio de Janeiro.; **108**(Suppl. I):11-17.

78. Ranson H., Burhani J., Lumjuan N. and Black W.C. (2010). "Insecticide resistance in dengue *vectors TropIKA.net Journal*, 1.

79. Reis S., Cornel A.J., Melo M., Pereira H. and Loiseau C. (2017). First record of *Aedes albopictus* (Skuse 1894) on SâoTomé island. *Acta Trop*., **171**, 86–89.

80. Russell A.B.M., Kay B.H. and Shipton W. (2001). Survival of *Aedes aegypti* (Diptera: Culicidae) eggs in surface and subterranean breeding sites during the northern Queensland dry season. *J Med Entomol*.; **38**(3):441-5.

81. Scott D. (1965). Epidemic disease in Ghana, 1901-1960. London, *Oxford University Press*, p.26-64.

82. Scott T.W. and Morrison A.C. (2010). Vector dynamics and transmission of dengue virus: implications for dengue surveillance and prevention strategies. Dengue Virus: *Springer* **1**:15-28.

83. Scott T.W. and Takken W. (2012). Feeding strategies of anthropophilic mosquitoes result in increased risk of pathogen transmission. *Trends in Parasitology* **28**:114–121.

84. Seixas G., Grigoraki L.,Weetman D., Vicente J.L., Silva A.C. Pinto J., Vontas J. and Sousa C..A. (2017). Insecticide resistance is mediated by multiple mechanisms in recently introduced *Aedes aegypti* from Madeira Island (Portugal). *PLoS Negl. Trop. Dis*., **11**: e0005799.

85. Semenza J.C., Sudre B., Miniota J., Rossi M., Hu W., Kossowsky D., Suk J.E., Van Bortel W. and Khan K. (2014). International dispersal of dengue through air travel: importation risk for Europe. *PLoS Negl Trop Dis*.**8**(12):e3278

86. Siriyasatien P., Pengsakul T., Kittichai V., Phumee A., Kaewsaitiam S., Thavara U., Tawatsin A., Asavadachanukorn P. and Mulla M.S. (2010). Identification of blood meal of field caught *Aedes aegypti* (L.) By multiplex PCR. Southeast Asian. *J Trop Med Public Health*. **41**(1):43-47. PMID: 20578481.

87. Smith D.L., Battle K.E., Hay S.I., Barker C.M., Scott T.W. and McKenzie, F.E. (2012). Ross, Macdonald, and a Theory for the Dynamics and Control of Mosquito-Transmitted Pathogens. *journal.ppat*.1002588

88. Stevenson B.J., Pignatelli P., Nikou D. and Paine M.J.I. (2012). "Characterization of cytochromes P450 associated with pyrethroid resistance in *Aedes aegypti*. *PLoS Negl. Trop. Dis*., **6** p. e1595.

89. Suzuki T., Osei J. H., Sasaki A., Adimazoya M., Appawu M., Boakye D., Ohta N., Dadzie, S. (2016). Risk of transmission of viral haemorrhagic fevers and the insecticide susceptibilitystatus of *Aedes aegypti* (linnaeus) in some sites in Accra, Ghana. *Ghana Medical Journal*, **50**(3),136–141.

90. Tabachnick W.J. (1991) Evolutionary genetics and arthropod borne disease: the yellow fever mosquito. *American Entomologist*. **37**:14-24.

91. Taylor M. and Feyereisen R. (1996). "Molecular biology and evolution of resistance of toxicants. *Mol. Biol. Evol.*, **13**:719–734.

92. Thomsen E.K., Strode C., Hemmings K., Hughes A.J., Chanda E., Musapa M., Kamuliwo M., Phiri F.N., Muzia L., Chanda J., Kandyata A., Chirwa B., Poer K. and Coleman M. (2014). Underpinning Sustainable Vector Control through Informed Insecticide Resistance Management. *PLoS ONE* 9(6): e99822.

93. van Regenmortel M.H.V., Fauquet C.M., Bishop D.H.L., Carstens E.B., Estes M.K., Lemon S.M., Maniloff J., Mayo M.A., McGeogh D.J., Pringle C.R. and Wickner R.B. (Eds.), (2000). Virus Taxonomy. Classification and Nomenclature of Viruses. Seventh Report of the International Committee on Taxonomy of Viruses. *Academic Press*, San Diego, CA. pp.xii. 1162.

94. Vazquez-Prokopec G, Galvin W, Kelly R. and Kitron U. (2009). A new, cost-effective, battery-powered aspirator for adult mosquito collections. *J Med Entomol*; **46**(6):1256-1259. PMID: 19960668.

95. Wan-Norafikah O, Nazni W.A., Noramiza S, Shafa'ar-Ko'ohar S.K., Heah S, Nor-Azlina A.H., Khairul-Asuad M. and Lee, H.L. (2012) . Distribution of *Aedes* mosquitoes in three selected localities in Malaysia. *Sains Malaysiana*. **41**(10):1309-1313.

96. Weaver S.C. (1997). *Vector biology in viral pathogenesis*. In: Nathanson, N. (Ed.), Viral Pathogenesis. *Lippincott-Raven*, New York, pp. 329–352.

97. Weaver S.C. (2017). Emergence of epidemic Zika virus transmission and congenital Zika syndrome: Are recently evolved traits to blame? *mBio*, **8**: e02063-16.

98. Weaver S.C. and Reisen,W.K. (2010). Present and future arboviral threats. *Antivir. Res.*, **85**: 328–345.

99. Weetman D., Kamgang B., Badolo A., Moyes C.L., Shearer F.M., Coulibaly M., Pinto J, Lambrechts L. and McCall P.J. (2018). *Aedes* Mosquitoes and *Aedes*-Borne Arboviruses in Africa: Current and Future Threats. *Int. J. Environ. Res*. Public Health, **15**: 220, 1-20.

100. Wetsman N. (2017). The missing pieces: Lack of Zika data from Africa complicates search for answers. *Nat. Med*., **23**: 904–906.

101. Womack, M. (1993). The yellow fever mosquito, *Aedes aegypti*. *Wing Beats*, Vol. **5**(4):4.

102. WHO (1995). Report of the Consultation on: Key Issues in dengue vector control towards the operationalization of a global strategy, WHO CTD/FIL (DEN)/IC/96.1: Geneva.

103. WHO (2009). Progress and Prospects for the Use of Genetically Modified Mosquitoes to Inhibit Disease Transmission; World Health Organization: Geneva, Switzerland.

104. WHO (2011). Global Insecticide Use for Vector-Borne Disease Control. A 10-Year assessment (2000–2009) fifth ed.

105. WHO (2014). "A global brief on vector-borne diseases". *World Health Organisation*. WHO/DCO/WHD/2014.1

106. WHO (2016a). Test procedures for insecticide resistance monitoring in malaria vector mosquitoes, 2nd ed.. *World Health Organization*. http://www.who.int/iris/handle/10665/250677

107. WHO (2016b). Monitoring and managing insecticide resistance in *Aedes* mosquito populations: interim guidance for entomologists. *World Health Organization*. http://www.who.int/iris/handle/10665/204588

108. WHO (2017a). "Denguecontrol".http://www.who.int/denguecontrol/en/.

109. WHO (2017b). Global Vector Control Response 2017–2030;*World Health Organization*: Geneva, Switzerland,; p. 53.

110. WHO (2018a). Yellow fever situation report. via http://www.who.int/emergencies/yellow-fever/situation-reports/8-july-2016/en/. *World Health Organisation* [accesed on 7th August, 2018].

111. WHO (2018b). Yellow fever in Ghana. http://www.who.int/csr/don/2012_02_03b/en/. *World Health Organisation* [accesed on 17th October, 2018].

APPENDICES

Appendix I: WHO Insecticide Susceptibility Bioassay Data sheet

	Replicate 1	Replicate 2	Replicate 3	Replicate 4	Control 1	Control 2
No. Exposed						
Number knocked down after exposure in minutes.						
	Exposure Tubes					
Time/minutes	Replicate 1	Replicate 2	Replicate 3	Replicate 4	Control 1	Control 2
10						
15						
20						
30						
40						
50						
60						
Number of dead/alive mosquitoes at the end of holding period (24 hours mortality)						
	Holding Tubes					
	Replicate 1	Replicate 2	Replicate 3	Replicate 4	Control 1	Control 2
No. Dead						
No. Alive						

Appendix II: Larval Sampling Form

Ecology of Arboviral Disease Vectors in Ghana - University of Ghana

LARVAL DISTRIBUTION SURVEY FORM

» Site/Cluster: _____ **» Survey Date** (DD/MM/YYYY):_____

	Habitat No.	Habitat Type	Veg. Cover (%)	Land use Type	Length (m)	Width (m)	No. of Dips	GPS Reading					Species		
								Longitude (N)		Latitude (E)		Elevation (m)	*Aedes*		
													L1-L2	L3-L4	Pupae
1															
2															
3															
4															
5															
6															
7															
8															
9															
10															

Habitat Type (A, B, …)

A. Drainage ditch
B. Foot print
C. Gold mine
D. Natural Pond
E. Swamp
F. Tyre track
G. Container
H. Rock pool
I. Fish pond
K. Other: _____

Land use Type (1, 2, …)

1. Farmland
2. Pasture
3. Swamp
4. Stream/River
5. Forest
6. Shrub
7. Road
8. Compound/Home
9. Water body (not 4)
10. Other: _____

Sample of GPS Reading

Latitude	Longitude
0.12345	34.98765

***Note**: report in decimal degree format*

Investigator:_____
Certified by _____
Supervisor:_____

Appendix III: Adult Mosquitoes Sampling Form

ENTOMOLOGICAL SURVEY DATA ENTRY SHEET

Biogent-2 Sentinel Mosquito Trap -Species Identification (Morphological)

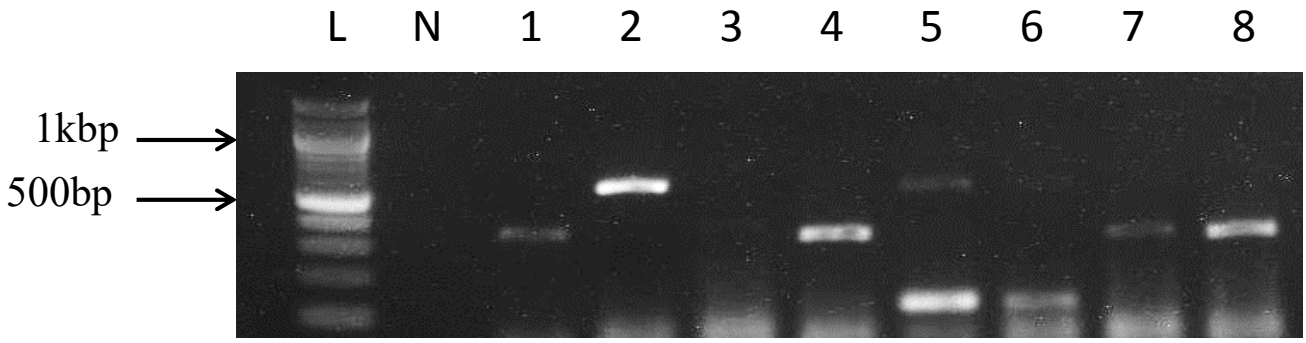
Community Name: _____ Habitat_____ (Indoor/Outdoor) _____ Sampling Round _____

Date of Collection: ___/___/_____ Date of Identification: ___/___/_____ Weather Conditions: _____

Time of Collection	Total Number Caught	SPECIES																							
		Aedes (aegypti):				*Aedes* (albopictus):				Other *Aedes* (Specify)				Culex				Mansonia				Anopheles			
		UF	BF	HG	G	UF	BF	HG	G	UF	BF	HG	G	UF	BF	HG	G	UF	BF	HG	G	UF	BF	HG	G
5-6 am																									
6-7 am																									
7-8 am																									
3-4 pm																									
4-5 pm																									
5-6 pm																									
6-7 pm																									

Keys: UF=UNFED, BF= BLOOD FED, HG= HALF GRAVID, G=GRAVID

Appendix IV: Agarose Gel Showing Blood Meal Analysis of *Aedes* Mosquitoes



L= NEB 100bp LADDER, NC= NEGATIVE CONTROL

(2% Agarose gel image showing PCR amplification of Blood meal)

Expected Band Size for the Species-specific Fragments

Human blood = 334bp, Dog blood = 680bp, Goat blood = 132bp, Cow blood = 561bp, Pig blood = 453bp

Appendix V: Ethical Clearance Form

UNIVERSITY OF GHANA
COLLEGE OF HEALTH SCIENCES
ETHICAL AND PROTOCOL REVIEW COMMITTEE

Ref. No.: CHS/EPRC/JULY/2018 July 11, 2018

Christopher Mfum Owusu-Asenso
Department of Medical Microbiology
School of Biomedical and Applied Health Sciences
Korle- Bu

ETHICAL CLEARANCE

Protocol Identification Number: ***CHS-Et/M.9 – P1.5/2017-2018***

The College of Health Sciences Ethical and Protocol Review Committee on June 28, 2018 reviewed and unanimously approved your research proposal.

Title of Protocol: **"Insecticide Resistance Status, Population Density and Carriage of Arboviruses in Aedes Mosquitoes in Ghana"**

Principal Investigator: **Christopher Mfum Owusu-Asenso**

This approval requires that you submit six-monthly review reports of the protocol to the Committee and a final full review to the Ethical and Protocol Review Committee at the completion of the study. The Committee may observe, or cause to be observed, procedures and records of the study during and after implementation.

Please note that any significant modification of this project must be submitted to the Committee for review and approval before its implementation.

You are required to report all serious adverse events related to this study to the Ethical and Protocol Review Committee within seven (7) days verbally and fourteen (14) days in writing.

As part of the review process, it is the Committee's duty to review the ethical aspects of any manuscript that may be produced from this study. You will therefore be required to furnish the Committee with any manuscript for publication.

This ethical clearance is valid till July 11, 2019.

Please always quote the protocol identification number in all future correspondence in relation to this protocol.

Signed: .....
Professor Andrew Anthony Adjei
Chair, Ethical and Protocol Review Committee

cc: Provost, CHS
Dean, SBAHS
Head, Dept. of Medical Microbiology

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