UNIVERSITY OF NAIROBI

INSECTICIDE RESISTANCE AND ITS IMPACT ON MALARIA TRANSMISSION POTENTIAL AMONG THE MAJOR VECTORS IN TESO SUB – COUNTIES, WESTERN KENYA

BY

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DECLARATION

I, Githinji Edward Kareithi, hereby declare that this thesis is my original work and has not been presented for a degree award in any other University.

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DEDICATION

This thesis is dedicated to my parents;

Mr. Geoffrey Githinji Ndei who throughout his life worked diligently as a lonely cook in Nanyuki and Nairobi far away from his Wang'ata Karĭmĭnû Mweiga Nyeri family and home; and denied himself a luxurious family life in order to see me through nursery, primary, secondary and university education and to Mrs. Hannah Thuguri Githinji who for decades led a poor, lonely, peasant separated life and also labored in other people's farms:

- "It has been a long journey but your prayers to God Abba Father, your zeal, hard work, honesty, sincerity, freshness, love, care and support always gives me the impetus, force, courage and momentum to carry on victoriously and fiercely".

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ACRONYMS / ABBREVIATIONS

ABTS	2,2'-Azino-Bis(3-Ethylbenzothiazoline-6-Sulfonic Acid
ACT	Artemisinin-Based Combination Therapies
ANC	Ante Natal Care
AR	Anopheles Arabiensis
BBI	Bovine Blood Index
BHC	Chlorinated Beta ß Hydrocarbons
Br_2CA	Calcium Bromide
CAS	Chemical Abstract Service
CDC	Centre For Disease Control
CGHR	Centre For Global Health Research
CI	Confidence Interval
CQ	Chloroquine
CSP	Circumsporozoite Protein
CYP450s	Cytochrome P-450
CYP691	Cytochrome P-691
DALYS	Disability-Adjusted Life Years
DCVA	2,2-Dichloro-Vinyl ACID
ddH_20	Double Distilled Water
DDT	Dichlorodiphenyltrichloroethane
DGPS	Differential Global Positioning System

DNA	Deoxyribonucleic Acid
EDTA	Ethylenediaminetetraacetic Acid
EIR	Entomological Inoculation Rate
ELISA	Enzyme-Linked Immunosorbent Assay
ER	Endoplasmic Reticulumn
ESACIPAC	Eastern And Southern Africa Centre Of International Parasite Control
F1	First Filial Generation
FAM	6-Carboxyfluorescein
FDA	Food Drugs Administration
FF	First Filial Mosquito generation
FREP1	Fibrinogen-Related Protein Family 1
GA	Anopheles Gambiae
GMAP	Global Map Of Environmental & Social Risk In Agro-Commodity Production
GMS	Greater Mekong Sub-Region
GNTS	Global & National Technology System
GPIRM	Global Plan For Insecticide Resistance Management In Malaria Vectors
GPS	Global Positioning System
GST	Glutathione S Transferase
GTS	Global Technical Strategy
H_20	Water

HBI	Human Blood Index
HEX	Hexachloro-Fluorescein
HLC	Human Landing Catch
HMS	His Majesty Service
HR	Hour
HRS	Hours
IgG	Immunoglobulin G
ILM	Information Lifecycle Management
IPTp	Intermittent Preventive Treatment Of Pregnancy
IR WHO	Insecticide Resistance World Health Organization
IRAC	Insecticide Resistance Action Committee
IRM	Insecticide Resistance Management
IRS	Indoor Residual Spraying
ITN	Insecticide-Treated Net
JH	Juvenile Hormone
JKUAT	Jomo Kenyatta University Of Agriculture And Technology
KD	Knock Down
Kdr	Knock-Down Resistance Gene
KEMRI	Kenya Medical Research Institute
KMIS	Kenya Malaria Indicator Survey
LD_{50}	Lethal Dose To Kill 50% Of The Test Population.
LD_{95}	Lethal Dose With 95% Confidence Levels

LL	Leucine Leucine
LLIN	Long-Lasting Insecticidal Net
LS	Leucine Serine
MAP	Malaria Atlas Project
MM	Master Mix
MoAB	Monoclonal Antibody
MOH	Ministry Of Health
mRNA	Messanger Ribonucleic Acid
N	Total Number Of Individuals Or Cases In The Population
NACOSTI	National Commission For Science, Technology & Innovation
NIRS	Near Infra Red Spectrum
NMCP	National Malaria Control Program
NPIC	National Pesticide Information Centre
NPTN	National Pesticide Telecommunications Network
NTP	Nucleoside 5-Triphosphates
OP	Organo Phosphates
OPC	Outdoor Pot Collection
Orange G	Orange Gelb
PAC	Policy Advisory Committee
PBA	Phenoxybenzoic Acid
PBO	Piperonyl Butoxide
PBS	Phosphate-Buffered Saline

PCR	Polymerase Chain Reaction
PDP	Pesticide Data Programme
Pf API	Annual Parasite Incidence
PfCSP-OD	Falciparum Circumsporozoite Protein - Optical Densities
PMI	President's Malaria Initiative
PSC	Pyrethrum Spray Catch
PST	Pacific Standard Time
RBM	Roll Back Malaria
RDTs	Rapid Diagnostic Tests
RfD	Reference Dose
RNA	Ribonucleic Acid
RNS	Reactive Nitrogen Species
ROS	Reactive Oxygen Species
RTS	Central **R**epeat Region Of *Plasmodium (P.) Falciparum* Circumsporozoite Protein (CSP); The **'T'** For The T-Cell Epitopes Of The CSP; And The 'S' For Hepatitis B **S**urface Antigen (Hbsag).
SAGE	Strategic Advisory Group Of Experts
SDG's	Sustainable Development Goals
SMC	Seasonal Malaria Chemoprevention
SP	Sulphadoxine-Pyrimethamine
SS	Serine Serine
TAE	Tris-Acetate-EDTA

TBE EDTA	Tris Boric Acid Ethylenediaminetetraacetic Acid
ULV	Ultralow Volume
UN	United Nations
UPAC	International Union Of Pure And Applied Chemistry
USAID	United States Agency For International Development
USDA	United States Department Of Agriculture
USEPA	United States Environmenta; Protection Agency
UV	Ultra Violet
WET	Window Exit Trap
WHO	World Health Organization
WHOPES	World Health Organization Pesticide Evaluation Scheme
WMR	World Malaria Report
X-LINKAGE	X-Linked Dominant Inheritance

ABSTRACT / SUMMARY

In Africa, knockdown resistance (*kdr*) is strongly linked to pyrethroid insecticide resistance in *Anopheles gambiae* s.l; which may have vital implications to the current up-scaled indoor residual spraying (IRS) and pyrethroid-treated bed net campaigns. This investigation proposed to evaluate the extent and levels of insecticide resistance among major vectors in Teso North and South sub counties, western Kenya, and its impact on entomological parameters of malaria transmission. For WHO phenotypic susceptibility tube assay, mosquito larvae were sampled using a dipper, reared into 3-5 days-old females which were exposed to 0.75% permethrin, 0.05% deltamethrin and 0.1% bendiocarb. Random sampling of adult mosquitoes [3448] was done using adult mosquito sampling methods. Abdominal statuses, parity rates, age grading and molecular species identification were recorded. *Kdr* East TaqMan PCR probed for the mutant allele and wild type. Sporozoite ELISA detected circum-sporozoite protein. *Anopheles gambiae* sensu stricto were the significant majority in terms of species composition at 78.9% as compared to *Anopheles arabiensis* (20.7%) [$t(4, 4) = 11.1268$, $p < .05$]. Highest proportion of SS alleles (86.1%) was also found among *Anopheles gambiae* sensu stricto mosquitoes. Susceptible Kengatunyi cluster had higher proportion of *An. arabiensis* than resistant Rwatama. Bendiocarb caused 100% mortality while deltamethrin had higher insecticidal effects (77%) on female mosquitoes than permethrin (71%). There was a significant difference in SS allele frequencies between Kengatunyi (57.8%) and Rwatama (93.4%). Of the parous vectors, 75.6% were homozygous resistant, 2.4% were heterozygous and 22% were homozygous susceptible. Mosquitoes in the resistant cluster had an average lifespan of 16 days compared to 5 days in the susceptible cluster. Resistant clusters had the largest number of infective bites per person per night at 0.4. Of the sporozoite positive

mosquitoes, 71.4% were homozygous resistant and the rest homozygous susceptible. Heterozygous LS allelic vectors had significantly higher affinity for bovine blood while SS allelic vectors had significant higher affinity for human blood. All SS, LS and LL carriers were biting more at dawn than at dusk. All results were significant at $p<0.05$. Most likely, the higher the selection pressure exerted indoors by insecticidal nets the higher were the resistance alleles. Use of pyrethroids impregnated nets and agrochemicals may have caused female mosquitoes to select for pyrethroid resistance, consequently impacted on species composition and behaviour patterns. Confirmed phenotypic and genotypic insecticide resistance in major malaria vectors in Teso North and Teso South sub counties, impacted significantly on vector species composition, age structure, density, infectivity, feeding and resting behaviours. Insecticide resistance management practices in Kenya should be fastracked and harmonised with agricultural sector agrochemical based activities and possibly switch to carbamate usage. The implication of such high resistance levels in the mosquitoes is that resistance is likely to persist and or even increase if mono-molecules of permethrin and deltamethrin or both continue to be used in all net and non-net based mosquito control purposes. Piperonyl butoxide (PBO) that prohibit certain metabolic enzymes within malaria vectors and has been combined into pyrethroid-LLINs to create pyrethroid-PBO nets, may be an extremely viable option against the spread of insecticide resistance in Kenya.

Key words: *Anopheles* species | Insecticide resistance | WHO tube assay | *kdr* | Genotypic frequency

CHAPTER ONE

1.0 INTRODUCTION

1.1. Malaria transmission dynamics and determinants

Malaria is caused by *Plasmodium* parasites (Phylum: Apicomplexa, Order: Haemosporida and Family: Plasmodidae) and transmitted to humans through the bite of an infected female *Anopheles* mosquito (Figure 1.1). Natural infections of *Homo sapiens* are caused by five species: *Plasmodium falciparum, Plasmodium knowlesi, Plasmodium malariae, Plasmodium vivax,* and *Plasmodium ovale* (Alout et al., 2017). *Plasmodium vivax* and *P. falciparum* pose the most significant threat among the five parasite species. *Plasmodium falciparum* in the African continent is the most prevalent, accounting for 99% of the approximated malaria incidents recorded in 2016 (Torre et al., 2002; Yawson *et al*., 2004). Outside of Africa, in the WHO region of America *P. vivax* is the predominant parasite, accounting for 64% ratio of malaria cases, 30% and above in the WHO Southeast Asia, and 40% in the Eastern Mediterranean regions (Pigott *et al*., 2012; WHO 2017b; Simon I Hay and Snow 2006).

The results of a study on conditions determining the breeding abundance of a mosquito vector while considering different habitats and preferences on the state of Oaxaca off the Pacific Ocean indicated that environmental conditions, geographical area, and climate are linked to differences in transmission of malaria among the localities (Hernández-Avila *et al*., 2006). These requirements appeared to designate the malaria infection receptiveness in the geographic area (Guyant *et al*., 2015). Human actions were linked to inception and spread of the malarial agents among and within selected villages (Endo & Eltahir, 2018). Socio-demographic and ecological factors determined the intensity of human vulnerability to mosquito bites (Sinka *et al*., 2011; Wiebe *et al*., 2017).

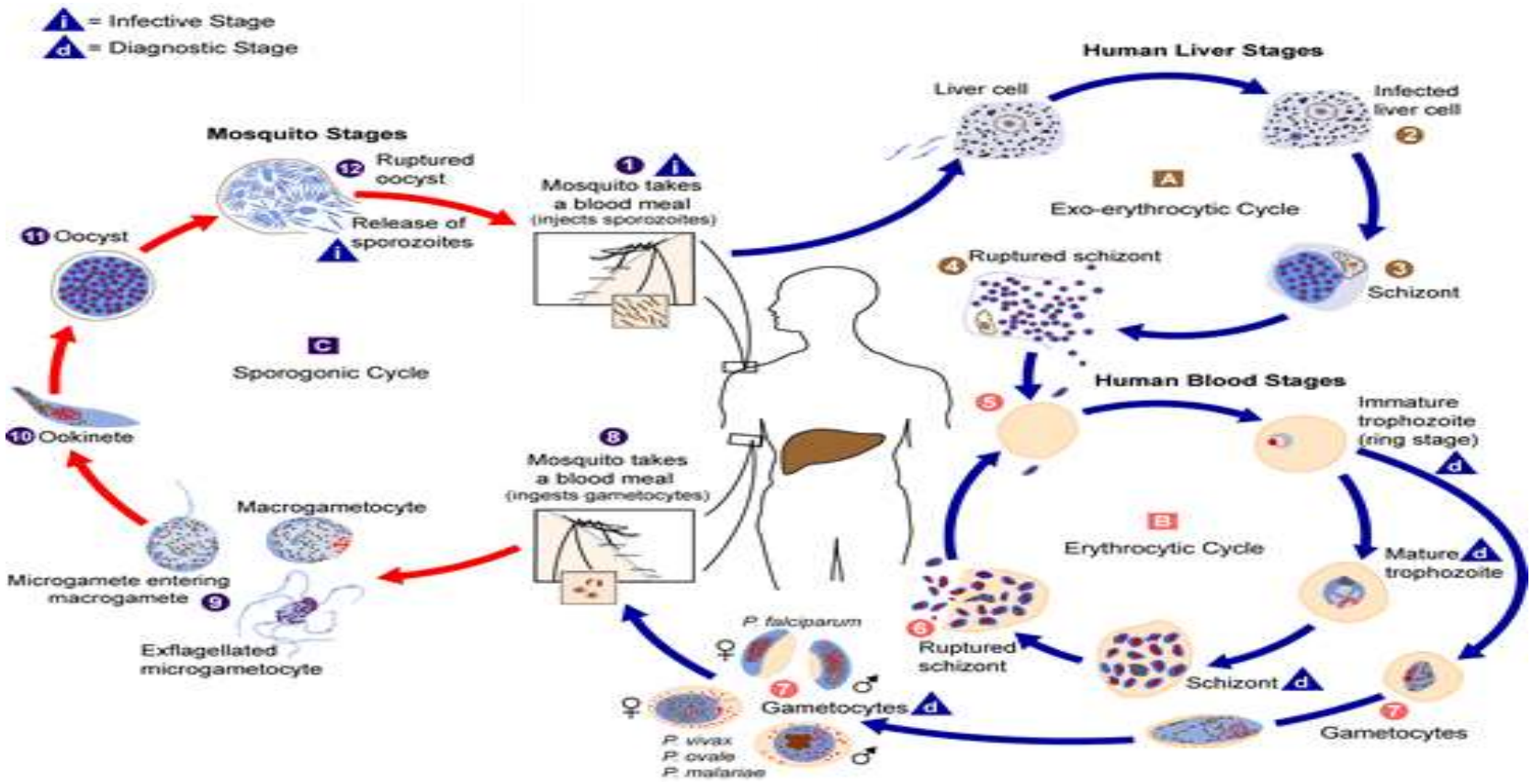


Figure 1.1: Transmission and development of malaria parasites between mosquito and humans (Content source: Global Health, Division of Parasitic Diseases and Malaria, 2020)

1.2 The worldwide burden of Malaria

Malaria is a global health risk (Shretta *et al*., 2017). In 2016, a total of 216 million incidents of malaria occurrence were recorded globally contrasting with 237 million incidences in 2010 and 211 million incidences in 2015 (Guyant *et al*., 2015; Mzilahowa *et al*., 2016b). Of the 216 million malaria cases globally, 90% were reported in Africa, 7% in South East Asia and 2% in Eastern Mediterranean regions (WHO, 2017b). In 2016, deaths linked to malaria were about 445,000 world wide, in comparison to about 446,000 demises in 2015 (WHO, 2017b). Notwithstanding the fact that malaria incidences have reduced internationally from 2010, the drop has delayed since 2014 and even regressed in various parts of the world. Also, the number of deaths has taken the same pattern (WHO, 2016d).

Of the 91 nations giving an account of native malaria incidences in 2016, 15 nations, all in sub-Saharan Africa, with an exemption of India bore 80% of the worldwide malaria load (Figure 1.2). The occurrence of malaria is approximated to have lessened by 18% internationally, from 76 to 63 incidences for every 1000 people at risk, between 2010 and 2016 (WHO, 2016d). The South-East Asia region reported the leading decrease (48%), trailed by the Americas (22%) and the African region (20%) (WHO, 2017b). Inspite of these decreases, between 2014 and 2016, considerable escalations occurred in America, and slightly in the Western Pacific, South-East Asia, and African regions (WHO, 2017b). Internationally, additional nations are headed for eradication: in 2016, 44 nations recorded less than 10,000 malaria incidences, up from 37 nations in 2010. In 2016, WHO certified Sri Lanka and Kyrgyzstan as malaria free states (WMR, 2017).

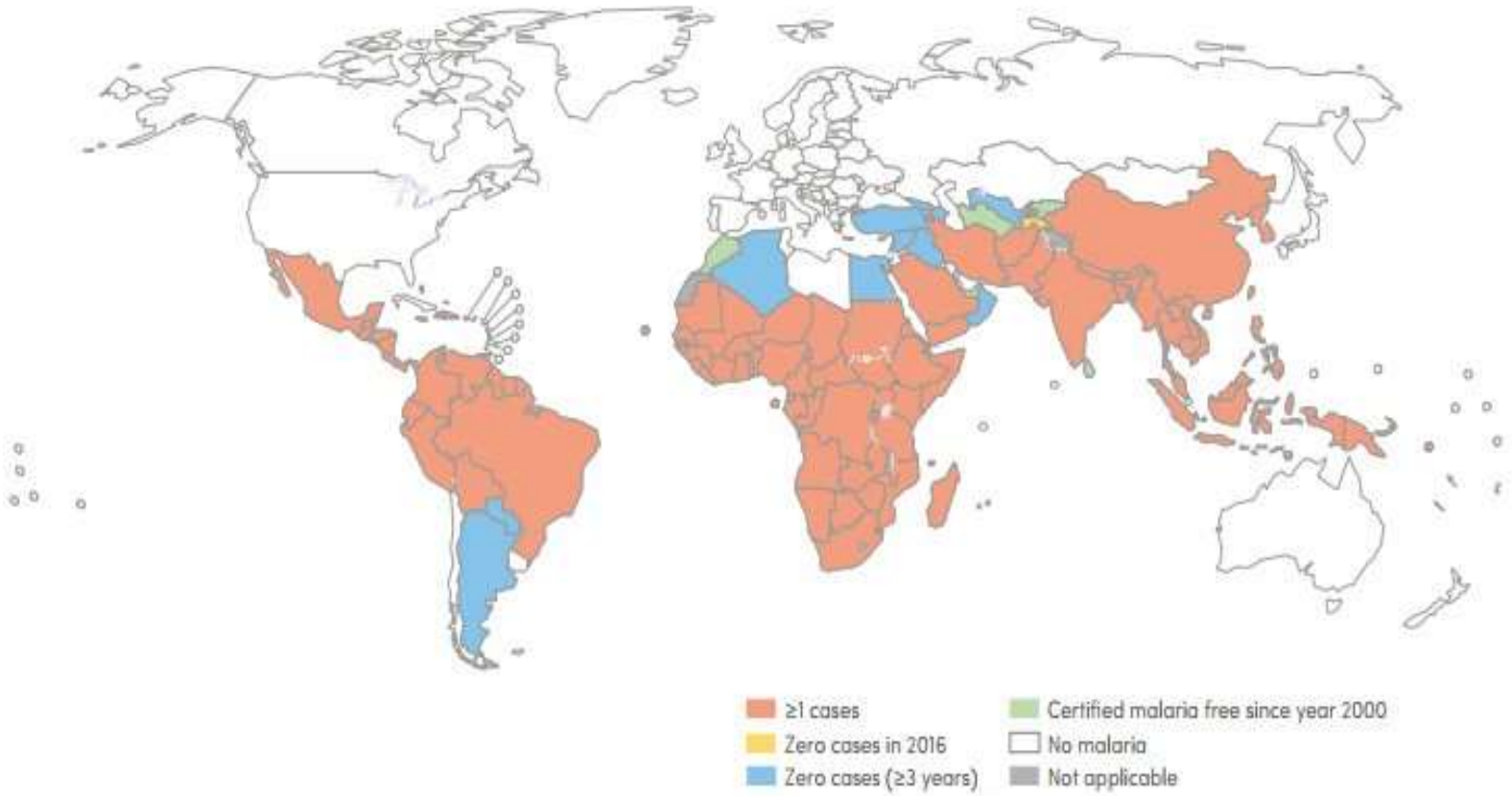


Figure 1.2: Confirmed malaria cases per 1000 individuals in 2016 (Courtesy: World Malaria Report , 2017)

1.3 Malaria in Africa

African sub-Saharan zone accounted for 88% of all malaria cases in 2015 besides 90% of all deaths being attributed to malaria (WHO, 2017a). Fifteen countries in Africa contributed heavily to the global malaria burden in 2015 (Newman, 2012; WMR, 2017). Jointly, these nations recorded an estimate of 80% of worldwide malaria cases and 78 percent of deaths. Advances in reducing malaria incident globally in these countries with high burden have trailed behind other states. Malaria places a socio-economic burden on African countries in addition to loss of human lives (WHO, 2016d).

Insecticide treated nets coverage has progressively been increasing over the last 15 years in the African sub-Saharan region (Camponovo *et al*., 2017; E. Ochomo *et al*., 2014). In 2014, 56% of the population were able to acquire an ITN, as contrasted to 2 percent or less in 2000 (WHO, 2016d). According to information from national malaria control programs, 75% of ITNs were given out during state organised mass net hand out campaigns between 2014 and 2016. From 2010 to 2016, one net per house ownership rose from 50% to 80% (PMI, 2017).

Around 2005 - 2014, malaria diagnostic testing increased sharply in the African zone from 36 to 65 percent of all malaria suspected cases (WHO, 2016d). An approximated 312 million rapid diagnostic test (RDTs) kits were supplied world wide in 2016, of which WHO Africa received 269 million RDTs (WHO, 2017b). The most effective drugs used against the highly lethal and widely diagnosed *Plasmodium falciparum* parasite are the Artemisinin-based combination therapies (Guyant *et al*., 2015; Shretta *et al*., 2017). Bell and Winstanley, 2004 reported that infection with malaria parasite during pregnancy causes

substantial risk to the fetus, newborn child and the mother. In Africa, the percentage of women who get preventive treatment against malaria during pregnancy has been rising, but the scores are persistently lower than the set national goals (PMI, 2017; WHO, 2016d). Among 23 African nations that gave accounts on intermittent preventive treatment in pregnancy (IPTp) coverage in 2016, an estimated 19% of entitled expectant women were given three or more IPTp doses, a slight rise from 18% in 2015 and 13 percent in 2014 (WHO, 2016d). Out of the fifteen nations where WHO approved therapies towards malaria prevention for every child under five years of age, only a single country, Chad has incorporated the approved prophylactic therapy for young children as a national policy (WHO, 2017b). In national-level assesments finalized between 2014 and 2016 in the sub-Saharan Africa, only 18 countries, hence 61% of at risk population had a median of 47% of infants with symptoms of a fever transported to a medical care facility and treated by trained health care providers (WHO, 2017b; WMR, 2017).

To identify critical zones or population age sets for targeting resources towards maximal preventive and treatment impact, proper records and analysis of malaria infection cases and deaths should be done through surveillance (WHO, 2003). At least 37 out of the 47 nations in the WHO African zone had channelled data on malaria through the country's health information system as given out by 80% of the public health facilities (PMI, 2017). However, the levels vary in other WHO zones (WHO, 2017b).

1.3.1 Declining malaria disease burden and changing epidemiology in Africa

Dramatic increase of malaria prevention and control campaigns since the year 2000 has been as a result of heightened significance in accurate measurements of impact on changes in malaria transmission and burden (Bhatt *et al*., 2015). Between 2000 and 2015, death rate due to malaria diminished by 66 percent whereas the number of malaria incidences decreased by 42 percent in the African zone (Ryan *et al*., 2006). To a greater extent, monetary contractual obligations to malaria control have risen twenty times from the year 2000 (Killeen, 2014). Even though these inadequate funding levels have stagnated, they have over the years increased access to preventative and curative drugs against malaria for millions of persons at risk, funded insecticide spraying activities in nearly all endemic African nations, facilitated distribution of more than half a billion insecticidal mosquito nets and made significant contributions to broader health system scale up measures (Guyant *et al*., 2015; WHO, 2017b).

With immense international effort yielding increased coverage of effective malaria control interventions, malaria transmission, case incidence, and deaths have been dramatically reduced (WHO, 2016d). But coverage of effective malaria disease control measures remains erratic and significantly below specified targeted goals. Effectiveness of any given control strategy will differ according to local vectorial, disease transmission and health system factors (Antonio-Nkondjio *et al*., 2015; Chanda *et al*., 2008; WHO, 2012). Further justification and modification of modeled predictions contrary to a current and geographically elaborate empirical rational evidence support platform is a requirement (Homan *et al*., 2016).

A fuller comprehension of the varying pattern of malaria disease transmission is becoming progressively more momentous for various reasons (Bousema & Drakeley, 2011; Ffrench-Constant, 2013; Hawkins *et al*., 2018a, 2018b; Kar *et al*., 2014; Pasteur & Raymond, 1995). Funding agencies are hard pressed to validate that investments have progressively impacted on diminishing disease burden and that this impact can be trailed decisively (Guyant *et al*., 2015). Layered approaches to malaria interventions in national programs necessitate a comprehensive overview of changing tendencies of risk in order to plan modifications as local conditions adjust (KNMIS, 2016; WHO, 2016b, 2016a, 2017b). Dependent on localized valuations means that local heterogeneities will not be ignored thus inspiring custom-made control measures to enhance effectiveness. Roll Back Malaria Global Malaria Action Plan underlines the need to appropriately stratify, specified and locally relevant measures towards clear perception and responding to changing heterogeneities in risk (Takken & Koenraadt, 2013; WHO, 2016d; WMR, 2017). Independently, WHO malaria Policy Advisory Committee has created and mandated Evidence Review Group on Malaria Burden Approximation to provide new focus on the drawbacks and future prospects of trailing changes in Africa (Pigott *et al*., 2012).

An increased installation and acknowledgement of a robust routine data base as African National surveillance systems should further support rapidly changing communication technologies, diagnostic capacity and enhanced access to healthcare (PMI, 2017). Adequate funding in support of significant malaria survey programs has given chance to several African nations to commence numerous rounds of cross-sectional household surveys, progressively with both cluster-level GPS coordinates and blood testing for

malaria parasites (Homan *et al*., 2016). During analysis of change process, growing availability of survey data gives chance to an unprecedented possibility to carry out spatiotemporally comprehensive assessments of shifting infection prevalence co-measured with control measures coverage. Corresponding raw information drawn from surveys, amplified by ongoing observational and theoretical evaluation of how control measures interact with the cycle of parasites transmission and the consequential human disease status, have the likelihood to change perception on how malaria phenomenon is changing in the African content (Killeen, 2014; Pigott *et al*., 2012). These crucial opportunities to fund quality data collection surveys need to be actualized, maintained, and transformed into active decision-making platforms (Killeen, 2014; Steinhardt *et al*., 2017).

Refreshed global commitments to fund and offer logistical support to survey and surveillance campaigns in Africa coupled with an initiative by the scientific community to come up with methodical geographically concordant strategies to analyze the resulting raw information will put to an end the excessive dependence on ad-hoc concoctions of findings from local studies (WHO, 2012). The important role of malaria disease modelling and effective communication of evolving assumptions and uncertainties to decision makers must be improved (Chanda *et al*., 2008). Future line up of monetary, climatic and evolutionary hazards against political and technological possibilities may merge and prolong successes hence the importance to come up with factual, timely and distictive mechanisms for determining shifts and are competent enough to address these disease control challenges (WHO, 2013).

1.4 Malaria in Kenya

Malaria has been the foremost cause of sickness and death in Kenya where over 70% of the people live in malaria vulnerable zones (KNMIS, 2016). The rate of malaria spread and chances of infection in the country is influenced largely by duration and amount of rain, height above sea level and temperatures hence malaria disease in Kenya is not uniform (Figure 1.3). The Indian Ocean coast and the Lake Victoria region record the greatest risk. Moreover, 18% of outpatient sessions and 10 percent of inpatient cases were attributed to *Plasmodium* infections according to statistics in the routine health information system (KNMIS, 2016). The four malaria parasite species of human *Plasmodium*: *P. vivax, P. ovale, P. falciparum,* and *P. malariae* are in Kenya (Olanga *et al*., 2015). According to Kenya National Malaria Indicator Survey (KNMIS) of 2010, 96% of the positive slides were *P. falciparum* while 80% were unmixed infections and 16% were composite infections with *P.malariae* or *P. ovale* or both. Another 2% were unmixed *P. malariae* infectious agents and 1% was *P. ovale*. No *P. vivax* was detected in this survey *(Sinka et al*., 2011). The foremost malaria vectors in Kenya are adherents of *An. gambiae* complex and *An. funestus* complex (Ondeto *et al*., 2017).

A lot has been achieved to decrease and eradicate malaria in Kenya and in the globe. The main goal of fighting malaria is fundamentally connected to majority of the UNs' Sustainable Development Goals (SGDs) which are in harmony with Kenya's Vision 2030 (PMI, 2017). With the aid of National Malaria Control Programme (NMCP), The Ministry of Health has applied logical measures and proven approaches towards malaria elimination (KNMIS, 2016).

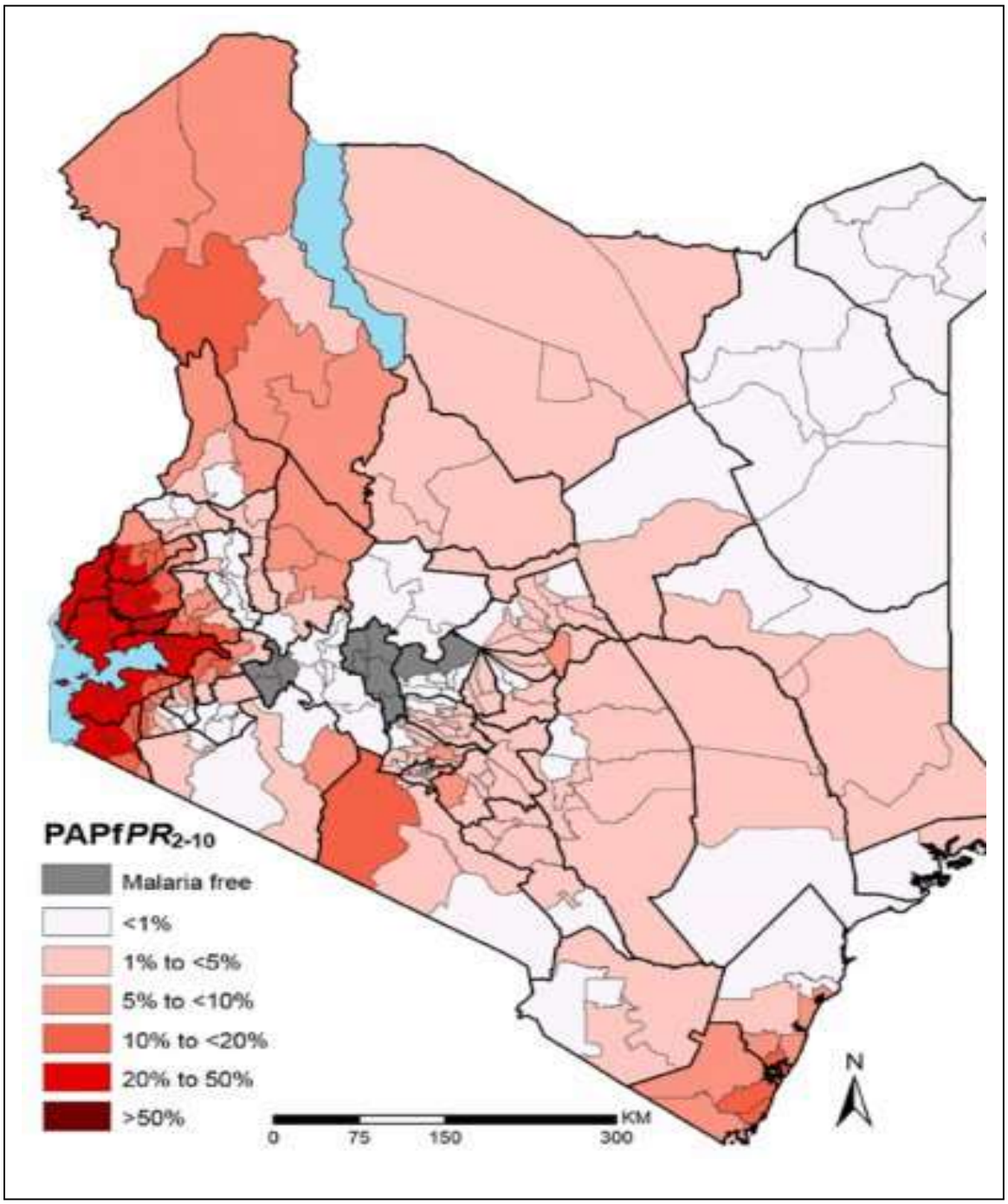


Figure 1.3: Kenya population adjusted malaria prevalence PAPfPR2-10 at 1 × 1 km spatial by sub-county (Source: KNMIS 2015)

1.4.1 Malaria endemic areas in Kenya

Endemic zones of stable malaria have height above sea level extending from 0 to 1300 meters in L. Victoria basin and coastal areas of Kenya (Figure 1.4). Precipitations, temperatures and moisture are the major determinants of the recurrent *P. falciparum* infections (Agegnehu *et al*., 2018). Mosquito's life span is short, yet it has a high survival rate due to satisfactory weather conditions with yearly entomological inoculation rates ranging between thirty and a hundred (Gimnig *et al*., 2003; Minakawa et al., 2012). The beginning of malaria spell in eastern Africa is notable at the close of the sixth month of the year especially after prolonged wet weather which is most favourable for vector propagation (Fillinger e*t al*., 2011). Even though, malaria has been dropping due to strengthened interventions as from mid 2000s, these alterations in the mosquito ecology might put at risk the inhabitants in the uplands of east Africa, which may lead to rampant malaria infections if the existing control measures are not continued (Olanga *et al*., 2015). Ecofriendly surroundings in the residual focus are ideal for the abundance of the *Anopheles* species within the neighborhood. Earlier research had showed a close relationship between mosquito propagation, land usage and gradient in the nearby villages (Wilke *et al*., 2015). A tricky balance of the malariogenic system with extreme ecological variations could lead to high mosquito densities thus trigger an eruption in new infections (Bastiaens *et al*., 2011; Pigott e*t al*., 2012). Closeness to roads increases human activities hence frequent inception of the malaria parasite, while remote places would have less malaria occurrences as a result of smaller number of people moving in and out of the villages (S.I. Hay *et al*., 2006; Noor *et al*., 2009).

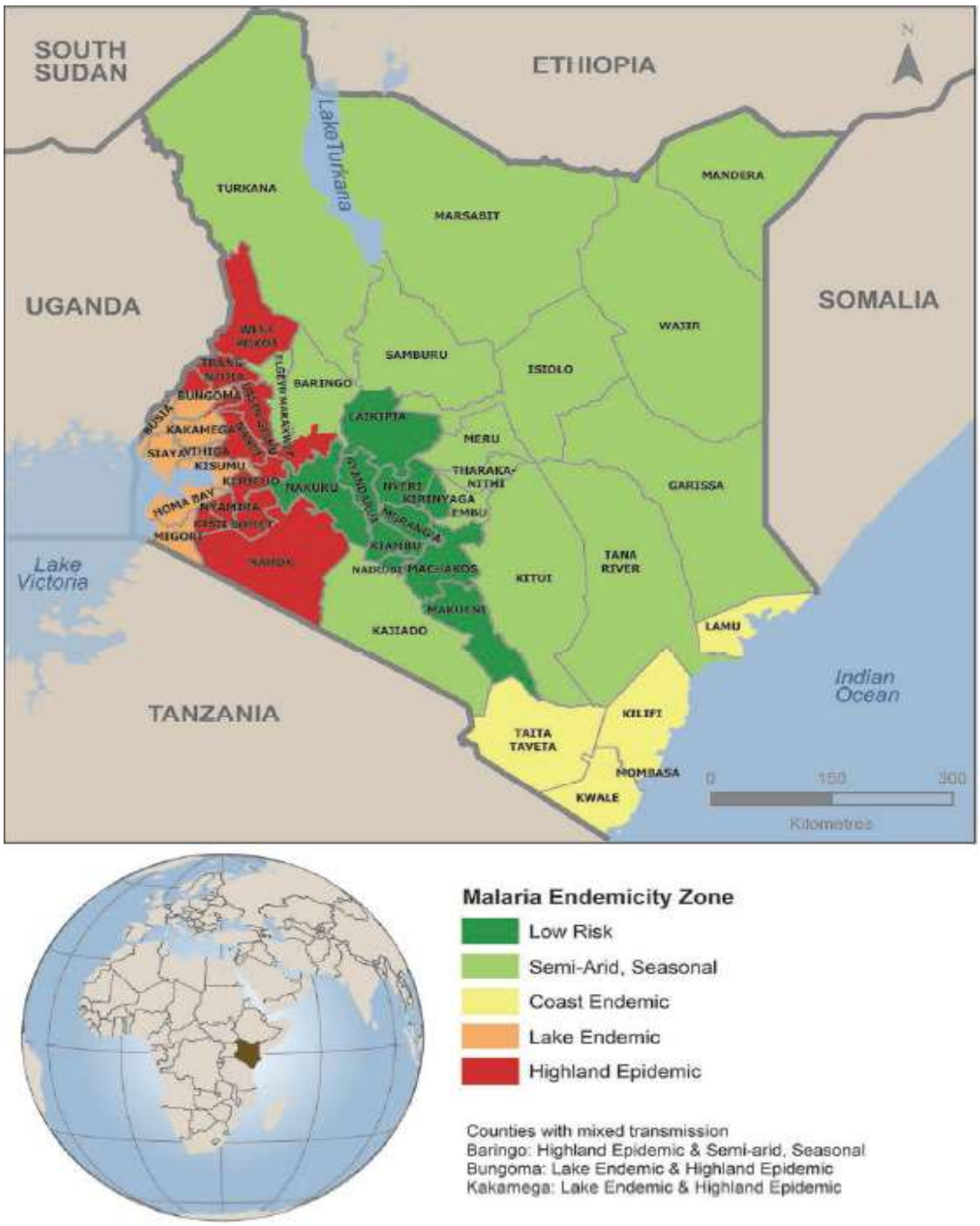


Figure 1.4: Kenya malaria endemicity map 2015 (Source: KNMIS 2015)

1.5 The Global and National Technical Strategy (GNTS) to control malaria

Responsiveness, coverage and usage of fundamental interventions are essential in the fight against malaria. In 2014, the fraction of households with ITN in the sub-Saharan countries was at 56% while 82% of people with ability to acquire the net actually slept under ITN (WHO, 2017b). Therefore, enabling reachability to ITNs has been crucial to raising the number of people spending nights under an ITN. For the nations in the sub-Saharan Africa, the approximated fraction of entire population spending the night under an ITN has inreased form 46% in 2014 to 55% in 2015. In 2015, 68% of infants aged less than five years spent nights under ITNs as compared to less than 2% in 2000 (WHO, 2017a). The percentage of the people spending nights under an ITN differs broadly among nations, with median percentage at 74% among five nations with the maximum proportions, and 20 percent among five nations with the minimum proportions (Newman, 2012).

The number of people vulnerable to 6malaria and are safeguarded by IRS has diminished worldwide from 5.7% to 3.4 % in 2010 and 2014 respectively, with the reduction evident in all areas excluding the Eastern Mediterranean WHO zone. Globally, 116 million persons were safeguarded by IRS in 2014 (WHO, 2016d). Out of the 53 nations that stated the type of insecticide sprayed in 2014, pyrethroid was used in 43 nations, while some nations used one or two other classes of insecticides (Figure 1.5). Merging figures on the population with an ITN within reach in a household and the persons protected by IRS, the projected population for whom mosquito control was made accessible in sub-Saharan Africa improved from 2% in 2000 to 59% in 2014 (Poché *et al*., 2015). This is still less than the worldwide goal of 100% availability as contained in the 2011 up to date information to the Global Malaria Action Plan (Newman, 2013).

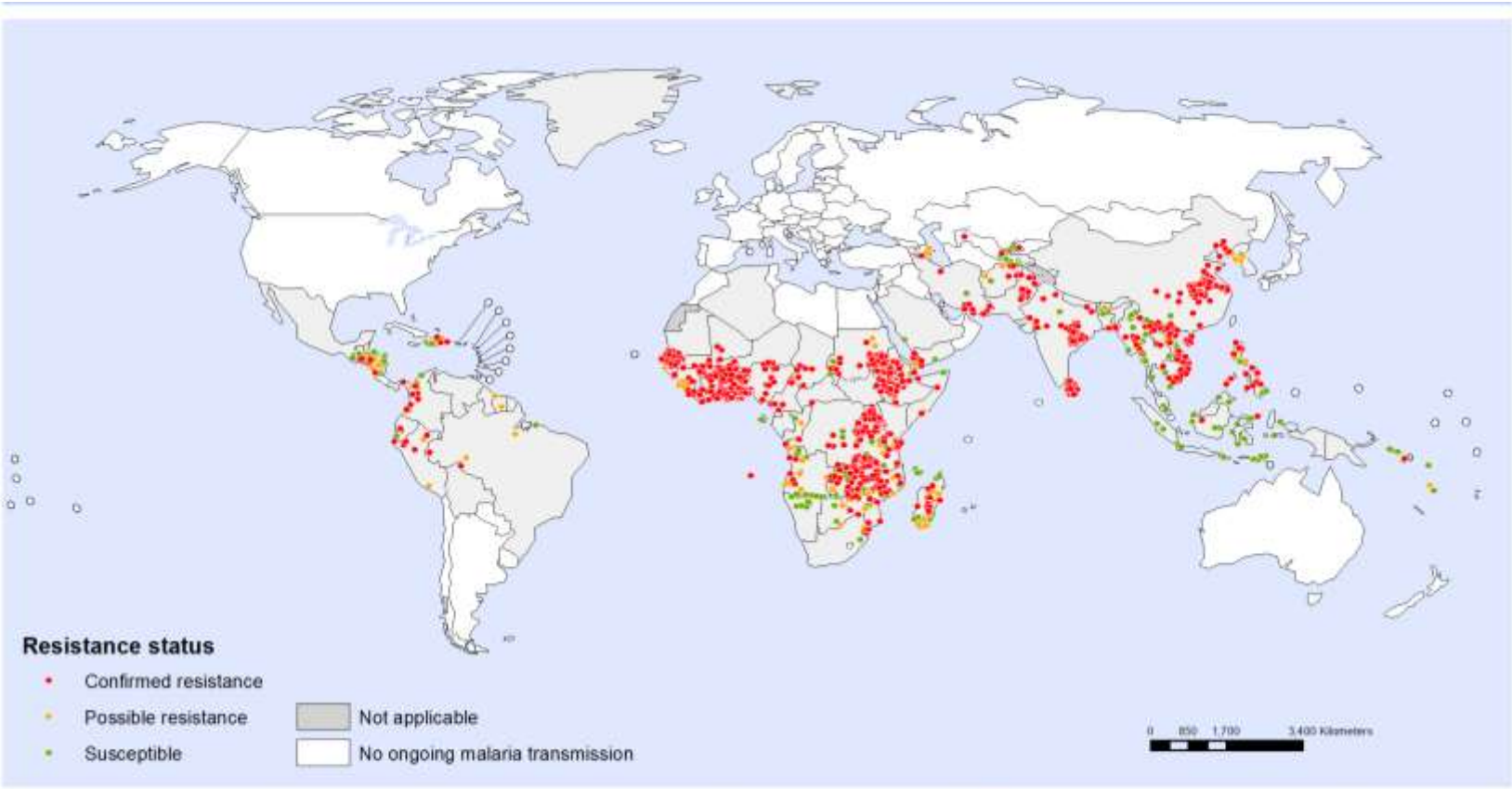


Figure 1.5 : Reported insecticide susceptibility status for malaria, 2010 – 2015 (Source: WHO 2017)

The number of expectant females getting at least three dosages of intermittent preventive treatment in pregnancy (IPTp) has escalated after WHO reviewed its recommendation in 2012 (WHO, 2016d). In 2014, a projected 52% of eligible expectant females got at least one dose of IPTp, 40 percent got two or more dosages, while 17% got three or more doses (KNMIS, 2016). The variance between the number of females attending antenatal care (ANC) clinics and the number getting the initial and succeeding dosages of IPTp indicates that the prospects to distribute IPTp at these clinics were not met (PMI, 2017). In sub-Saharan Africa, the number of females obtaining IPTp differed across the continent, with more than 60% of expectant mothers getting one or more doses in 10 nations, while nine nations reported more than 80% receiving one or more portions (Bradley et al., 2015; PMI, 2017; WHO, 2016d).

Acceptance and execution of chemoprophylaxis in offspring has been inadequate. In 2014, six of the 15 nations for which WHO approves seasonal malaria chemoprevention (SMC) – Senegal, Guinea, Chad, Gambia, Mali, and the Niger– had implemented the policy. Moreover, two other nations separate from the Sahel sub region – Togo and Congo – informed that the strategy had been implemented (WHO, 2017b). Only a single nation, Chad, informed implementation of an intermittent preventive treatment for infants (IPTi) program in 2014 (PMI, 2017; WHO, 2016d).

The vaccine against malaria (RTS, S/AS01) was favourably acknowledged by the European Medicines Agency under Article 58 (Camponovo *et al*., 2017; Gosling & von Seidlein, 2016). Preliminary implementation of the primary malaria vaccine was approved by WHO's Strategic Advisory Group of Experts on Immunization (SAGE) and the Malaria

Policy Advisory Committee (Gosling & von Seidlein, 2016; Guyant *et al*., 2015; WHO, 2017b, 2017b).

The number of assumed malaria incidences reported in the communal facilities supplied with diagnostic test kits for malaria has improved from 74% to 78% between the years 2005 and 2014 (Bradley *et al*., 2015; Kumar NK *et al*., 2014; Ondeto e*t al*., 2017; WHO, 2017b). In the worldwide trend, South-East Asia countries dominated, India to be precise, having procured a large number of diagnostic tests and performing over 100 million tests in 2014 (Kumar NK *et al*., 2014; WHO, 2017b). The WHO African countries experienced the leading rise in malaria rapid diagnostic test kits usage over the years; 36% in 2005, 41% in 2010 and 65% in 2014 (Steinhardt et al., 2017; WHO, 2016d). The number of tested malaria cases diagnosed was lesser in feverish children going for care in the private facilities as opposed to the ones going for consultation in the governmental communal facilities (WHO, 2017b, 2017b). In the midst of 18 country specific evaluations carried out in the sub-Saharan African region between 2013-2015, the medium range of feverish children who had gotten heel or finger prick stood at 53 % in communal health services, 36 % in the formal private facilities and 6% in the non formal private facilities (KNMIS, 2016; PMI, 2017; WHO, 2017b, 2017b).

The fraction of infants below five years that have malaria parasite *P. falciparum* and got treated using ACT improved from <1% to 16% in 2005 and 2014, respectively (Bell & Winstanley, 2004; Guyant *et al*., 2015; Terlouw *et al*., 2003). This fraction is way below the GMAP set goal for global availability of malaria case management. A major reason is because a high number of infants having fever does not receive treatment but instead use

informal private facilities, where they have few chances of receiving ACTs for treatment. The number of infants treated with ACTs has increased over the years while the fraction receiving alternative antimalarial drugs has declined (Amin *et al*., 2007). The number of ACT antimalarial medication was lesser when treatment was gotten from informal medical facilities that provided health-care, for example local retail shops market or roaming vendors (WHO, 2016d).

1.6 The challenges facing the malaria control

A lot of advancements to manage malaria have been achieved in a couple of nations in very endemic regions. African nations such as Zambia, Rwanda and Zanzibar have renewed interest in mosquito control with significant decreases in malaria disease burden (PMI, 2017; WHO, 2017b). In contrast to other zones of the earth, the percentage of people who had malaria confirmed through diagnosis and obtained medication is minimal in Africa (WHO, 2017b). The perception that anti-malarials could be used as medication for persons who had no malaria parasites is shifting cojointly with malaria epidemiology, particularly in parts where steps to decrease the *P. falciparum* infection parasitaemia levels had been taken. Incorrect administration of anti-malarials has contributed heavily to drug resistance which leads to extraneous costs (Camponovo et al., 2017; WHO, 2016d).

Malaria parasite *Plasmodium falciparum* is the most vilurent species which has evolved resistance to nearly all regularly approved anti-malarials, thus a principal impediment to the success in malaria treatment in endemic zones (Bhatt et al., 2015; Camponovo et al., 2017; Simon I. Hay et al., 2005; Simon I Hay & Snow, 2006; WHO, 2016d). Availability to treatment for high at risk people and difficult to access communities, lingering and out of door infective bites have made a major achievement to control malaria; more so with

the consumption of ACT (artemisinin-based combination therapy) which has considerably decreased gametocyte carriage hence diminished disease transmission. ACT medication to children and infants can be arduous and labourious (Bell & Winstanley, 2004; WHO, 2017b). To ease administration, necessary specially formulated drugs against malaria for this unresistant population will guarantee correct dosage and strict compliance *(Homan et al*., 2016; Newman, 2012).

As diagnostic guidelines undergo transformation, efficacious case management includes training of health personnel and village dwellers on averting malaria, testing and suitable medication (PMI, 2017; Thiévent *et al*., 2018). In the face of varying malaria epidemiology, it's a precarious task to stop and properly manage resistance to both artemisinin and artemisinin-based combination therapy and also in the insecticides used for vector control in the Greater Mekong Sub-region (GMS) as well as in African nations (Amin *et al*., 2007; Gosling & von Seidlein, 2016; WHO, 2017b). Indoor residual sprays and long lasting insecticidal mosquito nets tend to be protective against indoor infective bites, more so during the night (Brugman, 2016; Thiemann *et al*., 2011). Nevertheless, persons who spend their evenings, nights and early morning outside the houses are most vulnerable to be infected with malaria parasites since they are insufficiently equipped with anti-vector tools planned precisely to hinder out of door "residual" transmission (Hawkins *et al*., 2018b; Hernández-Avila *et al*., 2006). It is of paramount importance to ensure sustenance of monetary and governmental assistance towards malaria control measures particularly in regions of low parasitaemia and pre-elimination point (Bastiaens *et al*., 2011; Pigott *et al*., 2012).

1.7 Statement of research problem

Insecticide resistance in Anopheline mosquitoes in Kenya is currently a fundamental cause of concern to vector control program consumers, managers and designers. Mutation in genes selecting for resistance is a progressing, multifaceted and ever changing process which is threatening to reverse improvements in malaria control measures. Moreover, when the recurrence of resistant insects in a vector population rises, effectiveness of the pesticide declines until the replacement with another category of insecticide is put in place. Once vectors breed around or near agricultural crops, they might be made vulnerable to equivalent insecticidal mixtures, hence gradually select for the capacity to degrade the insecticidal properties of the compounds.

Additionally, numerous classes of insect killing chemicals are widely approved to control household vermin, thus applying more insecticide resistance selection force. Resistance against several insecticidal chemical compounds cause vector numbers to swiftly gain resistance to the new insecticides (Hawkins *et al*., 2018a, 2018b). Furthermore, IRM mechanisms, for example, alternations and combinations could be weakened by the cross resistance complications. The efficacy of insecticides used in ITNs and IRS may be rapidly diminished by the progressively developing reduced vulnerability to the closely related insecticides already in use. Pyrethroids based class of insecticides is singularly and heavily used in the world wide measures to control malaria, especially used in LLINs. Again indoor spraying activities utilize pyrthroids. Due to immense pressure on the same chemical compounds, insecticide resistance is thus created, and may have escalated levels in terms of spread and severity. The malaria parasite vertebrate and invertebrate hosts' behaviours

for example, biting outdoors and night long human activities may diminish anophelines contacts with the lethal treated nets and sprayed walls but increase their exposure to insecticides laden outdoor environments.

Since LLINs and IRS perform a major role in mosquito control programs, the present biological drawbacks may actually hinder the benefits achieved so far in the the malaria control, hence further reducing its achievements or lost altogether. Therefore, there is a pressing prerequisite to preserve the usefulness of an inadequate number of effective and obtainable insecticides. Innovative measures to counter development of non – vulnerability of mosquitoes to novel new insecticidal products are majorly at the initial steps of formulation and assessment. The results produced by this study will provide malaria interventions program managers with evidence based directions to boost informed planning of rational insecticide resistance management approaches. The purpose of this study was to investigate levels of insecticide resistance and its impact on malaria transmission potential among major malaria vectors in Teso North and Teso South sub – counties, Busia county, western Kenya.

1.8 Research questions

1. What is the level and the range of non – susceptibility to insecticides by the major Anopheline species in Teso sub – counties, western Kenya?
2. How does vector population age structure in areas with insecticides resistance compare with areas without?
3. What is the effect of the vectors non - vulnerability to insecticides on their densities and infectivity?
4. What is the effect of the mosquito non – vulnerability to insecticides on their feeding and resting behavior?

1.9 General objectives

The primary goal of this study was to determine the levels and ranges of insecticide resistance and its effect on various entomological parameters of *P. falciparum* infection in major *Anopheline* species in Teso sub – counties, Busia county, western Kenya.

1.9.1 Specific objectives

1) To determine the levels and ranges of insecticide resistance among *An. arabiensis, An. gambiae* complex, and *An. funestus* in Teso sub – counties, western Kenya
2) To determine the vector population age structure in areas with and without resistance to insecticides amongst key malaria vectors in Teso sub – counties, western Kenya: parity rates and life span
3) To determine the effects of resistance to insecticide on *Anophelines'* infectivity: abdominal status, densities, sporozoite rate and entomological inocution tates (EIR)
4) To determine the effect of resistance to insecticide on *Anophelines'* feeding and resting behavior: blood preference, human blood indices, indoor & outdoor resting

1.10 Justification of this research study

Recent reports from Busolwe and Tororo districts in eastern Uganda near the border with Kenya indicate elevated frequency of the *kdr* allele (1014S), similar to what has been observed in the Asembo study site, approximately 150-200 km to the southeast (Hawkins *et al*., 2018b). However, the occurrence of *An. gambiae* in mosquito collections is much higher than that observed in the Asembo study site and there are strong indications that the *kdr* allele is conferring both DDT and pyrethroid resistance particularly in homozygous resistant individuals. It was not clear whether this is the result of differences in ITN/LLINs ownership and use or whether resistance levels are higher in this area and leading to control failure. In addition, the impact of agricultural pesticides on insecticide resistance is unclear. Greater abundance of *An. gambiae s.s*., the elevated recurrence of the *kdr* genes and the substantiation of phenotypic non-vulnerability in this region, has prompted the establishment of the study site on the Kenya Uganda border in the sub county of Teso.

Given the close proximity of the current study site and districts in Uganda where resistance has been observed, it was interesting to investigate the *kdr* allele frequency in Teso North and Teso South sub counties. The study focused on characterization of susceptible and resistant mosquito populations in connection to vector population age structure, density, infectivity, feeding and resting behavior.

The adoption of insect killing chemical compounds in the malaria intervention activities is growing especially with widespread and up-scaled utilization of LLINs and IRS. Regardless of the vast input in LLINs and IRS, several nations do not carry out predictable anopheline and resistance surveillance surveys. Overseeing, assessment, supervision and

operational investigative activities are fundamental to tracing and enhancing improvements in malaria prevention and control accomplishments. There are four classes of insecticides approved by Pesticide Control Products Board (PCPB) for vector control namely; chlorine, organophosphates, carbamates and pyrethroids. Only pyrethroids are safe for use in bed nets impregnation. Resistance to each category of insecticide has been recounted in *Anophelines* and it is broadly acknowledged that, if the existing selection pressure on malaria transmitting mosquitoes is upheld, insecticide resistance will lead to intervention failure. The phase gauge for this consequence is a substance of consideration but there are already disturbing reports from Western and Southern Africa of operational substantial levels of insecticide resistance to pyrethroid by malaria transmitting mosquitoes. For East Africa, especially in Kenya, such information is lacking in the Global Insecticide Resistance Map. Therefore, information on insecticide resistance trends on enormous scales is essential for programme managers to appreciate the progress and distribution of insecticide resistance, hence plan corresponding intervention approaches that will reduce the development and range of insecticide resistance by choosing the most effective and appropriate insect killing chemical for vector control in a given area at a given time. To-date measures for handling evolution in resistance are costly and open ended, and their sustained usefulness is yet to be validated. To have rational and applicable malaria intervention schemes using insecticides, it is necessary that vector susceptibility status to major insecticide classes be clearly mapped out.

1.11 Null hypothesis

Presence of resistance in the main *Anopheles species* against insecticides does not affect entomological parameters of malaria parasites transmission.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1. Importance of malaria in Public health

Malaria illness is a problem in many regions of the earth, more so in the African sub-Sahara where about 90% of the incidences occur (Killeen, 2014; WHO, 2017b). In the world, about 3.4 billion persons in 91 nations are prone to get malaria infection and progressing into the disease. World wide, 1.1 billion individuals have a greater than 1 in 1000 chance of getting malaria every year (WHO, 2016d). In 2015, a total of 212 million incidences and 429,000 people died from malaria as reported in the 2016 World Malaria Report. The worst hit was African zone where about 92% of demises occurred; two thirds of which happened in babies or young children below 5 years of age. The illness is a danger to the well being of women and infants due to their weakened immune system. Malaria outbreaks upsurge the rate of one getting sick, especially in the highlands where their immune system of the inhabitants have not been exposed to malaria antigens before. Malaria and its vectors have progressively spread to areas formerly free from either (WHO, 2016d).

The disease tends to trigger an increase in premature deaths, monetary destitution to households stricken by poverty and it also consumes a lot of funds that could otherwise have been channeled for living standards improvement and commercial progression. The typical malaria impacts are: complication of pregnancy, severe pyretic infection, prolonged debilitation, and waning of the physical growth as well as learning capability of youngsters hence a negative impact on the society especially in the affected communities (Cohen *et al*., 2012; Craig *et al*., 1999; Noor *et al*., 2009; Takken & Koenraadt, 2013).

With increased disability adjusted life years (DALYS), individuals, their dependants, caregivers in the case of infants and children are often negatively affected (Murray *et al*., 2015). Social economic progression is often hampered as workers productivity decreases, visits by local or foreign tourists' drop, imported capital invesments dwiddle as well as difficulties in movement of goods and services (Killeen, 2014; Ondeto *et al*., 2017). Moreover one percent of economic budget is spent on malaria interventions hence a huge maroeconomic tax annually (Olanga *et al*., 2015; Pigott *et al*., 2012). The fee tend to add additional cost to the household head and the nation for the cure and control accomplishments such as purchasing of anti-malaria for home remedy; money needed to travel to and from health facility; time for work lost; school absenteeism and lack of concentration in class; costly intervention measures while in case of demises burial expenses and accompanying overheads are expended. National or local government expenditures include resources necessary to sustain public well-being, buying of anti-malaria drugs and medical supplies like nets and insecticidal sprays; public health measures against *P. falciparum* and its vector, production loss from absenteeism from work; and prospects lost for shared monetary ventures (WHO, 2016d). Such substantial losses and disruptions in trade and industry especially in malaria weighed down nations dampen their economic growth (Camponovo *et al*., 2017; WMR, 2017).

2.2 The Biology of Malaria

Malaria causing parasite in human is by means of the protozoan parasitic agents from the genus *Plasmodium* known to cause the disease in humans (Bastiaens *et al*., 2011; Noor *et al*., 2009). There are four kinds of *Plasmodium* that have the capability to cause malaria in man, while other parasites belonging to this and interrelated genera cause malaria in

reptiles, monkeys and birds. Among malaria symptoms, fever is usually the primary sign and the intensity differs depending on each particular parasite concerned. The four major malaria parasites transmisible to humans are: *P. malariae* Grassi and Felleti, *P. vivax* Grassi and Felleti, *P. ovale* Stephens and *P. falciparum* Welch (Kumar NK *et al*., 2014; Olanga *et al*., 2015; Ryan *et al*., 2006).

The illness is also identified by the frequency of its prime sign, fever: quotidian malaria in which the febrile flare ups happen at a time span of 24 hours; tertian malaria, in which it relapses each third day, further categorized as benign or simple (*vivax)* malaria, malignant tertian (*falciparum*) malaria or subtertian malaria (with low-grade fever between paroxysms) and quantuan (*malariae*) malaria, in which paroxysms revert each fourth day (Kumar NK *et al*., 2014). In North Africa, Central and sections of South America, the Indian sub-continent, and the Middle East, *P. vivax* predominates while *P. falciparum* is more extensive in Haiti, Papua New Guinea and Africa. In other parts of Oceania, East Asia, and South America, the occurrence of the two species is almost equal. *Plasmodium ovale* is scarce beyond West Africa, while *P. vivax* is sparse in sub-Saharan Africa. *Plasmodium malariae* is found in most regions but is comparatively rare outside Africa (Simon I. Hay *et al*., 2005; Simon I Hay & Snow, 2006; Wiebe *et al*., 2017).

With the exemption of quite a few cases of trans-placental and blood transfusion – linked transmission, malaria parasites in mankind are spread solely by the female mosquitoes of the genus Anopheles Giles, which feed on vertebrate blood. Infected mosquitoes retain their infection and can transmit the parasite the rest of their life. *Anopheles funestus* and *Anopheles gambiae* complex are the most dorminant malaria vectors in the Afro-tropical zone (Anto *et al*., 2009; Glunt *et al*., 2015; Spillings *et al*., 2008).

2.2.1 Breaking malaria transmission cycle

Despite availability of a range of new drugs and vaccines in future for malaria, majority of these are far from actual realisation and will not be adequate enough to discontinue the transmission cycle in most African environments (Kumar NK *et al*., 2014; Sinka *et al*., 2011). This arises from the fact that sub–Saharan Africa is where *P. falciparum* infection levels by the most competent malaria vector is thousand folds greater than the minimum needed to sustain endemicity (S.I. Hay *et al*., 2006; Simon I Hay & Snow, 2006).

An antigen, the portion of malaria parasite that notifies an individual's immune system has already been detected and isolated. This means that a vaccine may be made through mixing the antigen with either an innocous virus or a simulated protein to fake the immune system into perceiving an enormous *Plasmodium* attack hence trigger generation of a massive number of active immune cells over the extended period hence protect persons by producing colossal amounts of "unceasingly monitoring" CD8+ immune cells. (Torre *et al*., 2002). Malaria transmission can be interrupted if "jumping genes" can be used to change the *Anopheles* genome where mobile genetic components known as transposons which reside in the genome of the *Aedes aegypti* mosquito are used to alter the genome of two major species of *Anopheles* (Brooke & Koekemoer, 2010; Mathias *et al*., 2011; Spillings *et al*., 2008). A new antibiotic can be produced to invade the *Plasmodium* parasite in *Anopheles* mosquito by blocking a biological catalyst it needs for cell growth just like fumagillin clears infections in honey bees (Melnyk *et al*., 2014). Development of new procedures for obstracting a metabolic pathway crucial to many processes in the cells of the *Plasmodium* parasite has raised expectations as far as breaking malaria transmission cycle is concerned. Another way is by finding marked proteins in the mid gut of the

mosquito that may be utilized to block the mosquito's capacity to acquire the *Plasmodium*, develop it and transmit it to human beings. A particular protein, known as FREP1, in the midgut of mosquitoes' digestive systems makes the insects more susceptible to invasion by the parasite (Kelly-Hope & McKenzie, 2009; Kelly *et al*., 2003). Also a single normative protein, AP2-G, essential for triggering a group of genes that initialize the growth of gametocytes; the only stage of *Plasmodium* that are infective to mosquitoes, has been ascertained (Aly et al., 2009; Poché *et al*., 2015).

2.2.2 Bionomics of major malaria vectors in Kenya and Eastern Africa

An. gambiae complex has three intricate members that are accountable for *Plasmodium* transmission in Kenya (Sinka *et al*., 2011; Wiebe *et al*., 2017). The key malaria mosquitoes are adherents of the *Anopheles gambiae* complex and *An. funestus* with peak night biting between 23.00 and 05.00 hours (Bradley *et al*., 2015; Cooke *et al*., 2015a; Mathenge *et al*., 2001). Three species (*An. gambiae* Giles, *An. arabiensis* Patton and *An. quadriannulatus* Theobald) breed in freshwater whereas two species, (*An. melas* in West Africa and *An. merus* in East Africa) breed in salty water, hence the *An. gambiae* complex (Djènontin *et al*., 2017; Ebenezer *et al*., 2014; Wiebe *et al*., 2017) (Figure 2.1). A sixth member, *An. bwambae*, was found in the Semliki forest of Uganda related with water that contains high mineral component (Bousema & Drakeley, 2011). With the recent description of *An. quadriannulatus* B from Ethiopia, *An. gambiae* complex now includes seven species (Hiwat & Bretas, 2011; Poché *et al*., 2015). The anthropophilic patterns of behaviour of dorminant *Plasmodium* infectious species are a leading aspect to their effect on public health. Indeed *An. funestus* is placed as the first mosquito to have adjusted to human hosts (Ferguson & Read, 2004; Machani *et al*., 2019).

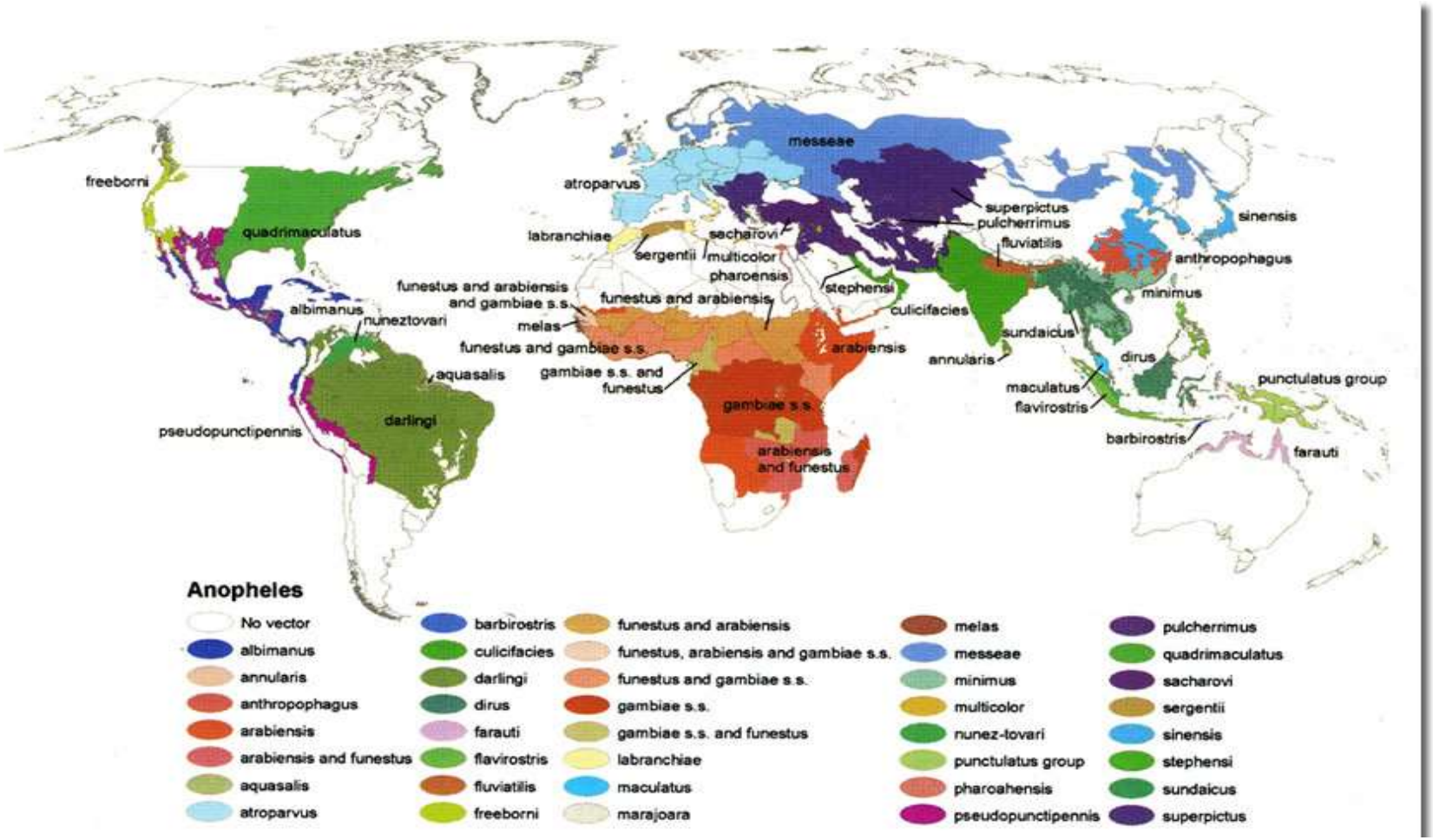


Figure 2.1: Global distribution of dominant or potentially important malaria vector (Source: Malaria Atlas Project)

Some mosquitoes in Kenya such as *An. gambiae* bite predominantly indoors whereas *An. arabiensis* bites predominantly outdoors (Ndiath *et al*., 2014; Poché *et al*., 2015; Stone & Gross, 2018). *An. arabiensis* shows both indoor and outdoor resting traits in the same individuals while *An. gambiae s*.s prefers to rest indoors (Bradley e*t al*., 2015; Cooke *et al*., 2015b). *An. gambiae* s.s and *An. funestus* were reported to be highly anthropophagic and endophagic while *An. arabiensis* was to a large extent zoophilic but endophagic (Afrane *et al*., 2012). The larval habitations of the *An. gambiae* are particularly small or medium sized, impermanent in muddy water collections, for example in ditches, puddles, small ponds, and pools while *An. funestus* can breed in considerably bigger and unflactuating waters like rice fields and also in small water bodies. Analysis was conducted on sources of blood meal in anophelines gathered in Kenya, particularly the coast region (Blackwell & Johnson, 2000; Childs *et al*., 2016; Wood *et al*., 2014). The results indicated that *An. merus* primarily relied on humans as their main source of food. Based on these findings, it was concluded that *An. merus* was exceedingly anthropophilic along the coastal region (Mutuku *et al*., 2011; Stone & Gross, 2018). But previous studies had shown that *An. merus* was majorly zoophilic, with a higher inclination to bite more when outside the houses than indoors (Mutuku *et al*., 2011; Ndiath *et al*., 2014; Stone & Gross, 2018). For An. *meru*, the maximal biting time occur between midnight and one in the morning (Bradley *et al*., 2015).

2.2.3 Environmental risk factors of malaria transmission

Factors in the environment that favour transfer of *Plasmodium* parasites from female *Anophelines* to humans, are highly concentrated in the tropics and substanttially in Africa hence the confounding worldwide burden of malaria (Alout *et al*., 2017; Hiwat & Bretas, 2011; Killeen, 2014; Kumar NK *et al*., 2014; Sinka *et al*., 2011; Steinhardt *et al*., 2017). Local mosquito ecology, hydrology and climate determines the resilience and rate in which malaria parasites are transmitted (Alout *et al*., 2017; Hiwat & Bretas, 2011; Killeen, 2014; Kumar NK *et al*., 2014; Steinhardt *et al*., 2017).

Insufficient health infrastructure, concomitant immunity and poverty permit persistent *Plasmodium falciparum* infections to live uninterrupted in human hosts hence a challenge to intervention measures put in place against malaria transmission in Africa compared to other zones in the globe (Strode *et al*., 2014a, 2014b; Takken & Verhulst, 2013). The risk of transmission is often characterized by means of the rate of entomological inoculation which is affected by the number of times that vectors feed on human beings and the sporozoite positivity. Anthropophilic vectors with long life spans are known to be the most proficient globally, in carrying out the transmission (Gu & Novak, 2005; Kar *et al*., 2014; Mutuku *et al*., 2011). Untreated and drug-resistant malaria infections can endure in a person for many years (Etang *et al*., 2004; Sainz-Elipe *et al*., 2010). African *Anopheline* mosquito with high survival rates, for example *An. arabiensis* Patton, *Anopheles gambiae* Giles and *An. funestus* Giles ingest gametocytic malaria parasites and then transfers them to a different victim (Simon I Hay & Snow, 2006; Kelly-Hope & McKenzie, 2009; Kelly *et al*., 2003). Eventually, persistent endemic malaria can manifest itself in the African sub-Saharan region where

individuals are vulnerable to less than one mosquito bite every week and new infections often go unnoticed (Beier *et al*., 1988; Mutuku *et al*., 2011).

Unbalanced spread of malaria vulnerability and disease burden is not only at worldwide and zonal levels but also at domestic levels. Poor education, housing and healthcare services lead to a savage cycle between exacerbated vulnerability, increased household medical expenses, diminished power to cater for treatment, and vice versa (Antonio-Nkondjio *et al*., 2006; Kumar NK *et al*., 2014). Additionally, malaria infection rate is often amplified in African sub-Sahara due to ecological degeneracy, clearing of vegetation and poor drainage freely enhance the multiplying of *Anopheles* species like *An. gambiae* which breed itself in small scale sunlit temporary shallow pools of water, more so unnatural habitations as a result of human activities (Simon I Hay & Snow, 2006; Sogoba *et al*., 2008). Currently, *Plasmodium* infections, ecological alterations and impoverishment are intimately bonded and persist to be related almost through out all the African states (Kar *et al*., 2014).

2.3 Common Malaria Control methods and challenges

Reduction in morbidity, avertion of mortality and socioeconomic loss through incremental enhancement and reinforcing of national and local capacities for malaria intervention measures remain the fundamental aim for malaria control (Billingsley *et al*., 2005; Cohen *et al*., 2012). In 2005, the President's Malaria Initiative (PMI) was started with an aim of decreasing deaths resulting from malaria by 50% in fifteen high-burden nations in African sub-Sahara (Shretta *et al*., 2017; WHO, 2017b). A swift scale-up of four verified and greatly operational malaria deterrence and treatment approaches were envisaged: indoor residual spraying (IRS); long-term insecticidal mosquito nets (LLINs); precise *P. falciparum* detection and immediate management using artemisinin-based combination therapies

(ACTs); and intermittent preventive treatment of pregnant women (IPTp) (Okumu *et al*., 2012; Olanga *et al*., 2015; WHO, 2017b). The prime measures in the managing of malaria consist of timely detection and prompt management, indoor residual spraying (IRS) and the use of long lasting insecticidal nets (LLINS) (Killeen, 2014; E. Ochomo *et al*., 2014). Regrettably, these strategic measures are pretty less operative due to the swift evolvement and proliferation of resistance against extensively utilized insecticides and drugs (Hemingway & Ranson, 2000; Mnzava *et al*., 2015; Strode *et al*., 2014a). Taking advantage of the genomic sequences of the malaria vectors and parasites may eventually result in new generations of insecticides and drugs, the upcoming of an efficient vaccine or genetically altered mosquitoes (Aly et al., 2009; Bell & Winstanley, 2004). Unfortunately, these novel intervention measures may be inaccessible for the next ten years.

2.3.1 Chemotherapy

Drugs against *P. falciparum* play a crucial part in malaria treatment and control (Bell & Winstanley, 2004; Noor *et al*., 2009). Many different types of anti-malaria medicines are within reach. However, their potency is being diminished by the emergence of drug resistant parasite strains (Okumu *et al*., 2012; WHO, 2012). The escalating resistance of the malaria parasites to inexpensive and efficacious drugs like sulphadoxine/pyrimethamine and chloroquine has rendered treatment less and less effective and therefore more expensive drugs are needed in the foreseeable future (Terlouw *et al*., 2003). The upsurge of chloroquine resistance in East Africa resulted in increased malaria deaths (Cohen *et al*., 2012; Guyant *et al*., 2015; Killeen *et al*., 2004). Equally, a substantial increase of malaria deaths in kids aged five years and below was recorded in Senegal, concurring with the rise of chloroquine resistance in the West African region (Strode *et al*., 2014a, 2014b). The cases of severe

malaria rose with escalating chloroquine resistance in Malawi and DRC (Cohen *et al*., 2012). Antimalarial drug resistance has also been incriminated in the rising frequency and fierceness of epidemics (Cooke et al., 2015a; Mzilahowa et al., 2016a; van den Berg et al., 2018; WHO, 2016d).

The eradication of malaria in North America and Europe as well as the collapse of worldwide malaria elimination program resulted in diminished enthusiasm towards malaria elimination for a period of around 25 years: between 1970s and 1990s. Between 1975 and 1996, only three out of 1223 new drugs developed were anti-malaria drugs (Fillinger *et al*., 2011; Sainz-Elipe *et al*., 2010; Shretta *et al*., 2017; Thiemann *et al*., 2011).

Plasmodium falciparum has built resilience to most accessible and inexpensive medications. In the 1950s, chloroquine (CQ) resistance was originally recorded in Southeast Asia and by late 1970s; it had extended and was detected in Africa (Bousema & Drakeley, 2011; Guyant *et al*., 2015; WHO, 2017b). Sulphadoxine–pyrimethamine (SP) has been adopted as the substitute of CQ. Unfortunately, its efficiency is currently extremely weakened by resistance (Guyant *et al*., 2015). In spite of this, various nations in Africa still depend on CQ or SP, or mixtures of the two, as typical initial choice of treatment. Quinine is set aside for cases where there are incidents of treatment lapse and for severe *P. falciparum* infection, but this drug is especially challenging to use (Bousema & Drakeley, 2011; PMI, 2017; Torre *et al*., 2002). The medicine is also quite costly at around $1 for every full dose of medication per person. Selecting for spontaneous mutations that bestow continued endurance benefit in the presence of the medication is considered to evolve drug resistance. This selection process is favored by antimalarial medications with extended terminal eradication stages (Nájera *et al*., 2011). During continued use of sub-therapeutic drug concentrations, new infectious parasites

harboring advantageous genetic alteration are specially selected. Sulfadoxine/Pyrimethamine and CQ both have extended half-lives, and this has undoubtedly promoted their extirpation. It is the acquirement of point genetic alterations in the genes *Pfcrt* and *Pfmdr1* that leads to the development of resistance to CQ (Bousema & Drakeley, 2011; Guyant *et al*., 2015; WHO, 2017b). In the case of resistance to SP, vital genetic alterations occur in the *dhps* and *dhfr* genes (Guyant *et al*., 2015).

The certain way to decelerate and delay the rate of resistance evolvement is through mixture chemotherapy where a number of drugs with varying sites and mechanisms of action are combined during treatment (Bousema & Drakeley, 2011; Cohen *et al*., 2012). Artemisinins have been proposed as perfect medicines for usage in admixture therapies; hence a switch from SP to artemisinin combination therapy in Kenya malaria treatment regulation (Bousema & Drakeley, 2011; Duffy *et al*., 2009) The greatest anti-*Plasmodium falciparum* action among all the antimalarial drugs, from the ring phase to early schizonts, and the resultant exponential decrease of parasites has been recorded in Artemisinin. Artemisinin is consumed as ACT, for three days, considerably decreasing parasite numbers and leaving the rest of the parasites to be exterminated by the number two medicine or the host immune system (Amin *et al*., 2007; Guyant *et al*., 2015; Shretta *et al*., 2017). Succeeding conveyance of gametocytes; the sexual phase of the parasite in charge of infection in the *Anopheles* mosquito, is reduced by treating *P. falciparum* malaria using artemisinins. Drug resistance against ACT has also been reported in Africa and Asia (Amin *et al*., 2007; Guyant *et al*., 2015; Shretta *et al*., 2017).

2.3.2 Long lasting insecticidal nets (LLINs) and Indoor residual spraying (IRS)

Killing adult mosquitoes by spraying living rooms with insecticides have been used successfully in the past (Awolola *et al*., 2007). The advent of dichlorodiethyltrichloroethane (DDT), which offered a standardized single attack, during the Global Malaria Eradication Campaign, led by the World Health Organization, gave great hope for malaria control. Recently, emphasis has been placed on the use of bed-nets that have been treated with a synthetic insecticide to enhance their protection against mosquitoes and the disease they transmit, principally malaria (Antonio-Nkondjio *et al*., 2006; Kerah-Hinzoumbé *et al*., 2009; Minakawa *et al*., 2012). Numerous research activities have been carried out on the efficacy of bed-nets (especially the insecticide treated ones) as a means of controlling malaria (H. W. Choi *et al*., 1995; K. Choi *et al*., 2013).

Regrettably, the outstanding capacity of insects to develop resistance to each group of insecticide that has been produced, time and again leaves control programs with limited insecticide alternatives (Knox *et al*., 2014). Widespread insecticide resistance in vectors of *P. falciparum* has been reported (Hawkins *et al*., 2018a; Kar *et al*., 2014; Xu *et al*., 2014). Thus, surveillance and controlling resistance to insecticides is important in malaria control schedules. The prompt detection of resistance is a vital part of resistance management because it may lead to the development of insecticide use strategies that would minimize the rate of evolution of resistance (Etang *et al*., 2004; Mathias *et al*., 2011; Pennetier *et al*., 2007).

2.3.3 Other malaria control options being explored

As a result of scientific and technological advancement in the 21st century, the entire genomes of *Plasmodium falciparum* and *Anopheles gambiae* have been sequenced (Antonio-Nkondjio *et al*., 2006, 2015; Machani *et al*., 2019). This opened a new era in malaria control

research as molecular biologists are questing for substitution of natural *Anopheline* populations devastating developing countries with "designer mosquitoes," genetically altered hence unable to transmit diseases such as malaria (Mavridis *et al*., 2018). There is hope that the sequential sorting of the *Plasmodium falciparum* genome will open up opportunities for anti-malaria drug and vaccine development (Pimenta *et al*., 2015). RTS,S/AS01 or Mosquirix, is comprised of hepatitis B surface antigen particles that look similar to virus, integrating a part of the *Plasmodium falciparum*- extracted circumsporozoite protein and a liposome-based adjuvant. The development of the malaria vaccine RTS,S/AS01 got into a crucial clinical phase in 2009 when over 15,000 children were recruited and significant results from tests were published in 2015 (Pimenta *et al*., 2015). Overall, the vaccine was regarded as safe. However, recipients aged between 5 and 17 months suffered from meningitis in contrast to children who were given the control vaccines.

Larval source management (LSM) offers a double advantage of not just decreasing numbers of mosquitos entering a house, but also female anophelines that bite outside the houses (Fillinger *et al*., 2011). Findings from trials in Africa confirmed that applying larvicides by hands can reduce transmission by 70-90% in situations where mosquito larval habitations are closely defined but is highly unsuccessful where habitations are prone to flooding or scattered hence cannot be covered on foot (Fillinger *et al*., 2011; Killeen, 2014). However, although these new technologies and approaches offer some hope for malaria control, new products are not likely to be available soon. Therefore, judicious use of the available malaria control tools should occupy center stage until alternative control tools are availed (Shretta *et al*., 2017; WHO, 2016c).

2.4 A general review of insecticides resistance in Mosquitoes

Dichlorodiphenyltrichloroethane (DDT) was initially introduced for control of mosquito and the initial incident of resistance happened in 1947 in *Aedes tritaeniorhynchus* and *Ae. solicitans* (Cohen *et al*., 2012). Turkey, Islamic Republic of Iran and Greece reported the initial resistance incident against DDT in malaria vectors in 1951 and resistance to dieldrin in 1956 (Cohen *et al*., 2012; WHO, 2017b). Non –vulnerability by female mosquitoes to DDT and dieldrin showed up later in Asia, Africa and America. In the year 1960, a total of 43 mosquito species were found to be resistant to a number of insecticides, and this number rose to 56 and 99 by 1970 and 1980, respectively (Cohen *et al*., 2012; Ondeto *et al*., 2017). Currently, over 100 species of mosquito are non-vulnerable to a number of insecticides, and anophelines account for over 50 of the species (Figure 6). Insecticides used for controlling malaria include Chlorinated Beta (β) Hydrocarbons (BHC), carbamates, organophosphates and pyrethroids, with the last type currently taking a dorminant market portion for both large-scale insecticide-impregnated bed net programs as well as inside the house residual spraying (Etang *et al*., 2004; Ondeto *et al*., 2017).

For the additional insecticide collections, like the benzylphenyl ureas and Bti (*Bacillus thurangiensis* sub-species-*israeliensis*) have experienced restricted utilization against mosquitoes. Resistance trends towards shifts in use of any given insecticides (Etang *et al*., 2004; Hiwat & Bretas, 2011; Mzilahowa *et al*., 2016a). BHC/dieldrin resistance is extensive in spite of non-use of these insecticidal agents for several years. Resistance to organophosphate (OP), in the form of wide-ranging OP resistance or malathion-specific resistance, emerged in the main vectors *An. culicifacies*, *An. Stephensi*, *An. albimanus*, *An. arabiensis* and *An. sacharovi* (Mzilahowa *et al*., 2016a). Resistance of organophosphorus

insecticide is prevalent in every main *Culex* vector and pyrethroid resistance happens in *C. quinquefasciatus*. Resistance to Pyrethroid has been observed in *An. albimanus*, *An. stephensi* and *An. gambiae* amidst others. Carbamate resistance has been detected in *An. sacharovi* and *An. albimanus* (Killeen, 2014; Steinhardt *et al*., 2017).

Resistance to pyrethroids is prevalent in *Ae. aegypti* and cases of OP and carbamate resistance has also been reported to this type of mosquito (Awolola *et al*., 2007). There has been particular focus by WHO as well as other groups on the utilization of pyrethroid-impregnated bed-nets as an intervention measure against malaria, in the light of pyrethroid resistance in *An. gambiae*. The evolvement of *kdr* pyrethroids resistance in *Aedes* mosquitoes in Mexico reveals the 'tipping-point' principle (Tabbabi & Daaboub, 2018). From 2000 to 2003, the rate of resistance occurrence was below (≤ 10%) at most sentinel sites (Li *et al*., 2008). However, at some point from 2003 to 2007, due to uninterrupted selection pressure from the similar insecticide, the tipping-point was attained, and resistance amplified considerably in six locations, attaining frequencies of ≤ 80% by 2007 (WHO, 2016d).

Both incidences of resistance to pyrethroid in *An. gambiae*, from Kenya and Ivory Coast are well documented (Alout *et al*., 2017; Vulule *et al*., 1999). The West African focal point seems to be bigger and has greater resistance levels compared to East Africa. However, studies on insecticides resistance in Kenya have concentrated mainly on synthetic pyrethroids and limited to very few areas of the country (Ondeto *et al*., 2017). Therefore, a systematic survey and documentation of the current levels of insecticides resistance among the main malaria vectors is desperately needed (Figure 2.2).

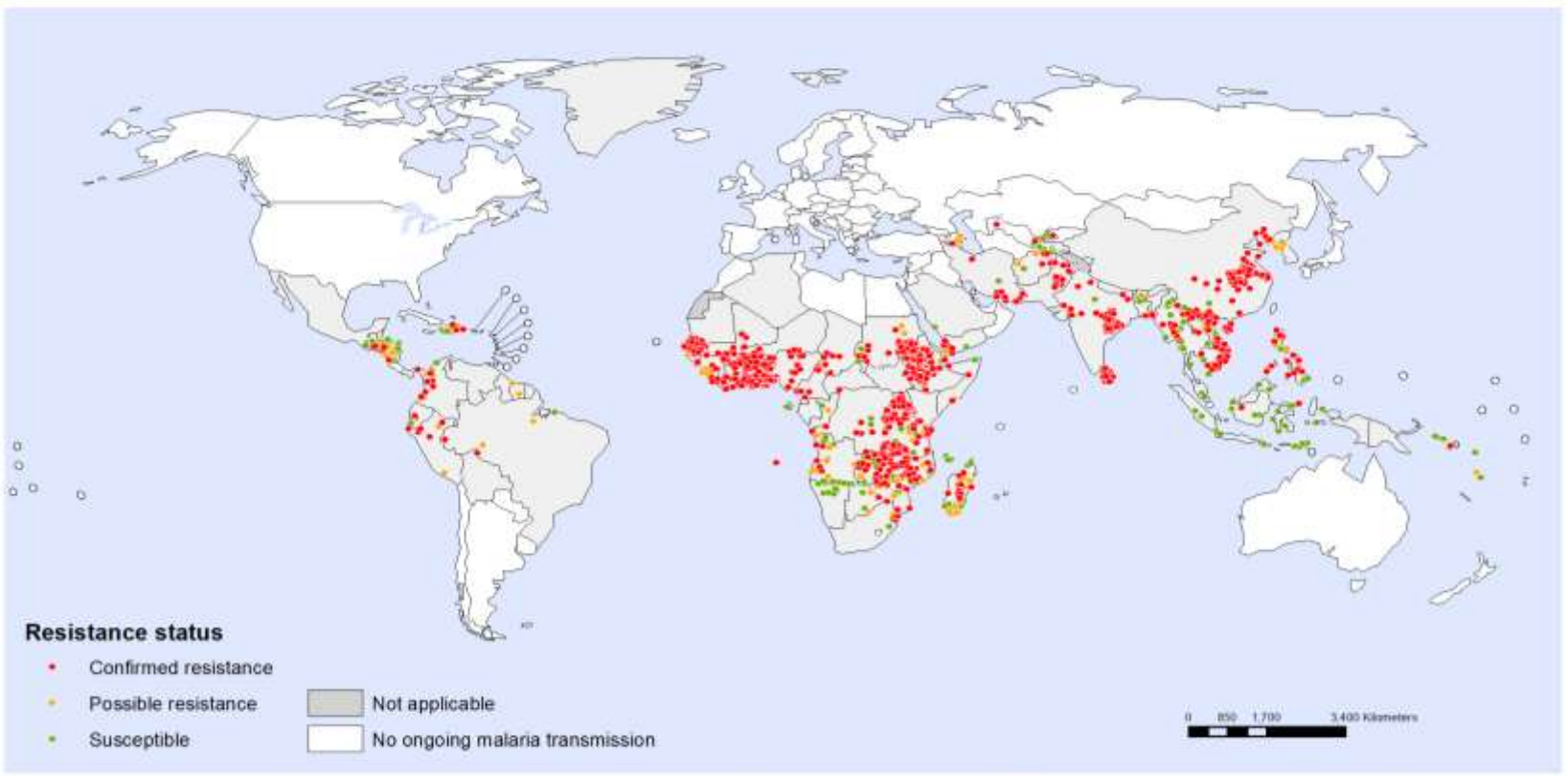


Figure 2.2 : Reported insecticide susceptibility status for malaria, 2010 – 2015 (Source: WHO 2017)

2.4.1 Mode of action in different classes of insecticides

When insects are exposed to insecticides, the active ingradient gets to the targeted body point after an insecticide active molecule rapidly penetrates through the insect's integument (the outer layer or cuticle) to the action site (Liu, 2015). Insecticide molecules combine with the point of action, which might be a crucial enzyme, receptor protein, or nerve tissue, and ultimately when they have achieved adequate threshold concentrations they knock down the insect and eventually it dies (Liu, 2015).

2.4.2 Mechanisms of insecticide resistance

Metabolic based resistance is often as a result of the altered activities of three main enzyme categories of oxidases, esterases, or glutathione S-transferases (GST) which prevent the insecticides from getting to their prospective target locations (Brooke & Koekemoer, 2010; Kaindoa *et al*., 2017). The overproduction of detoxification enzymes enables insects to resist the insecticides through break-down of the poisonous insecticides to less toxic or non-toxic compounds (Machani *et al*., 2019; Zhang *et al*., 2015). The enzyme pathways of an insect are linked to its metabolic resistance by enhancing the ability to render the foreign materials or insecticidal agents less toxic (Hawkins *et al*., 2018b). While it is important for the insect to detoxify foreign materials in the four insecticide classes, different enzymes affect different classes.

Altered target-site non-vulnrability happens when the insecticide and the target point can no longer bind (Awolola *et al*., 2007; Ondeto *et al*., 2017). Both target site and metabolic resistance can happen in the similar vector population and other times within the same mosquito. Target-site non-vulnerability is when the insecticide's action spot inside the

nervous system is genetically altered in resistant strains, in a manner that the insecticide does not bind successfully and thus the insecticide affects the insect slightly, or it is not affected at all (Hawkins *et al*., 2018b; Kumar NK *et al*., 2014). The voltage-gated sodium channels as the molecular target for DDT and pyrethroids, or acetylcholinesterase for carbamates and organophosphates can be affected by knock-down resistance (*kdr*) resistance mutations (Killeen, 2014; Strode *et al*., 2014b).

Penetration resistance happens when the exterior cuticle of an insect produces impediments which delay adsorption of the chemical agents into their bodies (IRAC, 2012; E. Ochomo *et al*., 2014). Resistant insects may take in the toxin less rapidly than susceptible ones hence protect insects from a variety of toxic chemical compounds. Resistance in penetration by insecticides is often times present together with other types of resistance, and decreased penetration aggravates the effects of those other modes of resistance (IRAC, 2012; E. Ochomo *et al*., 2014).

Behaviour based non-vulnerability is when a resistant insect dodges surface areas treated with insecticide by changing their typical behavior patterns (Killeen, 2014). Presence of behavioural resistance defines the modifications in vectors' resting or feeding behaviour to reduce interaction with insecticides. To avoid insecticide or pesticide sprayed indoors some mosquitoes transmitting malaria in Africa have developed the tendency to repose outdoors to evade the poison (Killeen, 2014; Okumu *et al*., 2012). For example *An. arabiensis* mosquito has altered its reposing manner to exophilic (resting outdoor) from endophilic (resting indoor) nature so as to evade the exposure or contact to indoor residual spray in Africa (Kar *et al*., 2014). Behavioral resistance is somehow an adjustment in behaviour of an insect that enables it to stay away from the deadly impacts of insecticides. *Anopheles*

farauti vectors in New Guinea and the Solomon Islands ceased to bite later in the night (23:00–03:00) after the indoor spraying of DDT and as an alternative they bit only at dusk before people were protected by sleeping in sprayed rooms (Guyant *et al*., 2015). In various cases, still, there are inadequate facts to evaluate if behavioural dodging traits are adaptive or genetic; if it is genetic traits, it can have core challenge in the interventions for vector control. However, all behavioral traits may not be bad, as they could cause mosquitoes to have alternatives to feed on other animals apart from humans (Ayele, 2016; Thiemann *et al*., 2011). Decrease of vector species may also be misunderstood as behavioral resistance. Non-vulnerable insects keep off the toxins on the upper surface of leaves to evade or feed or move to the underside of a sprayed leaf.

2.4.2.1 Selection pressure and insecticide regimes

In public health, minimal amounts of insecticides are used compared to farming (Githinji *et al*., 2020; Zhang *et al*., 2015). Moreover, insecticides used towards human health management are applied for specific purposes and approaches; persist for longer, thus letting substantial accumulation of selection pressure over many generations of insect populations (WHO, 2016d). The enormous increase in the use of long-lasting insecticidal nets (LLINs) and indoor residual spraying (IRS) for malaria vector management, particularly in the last 5 years, has led to escalated selection in malaria native countries. Resistance has also emerged in some zones where vector control interventions have been introduced not long ago, but with no agricultural use of insecticides (Cooke *et al*., 2015a; Kelly-Hope & McKenzie, 2009). After the distribution of LLINs to infants and expectant mothers in the year 2004 in one region of Sudan, an elevated frequency of resistance to pyrethroids was recorded, seven years later in 2011 (Alout *et al*., 2017; Ondeto *et al*., 2017; Onyabe & Conn, 2001). In other

places, the increased intensity in the use of vector control interventions apparently seems to be related to the escalating frequency of resistance genes, though possibly not being the primary cause (Alout *et al*., 2017; E. Ochomo *et al*., 2014).

There was a marked rise in the *kdr* allele frequency from 1996 to 2009 in western Kenya, possibly due to strong selection pressure in *An. gambiae* from LLINs use in the Asembo and Seme parts (E. Ochomo *et al*., 2014). However, while LLINs may have led to a substantial rise in resistance genes, extremely low levels of the *kdr* genes had already been detected in 1996, possibly from earlier DDT selection (Guyant *et al*., 2015; Xu *et al*., 2014). Likewise, in Niger and Benin, the wide spread dispensation and utilization of LLINs seemed to raise *kdr* genes frequency but did not cause its first emergence. In Niger, resistance had risen substantially in *An. gambiae* by 2007 after giving out of LLINs began in 2005 (Endo & Eltahir, 2018).

Likewise, instances have been witnesssed with IRS. In Burundi, 1% of *An. gambiae* had the *kdr* gene before an IRS campaign commenced in 2002 and had rose to 86% by 2007 (Alout *et al*., 2017; WHO, 2017b). This stresses the vitality of prompt and rational evidence based insecticide resistance management (IRM) as nations expand utilization of vector control interventions, especially IRS and LLINs which are likely to raise selection pressure for resistance genes (Alout *et al*., 2017; WHO, 2017b).

The contact between malaria vector and farming insecticides is mostly linked to market gardening, rice and cotton crops (Brown *et al*., 2017)(Mwangangi *et al*., 2006, 2007). The connection between insecticide non-vulnerability and cash_food crop farming pressure have been seen in some reports, with a clear link between periodic variations in resistance in key farming zones showing the relationship between application of insecticides to the main crops

and the highest resistance frequencies in the areas of intensive farming pressure. The *kdr* mutation in Burkina Faso is found in numerous locations where cotton is grown, and the greatest resistance frequencies detected in the 'cotton belt' (Anto *et al*., 2009; Chandre *et al*., 1999; Yawson *et al*., 2004). A six towns' research findings in Côte d'Ivoire displayed dichlorodiphenyltrichloroethane (DDT) and pyrethroids resistance in the areas where extreme farming was practiced through out the year (Asidi *et al*., 2005; Chandre *et al*., 1999; Mouhamadou *et al*., 2019; E. Ochomo *et al*., 2014).

Nevertheless, farming has been held responsible for the development of resistance, but more research reveal that application of malaria control interventions may have contributed a lot more to the non-vulnerability to insecticides by insects, including mosquitoes. For example, non-vulnerability to carbamates and organophosphates in Sri Lanka was not as a result of agrochemicals on rice crops for the malaria vector was not breeding in rice fields (Shretta *et al*., 2017; WHO, 2017b). Therefore it was concluded that selection pressure was because of attempts to control malaria and not agrochemicals use (Hawkins *et al*., 2018a, 2018b).

But it still remains unknown how much selection pressure is contributed by malaria vector control interventions and not agrochemicals application. Ending malathion usage in IRS resulted in a resistance levels reversion in Sudan, even when no alteration in the insecticidal agents' regime applied on cotton crops (Kerah-Hinzoumbé *et al*., 2009; Murray *et al*., 2015; Mzilahowa *et al*., 2016a; Sogoba *et al*., 2008). The relationship between non-vulnerabilty to insecticides and agricultural use of pesticides is linked to type mosquito behavior, crop and pesticide regime. A connection can be alleged only if substantial quantities of agrochemicals are used on a particular crop and the same crop is the reproducing and habitation grounds for

the malaria vector at the time when the insecticide is applied (Djènontin *et al*., 2017; Li *et al*., 2008).

Contemporary studies have shown other limited circurmstances that add towards selection pressure. Of utmost concern is current proof that metabolic based resistance can be evolved through presence of numerous contaminants in the environment (Kaiser *et al*., 2014; Killeen, 2014). Pollution of ground waters with hydrocarbons, and its usage as breeding grounds by *Anopheles gambiae* in Nigeria and Benin, has been linked to excessive oxidase-based metabolic resistance in the malaria vectors resulting to resistance to pyrethroids as well (Camponovo *et al*., 2017; Chandre *et al*., 1999). The large-scale use of aerosols, coils, insecticides for domestic reasons, inferior insecticides and larviciding in Latin America, India and Sri Lanka have become other contributors to selection pressure; but their utilization in Africa is constrained (Cohen *et al*., 2012; WHO, 2016d; WMR, 2017).

2.4.2.2 Theories of gene(s) selection process

Characteristic traits in an organism are defined by natural forces during natural selection process (Alout *et al*., 2017). Some individuals are more adapted to endure in the environment than others due to a given variation or allele of a specific trait. Insecticides are mostly novel synthetic chemical compounds, and yet target species are often able to evolve resistance soon after a new compound is introduced. Therefore, insecticide resistance provides an interesting circumstance of swift evolution under strong selective pressures, which can be used to consider underlying questions concerning the evolutionary origins of hereditary alterations to novel settings (Hawkins *et al*., 2018a).

2.4.2.3 Monogenic and Polygenic Resistance selection pressure theory

Molecular basis of resistance to insecticidal chemical agents has been unlatched using the humble fruit fly *Drosophila melanogaster* as a genetic model. Extended artificial selection using DDT in the laboratory for 25 years caused the fruit fly to be significantly resistant involving all the three major chromosomes and a vast number of genetic aspects of minor effect (Ffrench-Constant, 2013; Hawkins *et al*., 2018b). Successive mapping studies in the 1990s led Ogita and others to show that resistance to DDT was linked to one key factor on the left arm of chromosome II at map position 62–64 cM (Knox *et al*., 2014; Wang *et al*., 2013). The researches on resistance to DDT in *Drosophila* have characterized differences of opinion surrounding mono- *vs.* polygenic inheritance of resistance to insecticide. Single gene or major-gene or qualitative resistance where organisms are either susceptible or resistant without intermediate levels as one or a few specific, well-defined genes confer a high level of resistance to a specific class of insecticide. Polygenic resistance involves several or many genes (Aly *et al*., 2009; Martinez-Torres *et al*., 1998).

This inconsistency of mono- and polygenic resistance characterizes initial research on resistance to insecticide and demonstrates the advantages of observing lately derived field strains, which display one resistance factor on chromosome II, instead of depending on prolonged selection of single strains in the lab, which tend to display polygenic resistance (French-Constant, 2013). Among the first to detect *Drosophila* resistance to diverse chemicals and not only to DDT, was Weiner and Crow (1951); who proposed that target-site resistance was not likely, but instead an enzyme was downgrading a wide range of diverse classes of chemicals (French-Constant, 2013). This conclusion on cross-resistance proposes that, in spite of the fact that Crow's DDT resistant strain was polygenic, one of the

resistance hereditary materials was indeed the *DDT-R* gene. This dissimilarity between the results of selection pressure in the field *versus* selection pressure in the laboratory was explained by extra selection tests in the laboratory and their examination with a micro-array comprising all of the known P450 hereditary factors from *Drosophila*; which revealed that dissimilar laboratory selection modes can certainly select for varying P450s (Brooke & Koekemoer, 2010).

2.4.2.4 Field and laboratory based selection selection pressure theory

DDT and pyrethroid non-vulnerability is caused by sudden genetic change in the *Drosophila* gene *para*, which encodes the PARA-containing sodium channel, the target site for DDT and pyrethroids (Okumu *et al*., 2012). Regardless of its X – linkage which may suggest that males are competently homozygous for any new sudden hereditary changes, this target-site mechanism has not been detected in *Drosophila melanogaster* collected in the field. Though the reasons for this are far from clear, it could be connected to the success of CYP6G1 in downgrading such a wide range of pesticides. According to the theory selection within a uninterrupted phenotypic distribution, like a small laboratory population, support selection of a polygenic response while selection for phenotypes outside of the original phenotypic range, like selection of a field population with an insecticide, supports a monogenic response concerning a unusual variant (Brooke & Koekemoer, 2010). Thus, frequency and pattern of selection may be similar in the field and the laboratory (Afrane *et al*., 2012; Ffrench-Constant, 2013). But, only the heterogeneous nature of field populations permits the selection of the unusual variants that relate to resistance alleles and is expected to activate control failure.

2.4.2.5 Multiple mutations with multiple origins selection pressure theory:

Do upcoming sudden genetic changes associated with resistance emerge in single fields, counties, countries, or in fact only once on the planet; given the large extent of insect populations? Replacements of the two amino acids in the same encoded polypeptide or two mutations in the same allele are required for elevated intensities of pyrethroid resistance associated with the voltage – gated sodium channel encoding the *para* gene (Awolola *et al*., 2007; Martinez-Torres *et al*., 1998; Strode *et al*., 2014b). This rarity was primarily shown in *Musca domestica* where low levels of pyrethroid resistance were imparted by a single amino acid of knockdown resistance (*kdr*) substitution. But a second *knockdown resistance* (*kdr*) substitution of amino acid is needed in the similar polypeptide to confer greater resistance levels or *super-kdr* (Ffrench-Constant, 2013; Melnyk *et al*., 2014; Vulule *et al*., 1999).

2.4.2.6 Multiple mechanisms within a single genome selection pressure theory:

Originally, this idea of the vanishingly uncommon resistance sudden genetic change supported the impression that one mechanism might be essential in single genomes or single species (Awolola *et al*., 2007; Walton *et al*., 1999). For development of resistance in the field to take place, exceptional variants or new resistance associated sudden genetic changes are a pre-requisite. With the noteworthy oddity of pyrethroids, replication of E4 was forwarded as the single resistance mechanism in *Myzus persicae* and overexpression of the E4 esterase essential and adequate to catalyze and/or sequester a variety of dissimilar insecticide groups. That was after first finding that several copies of the E4 gene can be interconnected with the resistance intensities of a number of insecticides. Variants, for example Fast E4 described, were basically diverse replication occasions that had caused enzyme truncation and thus resulted in a more rapid and dissimilar electrophoretic mobility (French-Constant, 2013).

Additionally, DNA methylation changes were shown to be capable of turning off the elevated levels of E4 production which is connected with exceedingly replicated E4 – encoding genes in extremely resistant clones of *Myzus persicae* (Nkya *et al*., 2014; White, 2005). Energy cost associated with creating great amounts of esterase in the absence of pesticide was perhaps diminished. Demonstration in 1990 that both modified voltage-gated sodium channels (*kdr*) and unresponsive acetylcholinesterase were engaged in resistance to pyrethroids and organophosphorus/carbamates, respectively; decimated the impression that only carboxylesterase-associated gene replication can result to resistance (Nkya *et al*., 2014; White, 2005).

Some mechanisms were proposed to show elevated levels of fitness (when the pesticide was missing) or efficacy (when the pesticide was available) above others since relative frequencies over time had been changing according to resistance studies on the three mechanisms carried out in retrospect in the United Kingdom (Strode *et al*., 2014b; Watkins *et al*., 1993). Therefore, a succession of diverse mechanisms, which can substitute each other in a given time, occurred in aphids in United Kingdom and not just a single resistance mechanism. A proposal is made that numerous mechanisms in one pest or vector might be more prevalent than expected and that we must not be blindfolded by excessive focus on any single mechanism in seeking to explain the present heights of resistance in the field since a single aphid species has almost each resistance mechanism detected so far (Brooke & Koekemoer, 2010).

2.4.2.7 Multiple mutational events at a single locus selection pressure theory

The comparative significance of structural sudden hereditary changes (amino acid substitutions) versus regulatory (transcriptional) sudden genetic changes continues to be a

subject of great debate knowing that numerous mechanisms can be in one genome of an insect (Aly *et al*., 2009). Nevertheless, the potential of numerous diverse mutational activities (e.g., both insertions and replications) result to development of a variety of composite alleles at a single locus has mainly been ignored. The vulnerable progenitor allele (later termed the M allele) did not have a transposable component (Accord) insertion and thus has a basal level of Cyp6g1 transcription in the initial description of Cyp6g1 in *Drosophila melanogaster* DDT-R (Ffrench-Constant, 2013). A resistant allele (termed A for Accord) was then made after the insertion of the Accord retrotransposon.

Non-vulnerability in *Drosophila simulans* seems to be astonishingly involved in parallel evolution with the insertion of a dissimilar element, a Doc element, in the corresponding location upline of the Cyp6g1 homolog (Ffrench-Constant, 2013). DNA accompanying the Accord insertion was sequenced so as to analyse how many times the Accord insertion had taken place in the worldwide populations of *D. melanogaster*; the resulting phylogeny was persistent with having been a single Accord insertion, i.e., a single origin of resistance and a world wide spread of a single Accord allele (Awolola *et al*., 2007).

Four more resistance alleles also exist as shown in subsequent detailed work by Schmidt *et al*., 2010. Two non-vulnerable copies of Cyp6g1, each having the Accord insertion (termed the AA allele) seems to have been formed through replication of Accord allele carrying Accord insertion. Two Accord insertion spots have been additionally mutated either via the insertion of an HMS-Beagle element into one spot (to constitute the Beagle-Accord or BA allele) or via the succeeding insertion of a aphid's P-globin gene cluster element into the alternative Accord insertion site (to constitute a Beagle-P or BP allele) following the dramatic replication event. HMS – Beagle element was named after His Majesty Service

(HMS) royal ship used by Charles Darwin to travel widely. The P-element insertion with scrambled P-terminal repeats (termed BPΔ) has ultimately been found as the sixth allele (Ffrench-Constant, 2013).

2.4.3 Impact of insecticide resistance on effectiveness of vector control interventions

Non-vulnerability to insecticides is imparted by metabolic detoxification of the insecticide for it not extend to its target spot, changes in sensitivity of the target site or diminished cuticular penetration (Awolola *et al*., 2007; Hemingway & Ranson, 2000; IRAC, 2012). The utilization of permethrin-impregnated bednets has been connected to the development of permethrin tolerance in the mosquito populations, finally resulting to the problem of insecticide resilience (Ffrench-Constant, 2013). Gene conferring resistance to pyrethroid insecticides have been proliferating quickly over the last few years and are now in circulation in the major African mosquito vectors of malaria (Antonio-Nkondjio *et al*., 2006; Knox *et al*., 2014).

Kdr type of resistance has been spreading in *Anopheles gambiae* for some years, especially in central and West Africa. Metabolic type of non-vulnerabilty to insecticides have been detected in *An. gambiae* and *An. funestus* in scattered areas all over Africa (Anto *et al*., 2009; Brooke & Koekemoer, 2010). Metabolic types of resistance are sophisticated to track and, singly or together with *kdr*, may pose a significant menace. It has been demonstrated that at least some of these genes are capable of diminishing the efficacy of ongoing mosquito control measures (Asidi *et al*., 2005; Ranson *et al*., 2002). Hence, fewer options of insecticides for IRS and LLINs compelling the advancement of more vigorous insecticide resistance control approaches. No IRS has been conducted since 2013 due to limited choice of insecticide for IRS as a result of emergence of insecticide resistance (Antonio-Nkondjio *et al*., 2015).

2. 4.4 Insecticide resistance in Kenya

In Kenya, the first reported case of insecticide resistance was a decline in knock down rates detected in connection with ITN use in western Kenya in the early 1990s (Cohen *et al*., 2012; Stone & Gross, 2018). Full non-vulnerability of populations of *An. arabiensis* to DDT, fenitrothion, bendiocarb, lambdacyhalothrin and permethrin was reported from Mwea rice irrigation scheme, Central Kenya [(Mwangangi *et al*., 2006; Ondeto *et al*., 2017) (Figure 2.3) (Appendix 8, 9, 10 & I hereby write to confirm that in transit11)]. Extensive resistance to pyrethroids and DDT was detected across western Kenya (Kweka, 2016; Muturi *et al*., 2010; Mwangangi *et al*., 2006, 2007). *An. gambiae* s.l. displayed varying levels of resistance to bendiocarb, deltamethrin and lambdacyhalothrin in Kilifi, Malindi and Taveta districts in coastal Kenya (Kweka, 2016; Msami, 2013). There has been an account on pyrethroid resistance in *An. gambiae s.s* and *An. arabiensis* from four districts of Western Kenya (Msami, 2013).

Kamau and Vulule (2006) reported that *An. gambiae* s.l. and *A. funestus* from western, coastal and central Kenya were susceptible to bendiocarb, lambdacyhalothrin, fenitrothion, DDT and permethrin (Mutuku *et al*., 2011; Sogoba *et al*., 2008). The same research activity revealed the existence of leucine-serine (East African) *kdr* mutation in *An. gambiae* s.s. from western Kenya, but the leucine–phenylalanine (West African) mutation was missing in these mosquito numbers. Despite *kdr* mutation in East African being spotted in *An. gambiae* from western Kenyan, it had not happened in homozygous state. The occurrence of the L1014S *kdr* allele increased two fold in the ITN test village and its adjacent neighbor from 1987 to 2001, but not outside of this area (Kweka, 2016). This implies that ITN use has further selected for the *kdr* mutation in the anopheline population.

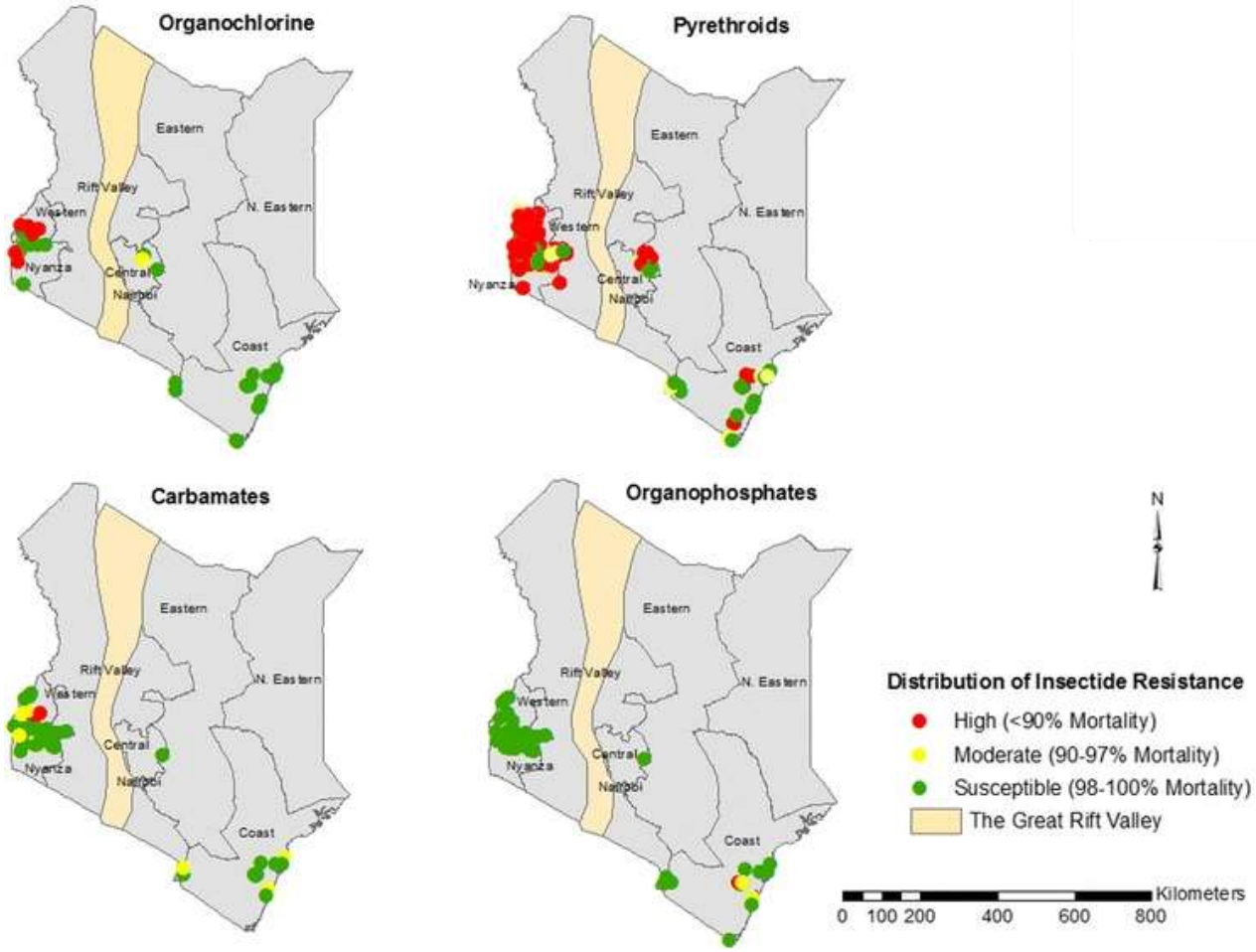


Figure 2.3: Malaria vector map in 2017 (Courtesy of Benyl *et al*., 2017)

2.4.5 Ways of managing insecticide resistance

The necessity for efficacious measures in resistance regulation is becoming urgent as the more insecticide-resistant species keep on rising globally while insecticide resources are decreasing (Awolola *et al*., 2007; Brooke & Koekemoer, 2010; Strode *et al*., 2014b). Projections for coming up with such approaches have improved due to recently upgraded information on ecology, biochemistry, molecular genetics, dynamics, monitoring, and other elements of resistance (Menze *et al*., 2016b). There are fundamental approaches to insecticide resistance managment: firstly, low selection pressure, supplemented by a strong component of non-chemical measures (that is, management by moderation); secondly, elimination of the selective advantage of non-vulnerable mosquitoes by increasing insecticide uptake through the use of attractants, or by suppression of detoxication enzymes through the use of synergists (that is, management by saturation); and thirdly, application of multi-directional selection by means of mixtures or rotations of unrelated insecticides or by use of chemicals with multi-site action (that is, management by multiple attack) (Ffrench-Constant, 2013).

A season-long management program can be created from each aspect since these approaches are mutually inclusive. The measures considered in chosing mused be o the basis of ones extensive information on reistant consequences of the candid The approach selected should be based on knowledge of the resistance implications of the chosen insecticides and of the ecology and biology of the mosquito species while applying all possible non-chemical control measures (Afrane *et al*., 2012; Chanda *et al*., 2008).

Controlling resistance is within reach due to the accessibilitiy of more sensitive and user friendly surveillance approaches. Use of insecticide-based strategies together with various

non-insecticidal vector-control approaches through integrated vector and pest management should be the first attempt towards resistance regulation (Chanda *et al*., 2008; Mosqueira *et al*., 2010; Wilke *et al*., 2015). Even though most of the programs intended for control can work properly during experimentation, they end up becoming impractical after scaling up to long-term intervention programs. Operationally, resistance management based on insecticide use is the most fundamental, and it can take a number forms (Melnyk *et al*., 2014).

2.4.5.1 Rotation

Rotational approaches are founded on the rotation over time of two or additional insecticide groups with varying modes of action (IRAC, 2012). Rotations are predominantly effective if the gene responsible for resistance has a related fitness cost. That is in other words, if resistant insects are at a selective disadvantage, compared to the susceptible 'wild type' insects, in the absence of the insecticide, therefore "back-selection". This approach assumes that if resistance to each insecticide is rare, then multiple resistances will be extremely rare. Therefore, any type of resistance established to the primary insecticide will deteriorate over time once the subsequent insecticide group is used (WHO, 2012). Rotation requires sufficiently short time span for resistance to relapse swiftly after it has been selected for (Hemingway *et al*., 2013).

2.4.5.2 Mixtures

The use of mixtures is a common practice to avoid the development of insecticide resistance. The theory behind this approach is that, suppose resistance to each of the compounds in the mix is uncommon, then multiple resistances to both compounds will be

exceedingly rare (IRAC, 2012). Nevertheless, for blends to function properly in practice, the two insecticides should be used at their full working target dose, and the persistence as well as efficacy of both must be largely similar (WHO, 2012). Even though not addressed during modelling, there are potential disadvantages such as promotion of resistance in secondary pests, interruption of biological control, and strong selection for cross-resistance (Pennetier *et al*., 2007).

2.4.5.3 Fine-scale mosaic

Spatially detached applications of diverse chemical agents against similar insect form a "mosaic" tactic to management of resistance. Fine-scale mosaics can be attained in vector-control programs for instance, by means of two insecticidal agents usage in various households in the same community (WHO, 2012). It forms the prospective for insects within one population to be exposed to both insecticides, and would lessen the degree of resistance selection – as long as multiple resistances contained in the vector population were exceedingly rare (Kweka, 2016). When such a fine-scale mosaic is to be used, careful records of which insecticide was used in each house are essential (Andriessen *et al*., 2015).

2.4.6 Global and country plan for insecticide resistance management in malaria vectors

The five basic pillars in any insecticide resistance programme in any country all over the world includes: design and establish strategies after baseline data and situation analysis, appropriate entomological and resistance surveillance, continued venture into insecticide reformlations, bridge insecticide resistance management information and efficiency gaps and crucial resources aquisation advocacy towards empowerment of each pillar.

Pillar I is to design and establish strategies towards the management of insecticide resistance in nations where malaria is rampant, through establishing a baseline data and carrying out situation analysis of insecticide resistance (WHO, 2012). For vector control and insecticide resistance management purposes, a system of eco-epidemiological stratification is used to divide a country into a few zones that are functionally different. During stratification, WHO approves that various issues be considered, comprising of eco-climatic regions, vector species, native malaria trends (such as interactions with agriculture, altitude, endemicity), demographic zones, and accessibility (Hemingway *et al*., 2013; Strode *et al*., 2014b).

Pillar II is to guarantee appropriate, well-timed entomological and resistance surveillance as well as efficient management of data. Most nations are limited in terms of monitoring resistance to insecticide. Therefore, they possess inadequate facts on the implication of the risk of resistance. This has affected their competence to manage insecticide resistance. IRM should be perceived in the framework of integrated vector management and be part of properly scheduled mosquito surveillance system. Nations should solve their resistance monitoring inconsistences to guarantee appropriate, timely entomological and resistance follow ups, and also proficient data handling by coming up with a resistance follow up plan, create and uphold capacity to gather and analyse the data on resistance, that will aid in decision making at a nation level, and for onward transmission to direct world wide WHO activities (Chanda *et al*., 2008).

Pillar III is to come up with new, advanced vector control tools. In order to come with novel active ingredients, continuous venture is required to progress development or

reformulations for insecticides with diverse modes of action. Because of the current overdependence on insecticide related intervention measures, certainty of insecticide resilience is guaranteed if selection pressure is continued (WHO, 2012).

Pillar IV is bridging of gaps that exist in the information regarding measures against insecticide resistance as well as on the effect of present IRM practices. The existing understanding of resistance to insecticide is adequate to validate instant activities for reserving the vulnerability of malaria vectors to pyrethroids and other categories of insecticides. Moreover, agricultural experience and scientific theory offer sufficient data on presently available IRM approaches to provide guidance on establishment of IRM approaches for malaria vectors (Strode *et al*., 2014b). However, there are significant gaps pertaining to our knowledge on insecticide resistance as well as resistance management techniques, and extra knowhow is required for effective delivering of IRM strategies. For instance, there is inadequate comprehension of ways to measure effect of resistance on the competence of vector intervention measures, delay resistance development and killing of resistant vmosquitoes in small-scale experiments (Strode *et al*., 2014b). Undertaking these queries is hindered by several factors, such as absence of perfect genetic indicators for some significant oxidase-mediated types of pyrethroids resistance. The responses to these queries would enable the development of improved IRM tactics and an evidence-based evaluation of their success (Chanda *et al*., 2008; Hemingway *et al*., 2013)

Pillar V is to make sure that crucial empowering mechanisms (financial and human resources, as well as advocacy) are implemented as a shared world wide obligation. Motivation of various shareholders at national, regional, as well as international levels is

crucial for IRM strategies to be satisfactorily implemented. Numerous teams of shareholders are inter-dependent, especially in terms of capacity building, funding, and coordination of responses in various sectors and nations. It is vital that associates recognize their responsibilities and roles as well as make sure that funds for IRM are sufficiently allocated. This sections also describes the suggested responsibilities of various teams of shareholders (WHO, 2012).

Awareness-raising is essential to involve all decision-makers at the beginning of designing IRM approaches while raising political profile of insecticide helps to improve resource allocation for IRM (Hemingway *et al*., 2013). For effective IRM plans, National malaria control programmatic approaches, WHO and the Roll Back Malaria Partnership ought to work as a team, and link up with governments and donors to secure funding (Muller, 2006). On research and evidence, WHO congregate experts to review evidence and science in order to make recommendations appropriately (IRAC, 2012). Despite research on resistance being conducted, continuous funding is required. Establishing research priorities is always among the initial steps in implementing the GPIRM (Roe, 2005). Nations depend on WHO to combine information and distribute latest guidelines regarding IRM strategy to all their national partners and agencies involved (WHO, 2012). Reinforced WHOPES and collaborative stations make sure there is a timely feedback to novel vector control tools (WHO, 2012).

2.4.7 Kenya's plan for insecticide management in malaria vectors

The rise of vector resistance to ordinarily applied insecticidal compounds is a serious threat to chemical–based vector control measures. Presently, DDT and pyrethroid resistance

levels are greatly elevated but little or no resistance at all, to cabamates and organophosphates (Kweka, 2016; Mathias *et al*., 2011). Sub-Saharan and East African malaria control programs largely depend on funding from donors for IRS implementation and LLINs distribution. However, monitoring of resistance and management as well as operational research remains unfunded. Further, East African nations have not implemented strategies to generate funds locally towards malaria control (Killeen *et al*., 2004). Consequenlty, it has made the entire vector control initiatives difficult, hence the risk of malaria renaissance in several foci along the evolution and spread of resilience in most regions of East Africa (Kweka, 2016). Generally, data involving resistance to insecticide in East Africa is patchy, and in various nations like Rwanda and Burundi such does not exist (Alout *et al*., 2017; Knox *et al*., 2014). It is therefore crucial that nations develop a database for storing resistance data collected in all the nations in a bid to understand the trend, enhance information sharing and make decisions in line with the WHO plan for IRM. Kenya has an obligation to implement and establish the global IRM strategy, the five pillars, IRS and IRM plans in order to offer guidance to IRS undertakings in the nation (WHO, 2016a). Kenya targets to establish capacity at the national level to put in place insecticide resistance monitoring as well as entomological surveillance. PMI has offered significant support in this exercise by conducting insecticide resistance monitoring and entomological surveillance in 16 locations all over western Kenya from 2008 (WHO, 2012).

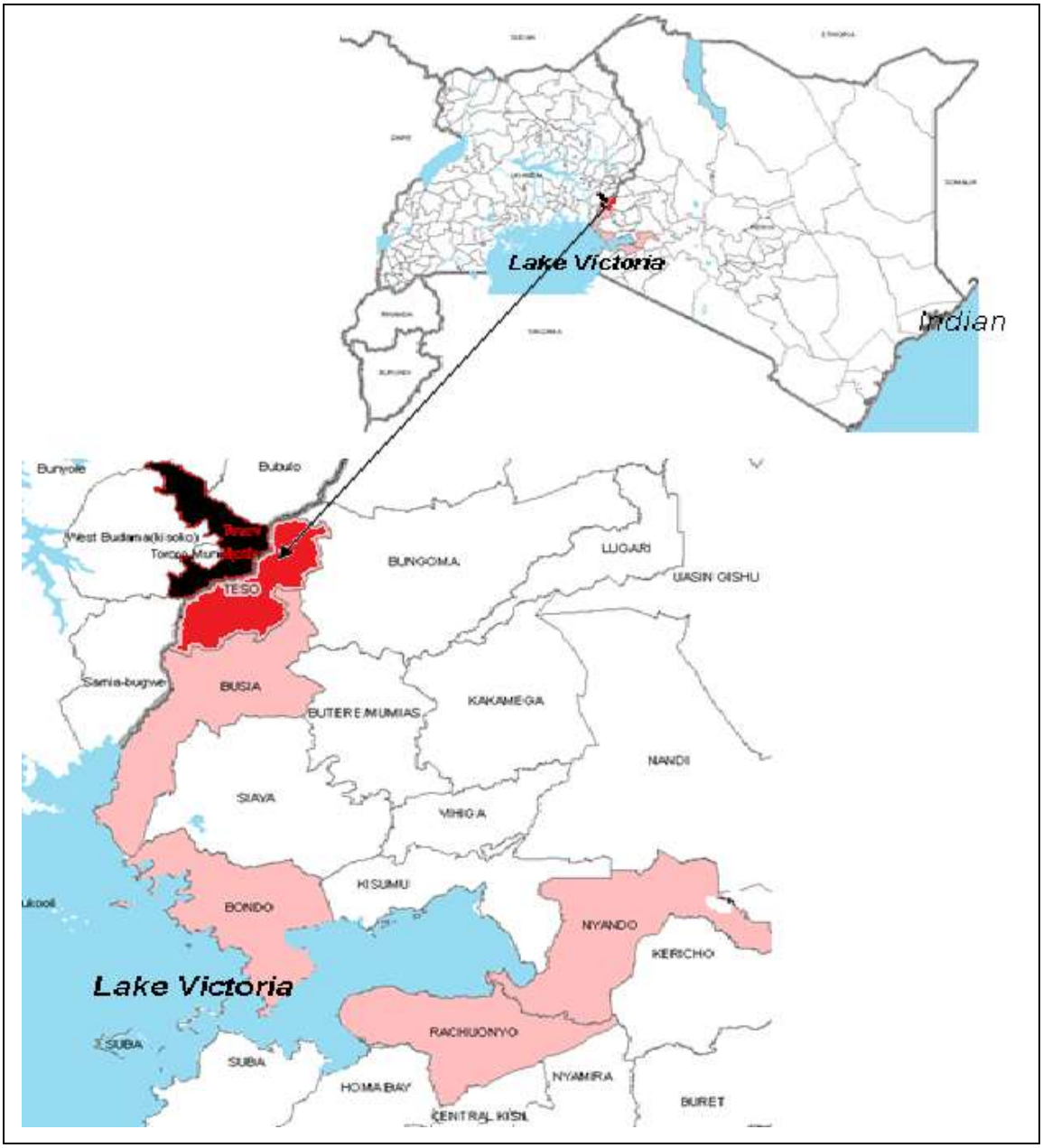
CHAPTER THREE

3.0 MATERIALS AND METHODS

3. 1 Study site

Teso North and Teso South sub counties is an administrative region in western Kenya. Population at risk is 252,884 people, of which 16.9% are between 0 and 4 years old, 40.5% occupy 5 to 15 years' age bracket and 42.6% are over 15 years.

Teso North and Teso South sub counties are found in the low land Lake endemic region which has malaria endemicity with throughout the year transmission and 88.1% LLIN universal coverage (Figures 3.1, 3.2 and 3.3). *Anopheles funestus, An. Arabiensis* and *An. Funestus* are the primary vectors of malarial agents. The principal malaria parasite is *Plasmodium falciparum*. The area has an annual rainfall of around 1700mm mean and minimum and maximum temperatures of about 17^{o}C and 32^{o}C respectively (KNMIS, 2016; Lindh *et al*., 2015). The communities mainly practice subsistence farming and health demographic characteristics include high infant mortality rates, neonatal and post natal mortality rates and crude death rates (Kakai *et al*., 2011; Takken & Verhulst, 2013).

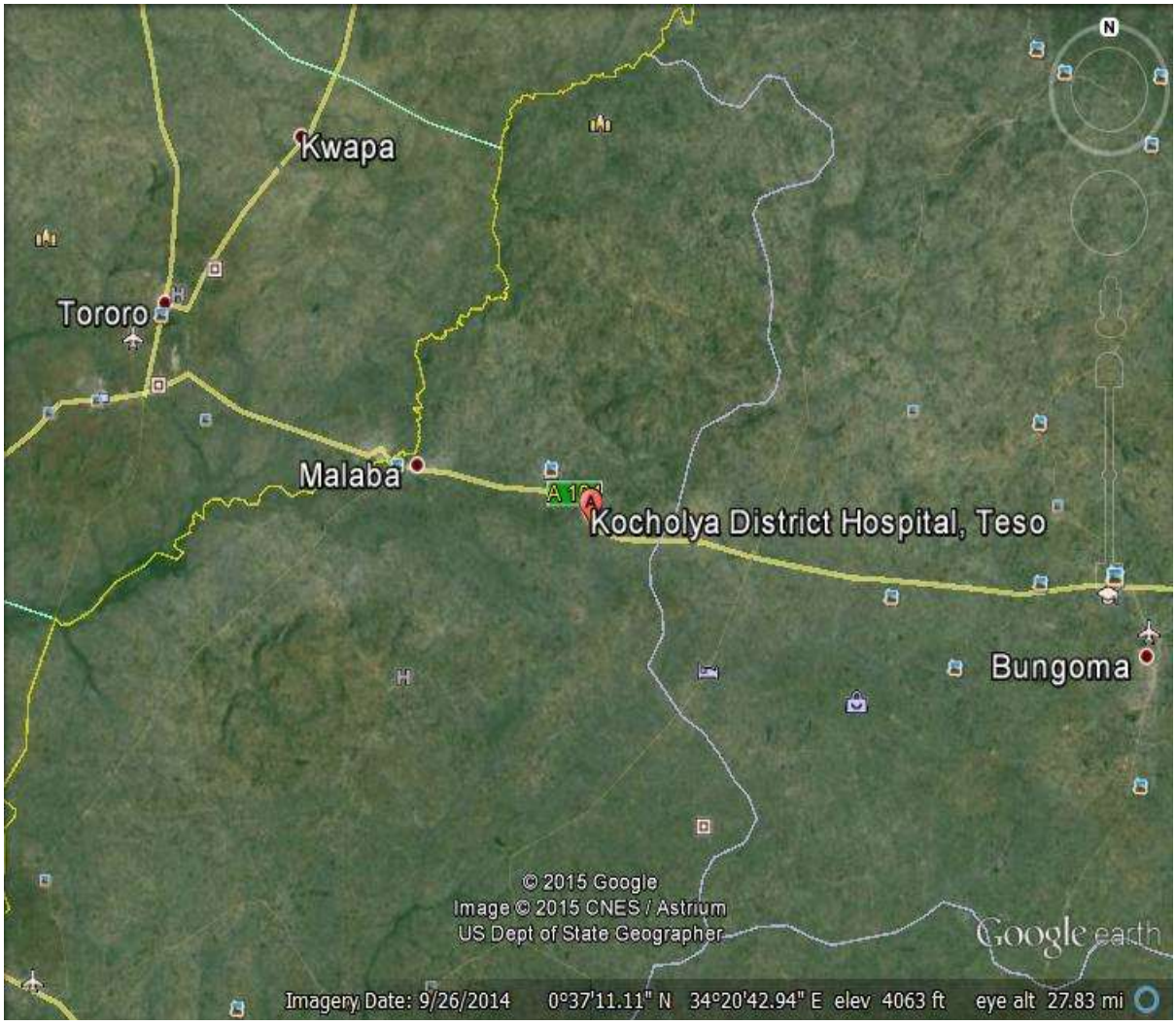


Key:

Red in colour – Formerly Teso district in Kenya

Black in colour – Neighbouring Tororo and Busolwe districts in Uganda

Figure 3.1: Map of Tororo and Busolwe districts in Uganda and the neighbouring Teso district in Kenya (Source: RCE Network)



Key:

Tororo and Kwapa towns are in Uganda

Malaba is a border town

Kocholya District Hospital is in Teso South Sub County

Figure 3.2: Teso North and Teso South sub counties - sandwiched between Bungoma county in Kenya and Tororo district in Uganda (Source: Google maps)

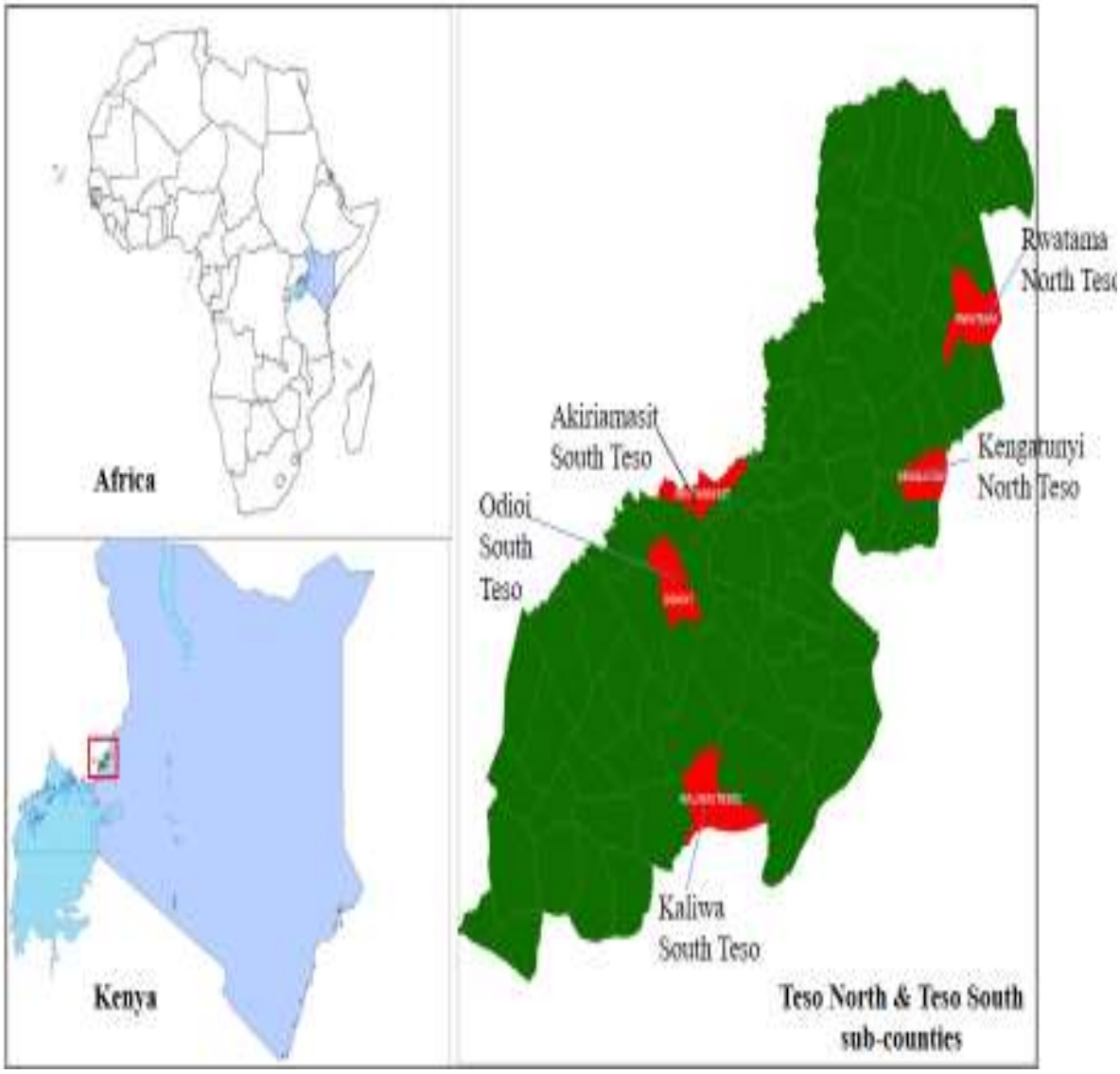


Figure 3.3: The five clusters randomly selected in North Teso and South Teso

(Source: Generated by this study)

3.2 Study Design

A longitudinal design was applied in the study, where data collection was done at different times on the same set of variables and cases. Raw data was collected, summarized and analysed into a useable form.

During base line, year two and three survey, wild larvae mosquitoes were collected and reared to produce 3 to 5 days old adult mosquitoes which were exposed to three types of insecticides, namely permethrin, deltamethrin and bendiocarb. Different clusters had different levels of insecticide resistance. Through pyrethrum spray, indoor aspiration, human landing catches and outdoor pot collection methods, adult mosquitoes were collected for age structure, vector density and infectivity, feeding and resting behavior studies. Adult mosquito collections had abdominal status classified into gravid, fed and unfed. Legs and wings of all reared and adult collected mosquitoes were used for species identification PCR. Fed mosquitoes were analysed for blood meal source. All adult collections' heads and thorax were analysed for circumsporozoite antigen through ELISA. Vector population age structure was determined through parity grading and near infra red spectrometer (NIRS) methods. Vector density was calculated after household data analysis was done while infectivity was investigated through sporozoite ELISA. Randomly selected mosquito samples from both larval and adult collections were analysed for *Kdr* gene. Exophilly, endophilly, exophagy and endophagy behaviours were determined by mosquito populations caught outdoor and indoor respectively. Anthrophagic and zoophagic behaviours were known through blood meal PCR.

The purposes of the study were to describe patterns of change and determine the direction (Figure 3.4).

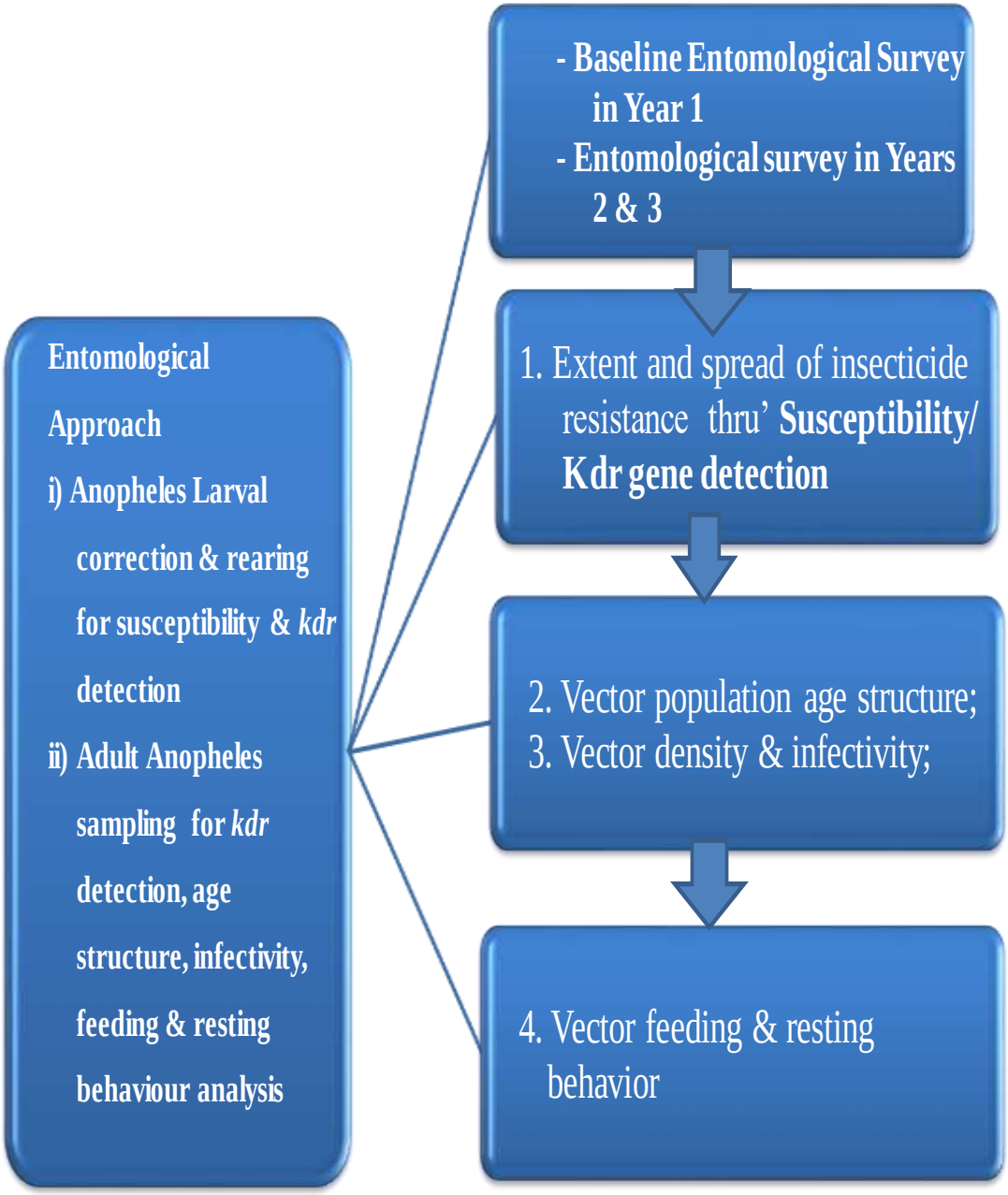


Figure 3.4: Study design

3.2.1 Conceptual frame work

The conceptual framework was structured into three variables (Figure 3.5). The Background Variables (climatic factors likely to cause fluctuations in vector populations, developmental stages, survival rates, feeding & resting behaviour) are hypothesized to significantly influence three Independent Variables (gene presence in the vector, age of mosquito, reproductive cycle & the sex of the vector) which in turn are hypothesized to influence the Dependent Variable (Malaria transmission potentiality) which has seven operational definitions.

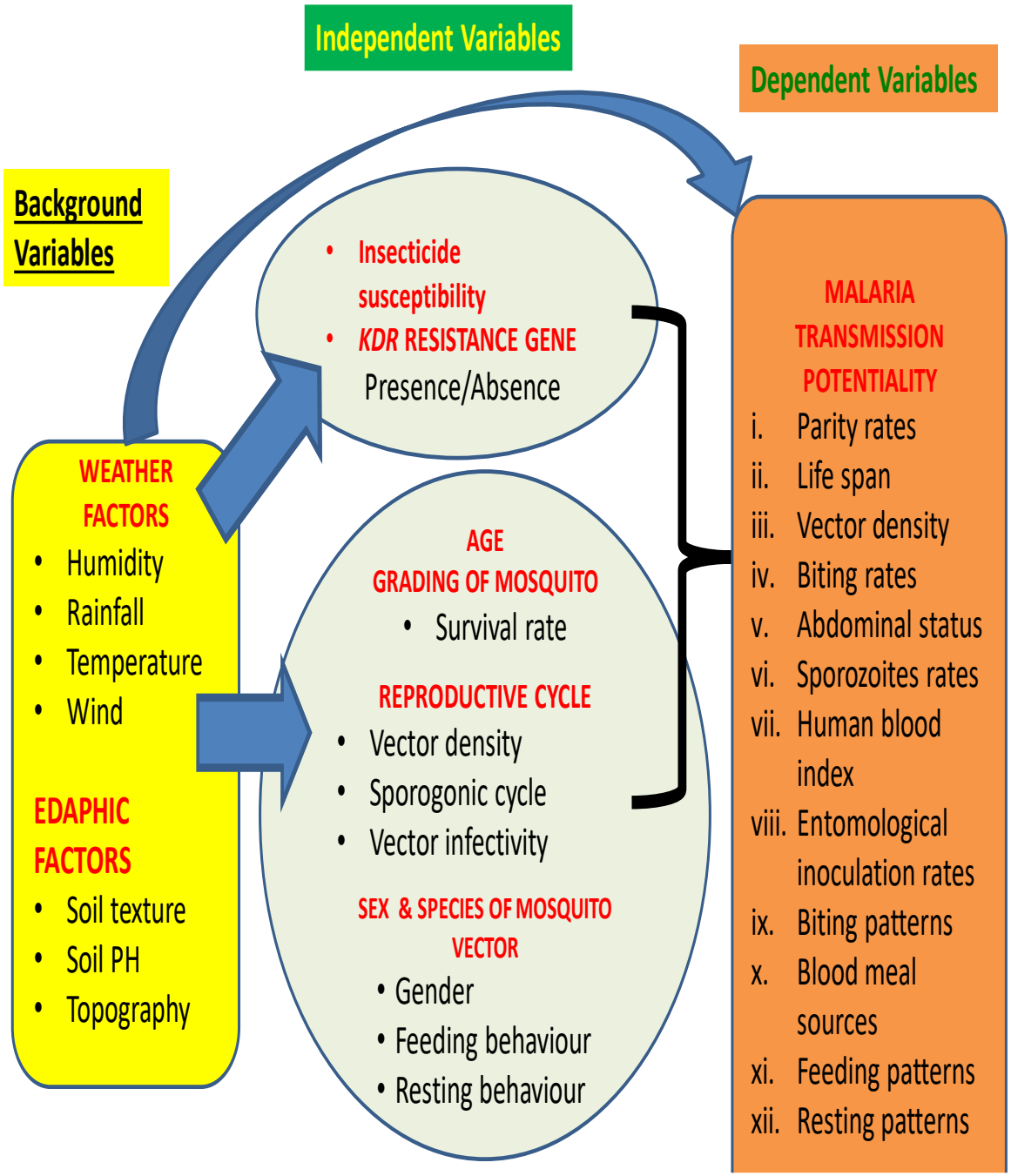


Figure 3.5: Conceptual frame work

3.2.2 Sample size determination

For larval collections reared into adult mosquitoes, sample sizes were dependent on WHO assays requirements whereby susceptibility examinations were conducted on 3 to 5 days old females that had not fed on blood. A total of 150 adult females were used, 100 of which were exposed to the insecticide in 4 replicates containing twenty five mosquitoes. The surplus of 50 served as "controls", that is 2 repeats, with each dublicate containing 25 female mosquitoes.

During adult mosquito collections, a sampling frame of household list from county registration files gave us the total number of house units in each randomly chosen compound, village and sub-location or cluster. A clustered probability sample was achieved with the help of computer generated tables of random numbers which were used to select sub-location, village, compounds and households where mosquito sampling and questionnaire administration was undertaken. Selected houses lay within 2km radius from larval collection sites.

An assumption used to work out sample size for malaria vector survey is that *Anopheles spp*. density will be normally distributed. Bearing in mind that the site had no previous data on parasite prevalence in females/house/night, a sample size with a 95% CI and precision level of 50% power was calculated (Abbott, 1987).

$$n = \frac{S^2 (t_\alpha + t_\beta)\, 2}{\delta^2}$$

Where n is the sample size, which is number of houses required to be sampled, tα is the critical value of the t-distribution at α =5% significance level, tβ is critical value of the t-

distribution at the 1-β = 50% power and δ is the precision level. The sample size obtained was 96 houses hence twenty houses per cluster or sub - location. Larval collection sites were randomly selected within 2-3 kilometers radius from selected households.

3.2.3 Ethical approval and secure storage of data

The study protocol received ethical approval from KEMRI's Scientific and Ethics Review Unit SSC NO: 2560: *Determination of insecticide resistance and its impact on malaria transmission potential among the major vectors in Teso district in western Kenya Version 2.1 dated 31st October 2013*). At house hold level, individual consent from house hold heads was sought. Information related to households was handled with strict confidentiality as provided by the law. All identity was hidden and published results were not associated with any individual. Information lifecycle management (ILM) involved data creation, data acquisition procedure, data storage in secured KEMRI server under password accessible by Principal Investigator. Data was de-materialised securely through deletion of soft copies, shredding and incineration of hard copies.

3.3 Mosquito collection methods

Both larvae and adult stages of mosquito were collected for two years after year one baseline susceptibility survey.

3.3.1 Larvae collection and rearing

Larvae were obtained from their natural breeding sites using standard dippers, set in plastic vessels (Figure 3.6) and transported to the insectary for rearing, species identification and susceptibility tests. Only anopheles larvae were retained in the containers as screening was undertaken on all collected larvae using morphological features for identification.

Figure 3.6: Larval collection using dippers in Akiriamasit cluster less than 200 meters from Uganda border with Kenya (Source: This study)

3.3.2 Adult mosquitoes collection methods

Adult mosquitoes were obtained using indoor resting vacuum aspiration, human landing catch; widow exit traps, pyrethrum spray catch method and outdoor pot collection. Each sample was morphologically identified and abdominal status recorded. Head and thorax were preserved in drierite and stored for use in Sporozoite ELISA. Blood fed abdomens were preserved in a freezer maintained at -18^{o}C and stored for use in blood meal PCR. All legs and wings were preserved in drierite and stored for use in species identification. Adult sampling was undertaken at the end of the long (May-July) and short rains (Oct-Nov).

3.3.2.1 Indoor resting vacuum aspiration

A vacuum aspirator was used; either a motor driven one or a manually operated using suction pressure in the mouth (Figure 3.7). Collected adult mosquitoes were selected and put in paper cups in a cooler box. Sucrose solution in moderately soaked cotton wool was placed on the paper cup net as food to the adult mosquitoes.

Figure 3.7: Collecting adult mosquitoes in a Teso grass thatched house using a back pack motor driven vacuum aspirator (Source: This study)

3.3.2.2 Window exit trap

To sample female mosquitoes that repose outdoors but bite indoors in a bid to identify the impact of resistance on the usual mobility and alimentation habits of mosquitoes, window exit traps were used. In each study cluster five houses were selected randomly for window traps. These traps were positioned over bedroom windows at 6.00pm. In the morning, a mouth aspirator was used to pick up trapped mosquitoes from the traps.

Window exit traps were most appropriate for fitting mainly in rooms that had few exit points and were adequately sealed for mosquitoes. Dark cartons or old clothes were used to cover additional exits apart from the windows to which traps were fitted. However, the eaves were left uncovered (Figure 3.8). Ordinarily the sleeping room was chosen and the trap fitted to a window. Sections of the window that the trap did not cover were concealed with dark hardboard, cartons, or clothes. The trap was positioned in a way that the collecting sleeve pointed outside. It was essential that the traps were fixed into windows before sun set. Mosquito collection was done the following day immediately after dawn. All anopheles mosquitoes were collected through the sleeve of the trap using a mouth aspirator. Individual paper cups were used to transport the live and dead mosquitoes collected from each house. Household information was also entered in a structured questionnaire. The paper cups were labeled clearly using a pen or pencil with at least the following important data: date, location, exit trap number, house number or householder code, time of collection, if mosquitoes were observed dead or live in the trap and name of the collector.

Figure 3.8: A window exit trap on a Teso thatched mud house with open eaves

(Source: This study)

3.3.2.3 Pyrethrum spray catch

To obtain the indoor resting densities of mosquitoes, pyrethrum spray collection method was done in all the houses that had window traps the previous night. During Pyrethrum spray collection, an index house was randomly selected and additional houses were selected based on proximity to the index house.

Collection took place between 6 am - 8 am once every month for three months during March, May, June and July long rains. During the procedure, occupants of the house were requested to wait outside. A record was taken of all individuals who slept in the house the preceding night, and existence of ITNs/LLINs recorded. Drinking and food utensils were removed from the household. White sheets were used to cover the floor and furniture inside the household.

Using 0.025% pyrethrum emulsifiable concentrate with 0.1% piperonyl butoxide (PBO) in kerosene, the eaves were sprayed with the help of two collectors. The inside collector then sprayed the walls and roof. After spraying, the house was closed for a duration of 10 minutes. Dead mosquitoes lying on the white sheets were collected (Figure 3.9) and moved to the laboratory on moist filter paper inside petri dishes.

Figure 3.9: Hand sprayer and collection of knocked down mosquitoes (Source: This Study)

3.3.2.4 Human landing catch

Female mosquitoes were attracted to humans as they quested for blood food. Malaria transmission is highly determined by the amount of mosquitoes biting or landing on humans.

The suitable locations for the night collections were selected in such a way that they were closer to the vector breeding sites in the area. In case of malaria cases in the village, it was better to select the house with a higher number of such cases. Direct collection of biting mosquitoes was performed during the night when malaria vectors were active for they take blood meal in the night.

Hourly collections were perfomed in a full night programme throughout the entire period. It was from 17.00 to 07.00 hours therefore from sunset to sunrise. Due to the laborious nature of the activity, two groups of collectors were used (Figure 3.10); each group doing collections half of the night. Inside the house and outside the house sampling were conducted to ensure that the usual sleeping and resting habits of the residents were accomodated. Rainy hours of the night were also recorded for it was not possible to collect human landing mosquitoes out doors as it rained.

Figure 3.10: Human landing set up inside the house (Source: This Study)

3.3.2.5 Outdoor pot collection

Residents in the study area largely relied on clay pots as storage for drinking water. The clay pots were locally designed, made and placed outside five houses per village. Capacity of every pot was approximately 20 litres, with a 20 cm width opening, a round bottom, and a maximum width of 45 cm. A one centimeter radius hole was made into the middle of the base to reduce theft, since the pot could not hold water. On the opening of the pot, a cloth mesh from a standard adult mosquito cage was put in place. Sampling of mosquitoes was conducted on the mesh. As a way of forcing the mosquitoes to get into the cage, one of the two samplers raised the pot, which exposed the mosquitoes to light. As a result, the mosquitoes were agitated. By using the opening at the bottom, the sampler blew the pot, which led to the mosquitoes flying and then entering the cage being held into place by the other sampler. After this process, the woven mesh was detached. The mosquitoes that remained inside were then collected by use of an aspirator. They were shifted to the cage, which marked the end of the collection process.

3.3.3 Questionnaire administration

House hold information was collected from heads in each of the houses where indoor resting, pyrethrum spray and human landing catches were undertaken. Information on the number of persons who were sleeping in the previous night, type of wall, type of eaves, type and ownership of net, frequency of net treatment and indoor residual spraying was collected for each of the houses randomly selected.

3.3.4 DNA extraction

Mosquito DNA were extracted using Chelex method (Appendix 1)

3.3.5 Species identification

Species identification PCR was done to differentiate samples for *A. arabiensis* and *A. gambiae* from *A. gambiae* complex. It involved amplification of DNA templates (extracted from the mosquito abdomens) by using the taq polymerase enzyme. The mosquito's DNA were amplified by using specific primers AR (for *An. arabiensis*), GA (for *An. gambiensis*) and a universal primer UN to help in the extension of the DNA after denaturation of the four nucleotide primers (dCTP, dATP and dTTP were used) (Appendix 2).

3.4 Extent and spread of insecticide non-vulnerability among major malaria vectors

Mosquito larvae samples were reared and exposed to WHO susceptibility kits impregnated with 0.05% deltamethrin and 0.75% permethrin and 0.1% bendiocarb insecticides.

3.4.1 WHO susceptibility assays

Mosquito larva samples were reared and exposed to WHO susceptibility kits impregnated with 0.75% permethrin, 0.05% deltamethrin, and 0.1% bendiocarb insecticides. The WHO protocol was used for testing susceptibility to permethrin, deltamethrin, and bendiocarb insecticides. Treated test papers with the WHO diagnostic dosages were supplied by the WHO Collaborating Centre in Kenya. Cohorts of 25 female mosquitoes were exposed (Figure 3.11) to different insecticides at temperatures of 25 ± 2^0C and 70–80% relative humidity following the standard World Health Organization (WHO) tube test protocol. Negative and positive controls were exposed to untreated and treated filter papers for 1 h,

respectively. Knockdown time was recorded after every 10 minutes during the 60-minute exposure. After 1 hr exposure, mosquitoes were transferred to recovery tubes and maintained on 6% sucrose solution for 24 hr. Mortality was recorded after 24 hr recovery period. Mosquitoes that were knocked down after 1 hr exposure and those that were alive after the 1 hr exposure and still surviving 24 hr later were collected and stored individually in 95% alcohol for subsequent molecular analysis. WHO pre-conditions for susceptibility are: – death rates between 98%-100% show full vulnerability; death rates between 90%-97% would undergo additional investigation while death rates < 90%, the population is considered resilient to the assayed insecticides (Appendix 3).

Figure 3.11: Working table set up during WHO Susceptibility tube assay 1 h exposure to deltamethrin (Source: This Study)

3.4.2 *Kdr* PCR

Mosquitoes from Teso were pyro sequenced for East-kdr (L1014S) mutations. The pyro sequencer unambiguously scored the homozygote resistant (SS), heterozygote resistant (LS) and homozygote susceptible (LL) genotypes for L1014S-kdr genotypes (Appendix 5).

3.5 Comparison between vector population age structure

Vector population age structure in areas with and without insecticide resistance was determined using parity grading and Near Infra-red spectrometer. Vectors were collected using human landing catch, window exit trap and indoor resting vacuum aspiration methods. Samples for NIRS method were preserved in RNA later while samples for parity grading were kept alive and dissected in the KEMRI Kisian Kisumu entomology laboratories.

3.5.1 Parity grading

Dissection of ovaries was done during parity and age grading process. Mosquito collections from each sampling station were immediately killed using dry ice in the field and Mosquito collections from each sampling station were immediately killed using dry ice in the field and immediately dissected. To determine the number of broods, dissection of the female mosquitoes was carried out on a microscope slide in few droplets of distilled water at 30X stereoscopic magnification. A small slit was made in the tip of the mosquito abdomen with minuten probes and the ovaries removed by applying gentle traction to the tip of the abdomen. All specimens were first examined by Detinova's method of ovarian tracheolation (Detinova 1962) to determine whether the mosquito had oviposited. Detinova

method of age grading classifies mosquito into parous (older with no nodulated skeins) and nulliparous (younger vectors with nodulated skeins).

One ovary from each of the females was removed to a separate microscope slide and air dried at room temperature. When completely dry, the slide was examined at X150 to determine the condition of the tracheoles. Coiled tracheoles were used as an indication of nulliparity; stretched and unwound tracheoles would indicate a parous specimen.

3.5.2 Spectra collection using the NIR spectrometer

Mosquitoes gathered in Teso region were preserved in RNA later and transferred to KEMRI Kisian Kisumu Centre for Geographic and Human Research for Near Infra Red Spectrometer (NIRS) analysis (Figure 3.12). Near Infra-red spectroscopy measures the levels of infra-red rays' absorbance which is directly proportional to amount of moisture in the mosquito tissues which is directly proportional to the age of the insect. Before scanning, paper towels were used to remove residual RNA from the mosquito specimens.

Calibration of the spectrometer was done using forty 1-28 day old laboratory reared *Anopheles gambiae* sensu stricto. A lowest number of fourty female mosquitoes at every age for the pyrethroid-exposed and non-exposed laboratory units, and 331 wild-collected composite-age *Anopheles* mosquitoes were scanned through a LabSpec 5000 NIR spectrometer (ASD Inc, Boulder, CO), based on the method defined by Mayagaya and associates (Mayagaya *et al*., 2009). A maximal 20 female mosquitoes were positioned ventral side up on a Spectralon plate. To collect the spectrum, the head and thorax of each mosquito was scanned one at a time under a 3-mm-forked fiber-optic probe enclosing four collection filaments and 33 illumination filaments.

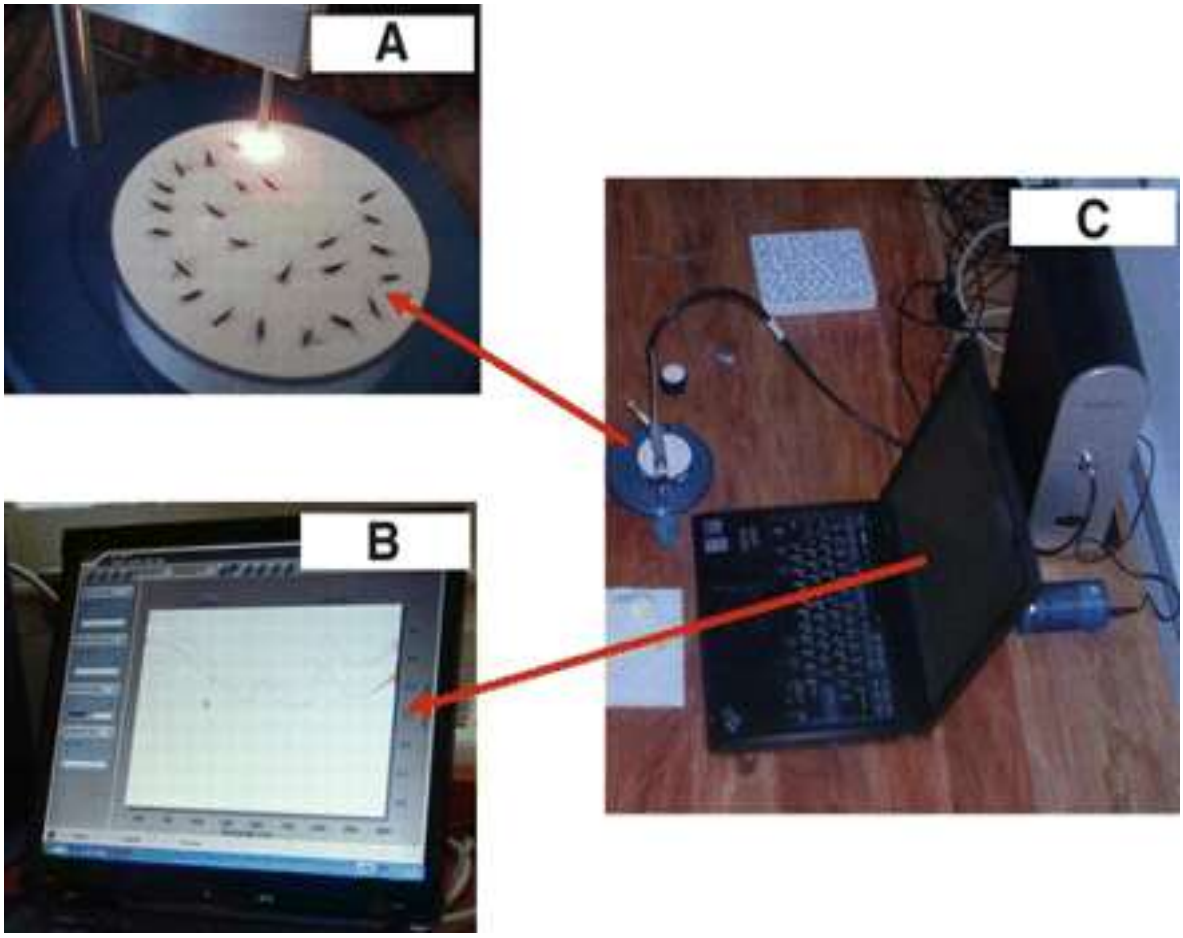


Figure 3.12: Mosquitoes scanned using a near-infrared spectrometer.

(A) Plate with anesthetized mosquitoes positioned for scanning.

(B) Near-infrared (NIR) spectra of mosquitoes.

(C) Complete NIR system including the spectrometer (ASD Inc., Boulder, CO).

3.5.3 Data analysis

Physiological age dissections of both susceptible and non-susceptible mosquitoes were grouped according to distance from the nearby breeding sites in order to find out whether differences in the age composition of the collections would be evident. Normality was checked using normal probability plot. A t-test was also used to determine if there is significant difference between the means of two groups of variables, which may be related. Anova carried out to separate the means. An analysis of variance of the three years data and multiple regression of the combined data were performed to indicate any statistically significant differences.

3.6 Vector density and infectivity

Collection of mosquitoes resting outside houses was conducted by using a large vacuum aspirator, like the Nasci Aspirator. Indoor-resting mosquitoes were sampled by PSC and hand-catch aspiration. Locations of the adult sampling were recorded by differential global positioning systems (DGPS). Mosquito sampling was undertaken twice a year within the study area, to obtain reliable data on the species composition and densities of both host-seeking and outdoor-resting mosquitoes. Five houses in a sub-location were randomly chosen for adult mosquito collections during the intervention phase. Also, the number of individuals who slept in the room the previous night was determined during sampling.

3.6. 1 Evaluation of density, Human Blood Index (HBI) and Entomological Inoculation Rate (EIR)

Further laboratory processing involved testing anopheline mosquitoes for *Plasmodium falciparum* circumsporozoite protein hence determine sporozoite rates (Wirtz *et al.*, 1987) (Appendix 4), and blood meal identification by PCR (Appendix 6). Combined with estimates of mosquito contact, the sporozoite rates were used to compute and trace temporal and spatial variation in EIRs for the study sites in a sub-location.

3.6.2 Data collection, management and analysis

Exit rates were computed as the number of female mosquitoes caught by the traps divided by all mosquitoes collected through window trap, indoor resting aspiration and PSC, expressed by site and per sub-location. A contrast between susceptible and resistant clusters was made in terms of species density, biting rate, physiological status, parity, infection rate, human blood index and vector biting behaviour. Vector density was derived by dividing the number of vector species with the number of houses sampled. Biting rate on the other hand was computed as number of mosquitoes per individual. Physiological status was analyzed by determining the proportion of fed, gravid and unfed mosquitoes expressed per sites and per sub-county. Parity rate was computed by dividing the number of parous mosquitoes with the number of mosquitoes tested expressed per sites and per sub-county.

Infection rate referred to the fraction of mosquitoes positive for *Plasmodium* sporozoite. The Sporozoite rate was derived by dividing the sum of sporozoite positive mosquitoes by all mosquitoes assayed. Human blood index refered to the number of mosquitoes that fed on people's blood. The proportion of samples containing human blood was derived as the

number of samples that tested positive for human blood divided by the number of mosquitoes that were assayed.

Vector biting behaviour took into consideration all mosquitoes collected during the human landing catches computed by hour of collection to determine the time of night that most biting occurred; summarized by sites in the sub-county. Entomologic Inoculation Rate (EIR) was computed as the number of infectious bites/person/night. Monthly and annual EIR was extrapolated from these estimates.

3.7 Vector feeding and resting behaviour

To assess the changes in vector behaviour influenced by insecticide resistance and susceptibility, house entry and exit behaviour, resting behaviour and blood meal sources were investigated. These studies were carried out once every year during the periods of high mosquito density. For each sampling method used (PSC, indoor aspiration, human landing catch and outdoor pot collection) in the five clusters, collected mosquitoes were identified morphologically and categorized by abdominal status. To determine parity rates disessection of subsamples of unfed mosquitoes was performed. All mosquitoes sampled were separately preserved in eppendorf tubes having a desiccant for the succeeding laboratory assays for frequency of resistance genes, blood meal source and sporozoite infectivity.

3.7.1 Molecular identification of mammalian blood meals from mosquitoes

The PCR and ELISA protocols differentiated between the bloods of 7 potential mammalian hosts in engorged anophelines.

3.7.1.1 Blood meal PCR

Livak method was used to extract the genomic DNA from engorged mosquito abdomens (Appendix 6). The head and thorax were removed before the extractions were carried out.

3.7.1.2 Blood meal ELISA

Abdomen of every mosquito was crushed in 50 µl of phosphate-buffered saline (PBS), and then 950 µl of PBS were added after grinding. Blood meals were analysed by direct ELISA by means of antihost (IgG) conjugate against donkey, human, dog, goat, cat, and chicken proteic compounds in a single-step assay (Beier *et al*. 1988). Chicken and goat IgG was used to test non-reacting samples. ELISA results were read visually (Beier *et al*., 1988) (Appendix 7).

CHAPTER FOUR

4.0 RESULTS

4.1 Extent and spread of insecticide resistance in Teso North and Teso South sub counties

From the five clusters, species composition, phenotypic and genotypic resistance results were obtained as follows.

4.1.1 Species composition sampled in Teso North and Teso South sub counties

Anopheles gambiae sensu stricto were the majority in terms of species composition in both Teso North and Teso South sub counties and also in all the clusters randomly selected (Table 4.1). Highest proportions of *Anopheles gambiae* sensu stricto and *An. arabiensis* were found in Rwatama and Akiriamasit clusters, respectively. Only Teso North sub county had traces of *Anopheles funestus*. Overall, the predominant *Anopheles gambiae* comprised of 78.9% of the female mosquitoes sampled in Teso sub counties. Highest proportion of *Anopheles gambiae* ss were collected using pyrethrum spray catch method while most *Anopheles arabiensis* were collected using window exit method. Only Outdoor pot collection method was able to sample *Anopheles funestus*. *Anopheles arabiensis* were significantly fewer than the majority species, *Anopheles gambiae* [$t(8) = 11.1268$, $p < .05$].

Table 4.1: Proportions of different members of *Anopheles* species and resistance alleles in different mosquito collection methods carried out in Teso North and Teso South sub counties, Busia county, Western Kenya

		Anopheles gambiae sensu stricto	Anopheles arabiensis	Anopheles funestus
Cluster	n	Proportions %	Proportions %	Proportions %
Kaliwa	491	80.6	19.4	0
Odioi	1041	81.6	18.4	0
Akiriamasit	738	65.7	34.3	0
Kengatunyi	177	79.1	20.9	0
Rwatama	1724	87.2	10.7	2.1
Mean	**4171**	**78.9**	**20.7**	**0.4**
Teso North	2639	77.3	22.0	0.7
Teso South	1532	81.1	18.9	0
Collection method				
Larval collections	2588	77.2	22.8	0
PSC	338	83.3	16.7	0
WET	722	64.1	35.9	0
OPC	36	25	25	50
HLC	605	81.6	18.4	0

4.1.2 Phenotypic resistance in mosquitoes collected in North Teso and South Teso

Bendiocarb caused 100% mortality in 24 hours after exposure to the insecticide (Table 4.2). Deltamethrin was more potent than permethrin. Kengatunyi cluster mosquitoes were equally knocked down by both permethrin and deltamethrin. Mortality rates caused by permethrin as compared to deltamethrin were insignificantly higher in 2012 but reversed in 2013 and 2014 [t (13) = 0.3330, p > .05]. All vectors were resistant to permethrin and deltamethrin but susceptible to bendiocarb.

Table 4.2: Proportions in species composition in each major malaria vector species per cluster and method of collection in Teso North and Teso South sub counties, western Kenya between 2012 and 2014

Year	**2012**		**2013**			**2014**		**Mean** ^{SD}		
	Different types of insecticides on WHO treated test papers for tube assay									
Clusters	Permethrin	Delta-Methrin	Permethrin	Delta-methrin	Bendio-carb	Permethrin	Delta methrin	Perme-thrin	Delta-methrin	Bendi-ocarb
Kaliwa	75^{*101}	63^{*16}	74^{*118}	93^{*100}	-	76^{*67}	79^{*52}	$75^{\pm5.4}$	$78^{\pm15.6}$	-
Odioi	37^{*99}	56^{*34}	79^{*103}	89^{*100}	100^{*61}	56^{*101}	53^{*100}	$57^{\pm19.4}$	$66^{\pm19.1}$	100
Akiriamasit	81^{*89}	-	78^{*117}	96^{*107}	100^{*100}	53^{*19}	76^{*84}	$71^{\pm15.4}$	$75^{\pm11.8}$	100
Kengatunyi	87^{*107}	66^{*44}	91^{*102}	99^{*116}	-	80^{*92}	93^{*14}	$86^{\pm5.7}$	$86^{\pm18.3}$	-
Rwatama	86^{*103}	70^{*38}	75^{*116}	87^{*101}	-	38^{*8}	78^{*63}	$66^{\pm23.1}$	$78^{\pm8.0}$	-
Mean ^{SD}	$73^{\pm19.5}$	$64^{\pm10.5}$	$79^{\pm8.2}$	$93^{\pm6.9}$	100	$61^{\pm16.7}$	$76^{\pm14.5}$	71	77	100

*Superscripted numbers represented the number of 3-5 days old female mosquitoes assayed - Where 3-5 days old female mosquitoes were not available for exposure to that particular insecticide. WHO criteria for susceptibility are: Mortality rates between 98%-100% indicate full susceptibility; Mortality rates between 90%-97% require further investigation while Mortality rates < 90%, the population is considered resistant to the tested insecticides

4.1.2.1 Knock down patterns

The knock down patterns were dependent on resistance levels in the mosquitoes and the cluster, rainy or dry season and the type of insecticide (Figure 4.1). Deltamethrin insignificantly knocked down the female mosquitoes earlier and faster than permethrin [$t(12) = 1.6238$, $p > .05$). During dry season the mosquitoes were not significantly as readily knocked down as compared to mosquito samples collected during wet season [$t(14) = 1.1708$, $p > .05$).

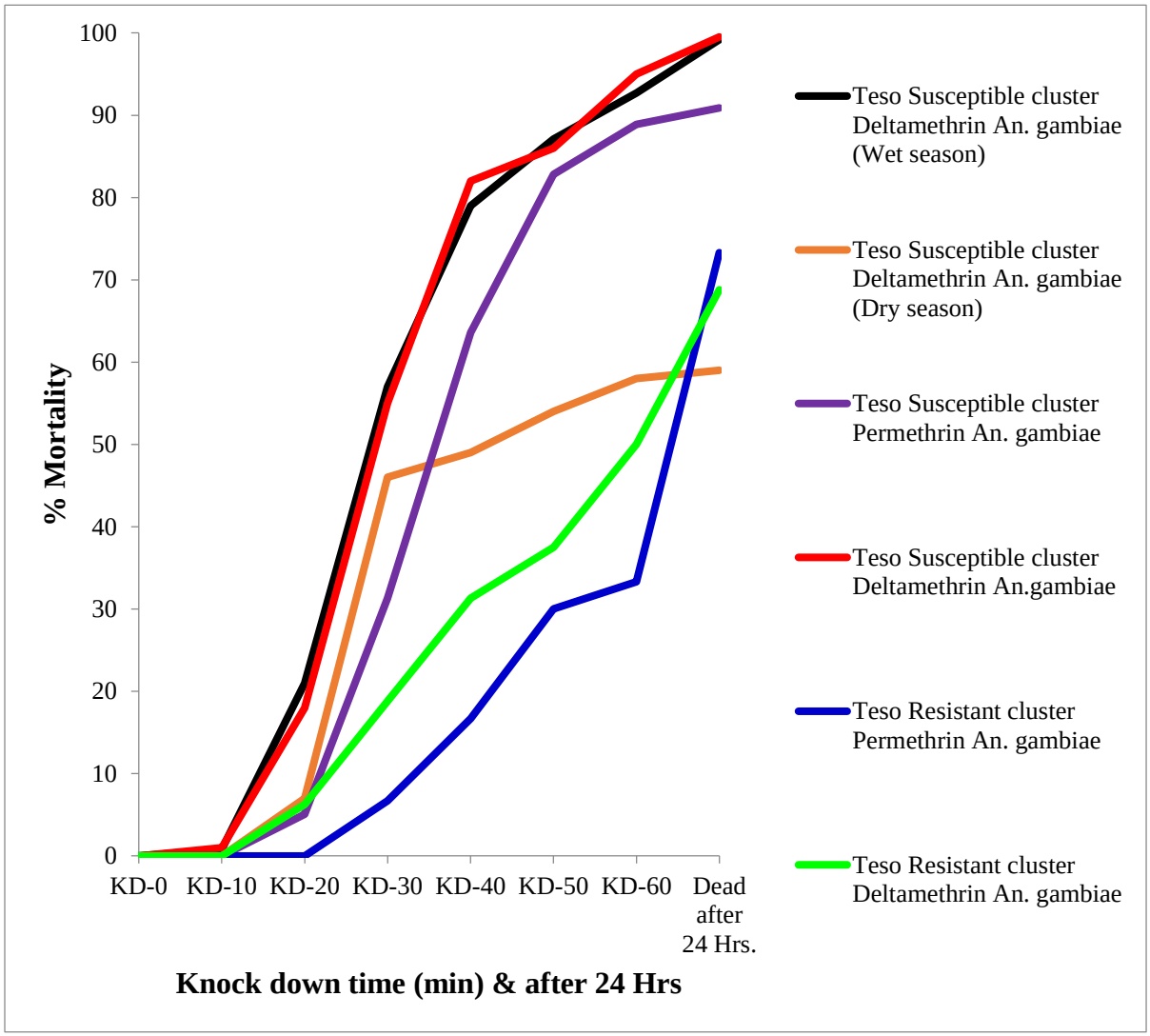


Figure 4.1: Knock down curves showing effects of types of insecticide, wet or dry season and resistance levels on knock down trends in 3-5 days old female mosquitoes collected in Teso North and Teso South sub counties, Western Kenya between 2012 and 2014

The Kisumu Asembo Anopheles gambiae strain was fully susceptible to the three insecticides (Table 4.3). The knock down times for 50 % (KDT_{50}) of mosquitoes sampled from Teso North and Teso South sub counties were between 24-47 min in contact with permethrin, 22-36 min with deltamethrin and 20-25 min with bendiocarb. For the three insecticides, KDT_{95} were less than 190 min while less than 86 % of exposed mosquitoes were dead at 24 hrs post exposure to permethrin and deltamethrin. Mosquitoes exposed to bendiocarb experienced 100% mortalities after 24 hrs post exposure. Susceptible Kengatunyi mosquitoes exposed to deltamethrin had the highest KDT_{50} R of 8.2.

Table 4.3: Knock-down times for 50 and 95% of the Susceptible Kisumu *Anopheles gambiae* strain and the Teso North and Teso South F_o *Anopheles gambiae* to Permethrin, Deltamethrin and Bendiocarb diagnostic concentrations

		Susceptible Kisumu Asembo *Anopheles gambiae* strain				Teso North and South $_0$*Anopheles gambiae* samples				
Diagnostic concentrations	Clusters	n	KDT_{50} [CI] (min)	KDT_{95} [CI] (min)	Mortality after 60 min (%)	n	KDT_{50} [CI] (min)	KDT_{95} [CI] (min)	Mortality after 24 h (%)	KDT_{50} R
0.75% permethrin	Kaliwa	100	14.155 [6.207-20.372]	32.462 [25.469-50.817]	100	95	41.285 [38.579-44.369]	103.363 [88.043-129.284]	75	2.9
	Odioi	100	17.658 [4.133-27.790]	58.565 [34.896-1259.225]	100	100	46.607 [36.379-71.682]	189.492 [104.482-1062.44]	57	2.6
	Akiriamasit	100	13.977 [3.707-21.112]	61.544 [37.499-466.702]	100	75	24.689 [19.390-30.196]	66.066 [48.540-128.794]	71	1.8
	Kengatunyi	100	8.189 [5.947-10.563]	27.813 [21.837-39.676]	100	100	36.397 [34.402-38.442]	74.654 [67.294-85.557]	86	4.4
	Rwatama	100	14.982 [12.625-17.192]	35.886 [29.288-48.448]	100	76	37.085 [34.536-39.788]	84.719 [73.667-102.885]	66	2.5
0.05% deltamethrin	Kaliwa	100	17.590 [15.962-19.036]	26.432 [23.914-30.975]	100	76	30.930 [23.644-41.566]	86.182 [56.925-292.337]	78	1.8
	Odioi	100	5.780 [3.534-7.794]	31.050 [25.978-39.834]	100	78	31.757 [29.521-34.007]	71.317 [63.207-83.777]	66	5.5
	Akiriamasit	100	13.256 [4.132-19.714]	62.378 [38.849-328.090]	100	96	35.448 [30.809-40.298]	70.829 [58.069-102.798]	75	2.7
	Kengatunyi	100	2.698 [0.722-4.916]	24.154 [18.974-32.751]	100	71	22.204 [20.821-25.139]	49.690 [46.172-52.962]	86	8.2
	Rwatama	100	17.012 [15.200-19.304]	50.773 [31.357-402.770]	100	67	30.423 [27.859-33.198]	80.692 [70.893-97.137]	78	1.8
0.1% bendiocarb	Odioi	100	5.432 [0.679-10.006]	51.612 [33.976-172.401]	100	61	20.543 [18.570-23.086]	58.895 [53.951-67.980]	100	3.8
	Akiriamasit	100	5.439 [0.258-10.444]	39.386 [25.578-168.087]	100	100	24.886 [17.223-31.783]	48.181 [36.636-100.030]	100	4.6

n: : sample size; CI: confidence interval at 50 and 95%; KDT_{50}: knockdown times for 50% of exposed mosquitoes; KDT_{95}: knockdown times for 95% of exposed mosquitoes; KDT_{50} R: ratio KDT_{50} Teso North and Teso South $_0$ female mosquito samples/KDT_{50} susceptible Kisumu Asembo *Anopheles gambiae* strain; min: time in minutes.

4.1.3 Genotypic resistance

Rwatama scored the highest levels of homozygous SS alleles for resistance at 93.9% while Kengatunyi had the lowest levels of SS alleles at 57.6% (Table 4.4). Conversely, Kengatuny showed the highest levels of homozygous alleles (LL) for susceptibility at 35.3%. All clusters registered over 50% homozygous SS alleles. Akiriamasit had the highest levels of genetically transitioning heterozygous LS alleles at 13.9%.

Teso South which is a bit further from Kenya - Ugandan border had a higher S allelic frequency than Teso North which borders Uganda. All female mosquito samples collected through pyrethrum spay catch method and tested for *Kdr* gene had a 100% SS genotypic frequency. There was no significant difference in allelic frequencies between *Anopheles gambiae s.s.* and *Anopheles arabiensis*. Mosquito samples collected in the year 2013 had the highest S allelic frequency. Mosquito sampling method had significant difference to genotypic insecticide resistance levels [$F(2) = 27.0$, $p < .05$]. Means of k*dr* genotypic levels also had significant difference with species of female mosquitoes sampled [$F(2) = 10.11$, $p < .05$].

Table 4.4: *Kdr* genotypic and allelic frequencies across the clusters, mosquito collection methods, species and year in mosquito samples collected from Teso North and Teso South sub counties, Busia county western Kenya

Cluster	n	SS genotypic frequency Proportions %	LS genotypic frequency Proportions %	LL genotypic frequency Proportions %	S allelic frequency	L allelic frequency
Kaliwa	223	91.5	2.7	5.8	0.93	0.07
Odioi	424	76.9	4.7	18.4	0.79	0.21
Akiriamasit	216	76.9	13.9	9.2	0.84	0.16
Kengatunyi	85	57.6	7.1	35.3	0.61	0.39
Rwatama	148	93.9	3.4	2.7	0.95	0.05
Mean	**219.2**	**79.4**	**6.9**	**13.7**	**0.82**	**0.18**
Teso North	449	78.6	9.4	12.0	0.83	0.17
Teso South	647	82.0	4.0	14.0	0.84	0.16
Collection method						
Larval collections	556	80.6	6.1	13.3	0.84	0.16
PSC	9	100	0	0	1.0	0
WET	348	79.0	4.0	17.0	0.81	0.19
OC	-	-	-	-	-	-
HLC	183	83.0	10.4	6.6	0.88	0.12
Species						
Anopheles gambiae s.s.	868	81	5.2	13.8	0.84	0.16
Anopheles arabiensis	228	80.6	6.2	13.2	0.84	0.16
Anopheles funestus	-	-	-	-	-	-
Year						
2012	106	66	5.7	28.3	0.69	0.31
2013	230	83.9	7.0	9.1	0.87	0.13
2014	760	81.6	6.1	12.3	0.85	0.15

Anopheles gambiae was resistant to permethrin, partially resistant to deltamethrin but completely susceptible to bendiocarb (Figure 4.2). Homozygous SS genotype carrying mosquitoes were the significant majority as compared to homozygous LL genotypes [t (8) = 7.6685, p < .05].

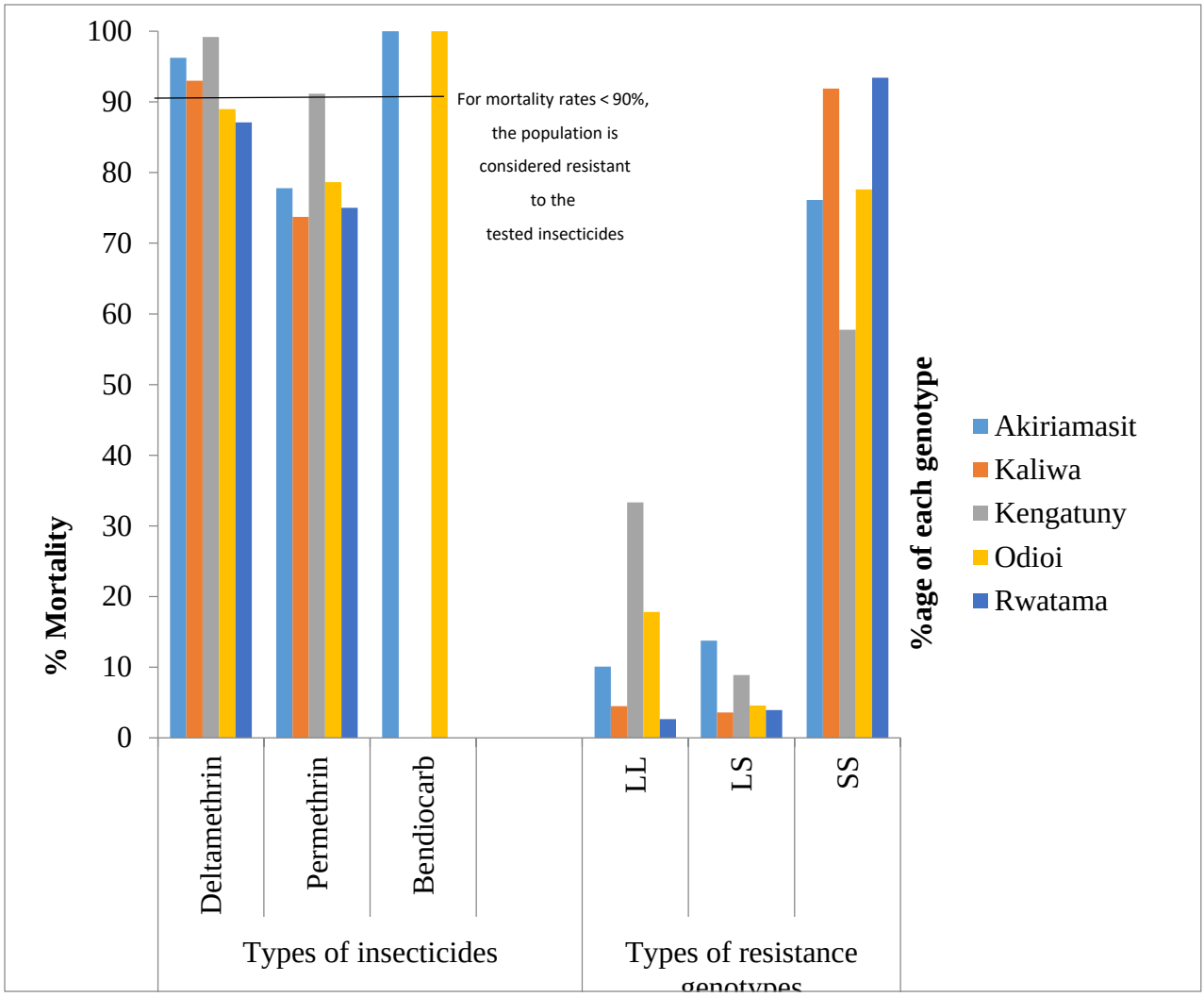


Figure 4.2: Over all phenotypic and genotypic resistance levels in *Anopheles gambiae* collected from five clusters in Teso North and Teso South sub-counties, Western Kenya

There was no significant diferrence between resistance and suseptible allele in Kengatunyi (Table 4.5). Rwatama cluster had significantly ($p \leq 0.05$) higher proportion of SS genotypic and S allelic frequencies as compared to Kengatunyi cluster.

Table 4.5: Significant levels between resistant and recessive alleles in each cluster in Teso sub counties, Western Kenya

Cluster	Mean SS alleles	DF	F value	ρ value
Kaliwa	91.9	258	4.29	0.0081
Odioi	77.6	561	33.84	<.0001
Akiriamasit	76.1	379	41.91	<.0001
Kengatunyi	57.8	76	0.05	*0.9845
Rwatama	93.4	266	2117.96	<.0001

*No significance difference between susceptible and resistant alleles

Overall levels of homozygous resistant alleles, heterozygous resistant alleles and homozygous susceptible alleles were 79.4, 6.9 and 13.7% respectively (Figure 4.3).

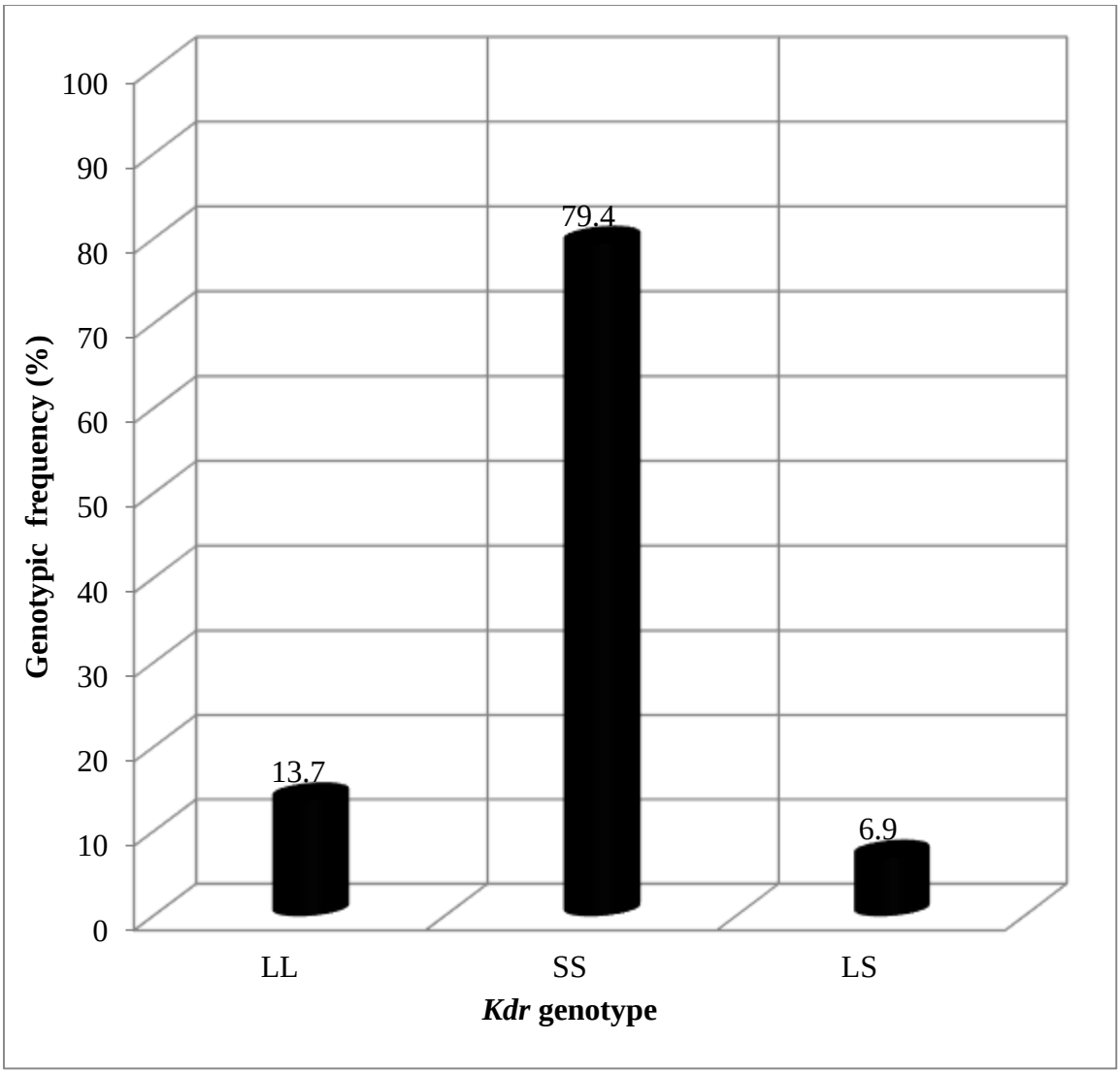


Figure 4.3: Teso sub – county's overall wild and mutant alleles levels

4.2 Comparison of age structure between resistant and susceptible populations

Parity rates and mean life spans were used to determine the age structure of mosquitoes collected in Teso North and Teso South sub counties.

4.2.1 Relationship between mosquito parity rates and SS allele frequency

Resistant cluster had the highest parity rates while the susceptible one had the lowest. Proportions of female *Anopheles* with highest SS alleles were found in Rwatama and Akiriamasit clusters at 100% (Table 4.6). Hence the higher the parity rate, the higher was the proportion of SS alleles. Parity rate had significant difference to *kdr* genotypic levels [$F(3) = 2.81$, $p < .05$].

Table 4.6: Parity rates and proportion of SS alleles in female mosquitoes collected from various clusters in Teso North and Teso south sub counties

Clusters	n	Parity rates (%)	SS positives in the dissected HLC mosquitoes (%)	Overall SS frequency (%)
Kaliwa	79	72	95	92%
Odioi	91	67	74	77%
Akiriamasit	21	86	100	76%
Kengatuny	39	26	32	58%
Rwatama	55	88	100	93%

*HLC: Human landing catch

4.2.2 Homozygous SS allele's frequencies and age structure as determined through NIR spectroscopy

Longevity was highest in female mosquitoes collected from high resistance Kaliwa cluster having an average life span of 17 days (Table 4.7). Lowest longevity and proportion of SS alleles were found among samples collected from low resistance Kengatunyi cluster. Therefore lower proportion of SS alleles was found in the shorter life spanned female mosquitoes sampled. There was significant difference in age between low resistance Kengatunyi and the other four clusters. There was significant difference between the female mosquito life span and *kdr* homozygous SS genotypic levels [t (8) = 9.9506, p < .05).

Table 4.7: Mean life span in relation to proportion of SS alleles in malaria vectors collected from five clusters in Teso north and south sub counties

Clusters	Mean life span (days)	SS positive (%)	n	F value	ρ value
Kaliwa	$17^{\pm 2.0235}$	92	95	0.18	<0.0001
Odioi	$12^{\pm 5.117}$	77	65	3.29	0.0380
Akiriamasit	$9^{\pm 1.9946}$	76	143	68.26	<0.0001
Kengatuny	$5^{\pm 4.165}$	58	95	19.51	0.8393
Rwatama	$16^{\pm 1.8074}$	93	18	102.0	<0.0001
Overall	$13.4^{\pm 6.2803}$		416		
Calibration sample	$13.6^{\pm 0.8067}$		130		

4.3 Impact of insecticide resistance on vector density and infectivity in major mosquitoes species sampled in Teso North and Teso South

Vector density and infectivity was determined in both areas of high as well as low insecticide resistance levels.

4.3.1 Impact of insecticide resistance on vector density

Highest mosquito densities were found after human landing catch (HLC) and the least during pyrethrum spray catch (PSC) (Table 4.8). Medium resistance Akiriamasit cluster had the highest malaria vector density in HLC collections. Only outdoor feeding mosquito densities in susceptible Kengatunyi cluster were higher than densities in resistant Rwatama cluster. There was no significant difernce in densities of msquitoes from resistant and susceptible clusters [$t(8) = -1.0527$, $p > 0.05$].

Table 4.8: Densities (number of female vector mosquitoes per house) of female mosquitoes per cluster and collection method in Teso sub counties

			Human landing catch			
	PSC	WET	Indoor	Outdoor	Overall	SS frequency (%)
Kaliwa	4.7	16	12.5	14.0	26.5	92
Odioi	9.2	22.4	34.0	85.0	119.0	77
Akiriamasit	6.6	19.0	73.0	65.0	138.0	76
Susceptible Kengatuny	0.0	5.9	1.5	7.0	8.5	58
Resistant Rwatama	3.2	21.6	5.0	4.0	9.0	93
Overall	4.74	17.0	25.2	35.0	60.2	79

Odioi cluster from Teso South had the highest vector density at 18 female mosquitoes per house during human landing and pyrethrum spray catches and outdoor pot collections (Table 4.9). Rwatama cluster vector density was significantly different from the susceptible Kengatunyi cluster [$t(8) = 4.9543$, $p < .05$). Highest biting rate was recorded in resistant Odioi cluster and lowest in susceptible Kengatunyi after human landing and pyrethrum spray catches.

Table 4.9: Vector densities and biting rates of female *Anopheles* mosquitoes in selected clusters, Teso sub counties Western Kenya

Cluster	Vector density	Biting rate	SS frequency (%)
Kaliwa	14	3.6	92
Odioi	18	7.6	77
Akiriamasit	17	4.5	76
Susceptible Kengatunyi	4	1.4	58
Resistant Rwatama	13	3.6	93

*Vector density = number of vector species divided by number of houses sampled
*Biting rate = number of mosquitoes per person

4.3.2 Impact of insecticide resistance on abdominal status and vector infectivity

The clusters with the highest proportions of fed; unfed and gravid are Akiriamasit, Odioi and Rwatama respectively (Table 4.10). The susceptible cluster Kengatunyi had a higher proportion of unfed while the resistant cluster Rwatama had the higher proportion of fed and gravid female mosquitoes. Exiting mosquitoes were mostly unfed. There was a significant difference between the mean proportions of sporozoites rate on fed, unfed, gravid abdominal statuses of mosquitoes collected per cluster [$F(3, 16 = 13.781, p < 0.05)$].

Table 4.10: Percentages of abdominal statuses of female mosquitoes as per cluster, method of collection and sub counties sampled from Teso North and Teso South sub counties in western Kenya between 2012 and 2014

Clusters	n	Fed	Unfed	Gravid	Sporozoite rate (%)
Kaliwa	266	6.2	49.4	44.4	12.9
Odioi	76	8.2	74.3	17.5	6.0
Akiriamasit	379	19	48	33	6.9
Kengatunyi	561	6.6	63.2	30.2	7.8
Rwatama	258	9.2	31.5	59.3	28.1
Mean		9.84±5.2619	53.28±16.2599	36.88±15.7675	12.34±9.2072
Methods of collection					
PSC	238	32.5	24.4	43.1	16.0
WET	699	1	62.9	36.1	14.2
HLC	603	0	53.7	46.3	6.5
Sub counties					
Teso North	819	1.5	39.7	58.8	17.95
Teso South	721	11.2	60.5	28.3	8.6

The susceptible malaria vectors had the largest proportion of fed, unfed and gravid (Table 4.11). Resistant Rwatama had higher proportions of gravid and fed while susceptible Kengatuny cluster had the higher percentage of unfed. The Abdominal status is significant at F-Value = 10.73, DF=2, in the genotypic KDR status model at < .05 level of significance.

Table 4.11: Abdomen status and sporozoite rates among malaria vectors in resistant and susceptible clusters in Teso North and Teso South sub counties, Busia county, Western Kenya

	Fed	Unfed	Gravid	Sporozoite rate	DF	ρ value
Susceptible						
Kengatunyi	6.60	63.20	30.20	7.8	258	<.0001
Resistant						
Rwatama	8.8	21.20	59.50	28.1	561	<.0001
Akiriamasit	19	48.00	33	6.9	379	0.0223
Odioi	8.20	74.30	17.50	6.0	563	0.6496
Kaliwa	6.20	49.40	44.40	12.9	380	<.0001
Overall	10.60	55.70	33.70	12.3	2141	

Among susceptible (LL) and heterozygous resistant (LS) female mosquitoes, most of them were unfed and fed, respectively. Female mosquitoes carrying the homozygous susceptible genotype (LL) had lower sporozoite rate (4.2%) than homozygous resistant SS containing ones (27.5%) (Table 4.12). There was significant difference between genotypic *kdr* status and sporozoite rate [$F(1) = 14.59$, $p < .05$].

Table 4.12: Abdomen status and sporozoite rates among malaria vectors after *Kdr* PCR

	FED (%)	UNFED (%)	GRAVID (%)	n	SPOROZOITE RATE (%)
LL	6.10	15.70	8	71	4.2
SS	84.80	75.20	86	435	27.5
LS	9.10	6.10	6	34	5.3

4.3.3 Human blood index in different study clusters

Higher resistance clusters had higher proportions of female anopheles mosquitoes which fed on human (Figure 4.4). Therefore, insecticide resistance was directly proportional to human blood index ($\hat{y} = 1.23259X - 15.21625$). Overall, 79% of female blood fed mosquitoes assayed for blood meal source was positive for human blood. There was no significant difference between human blood mean indices and means of SS proportions in the five clusters in Teso north and Teso South sub counties[$F(1, 8) = 0.1053$, $p > 0.05$].

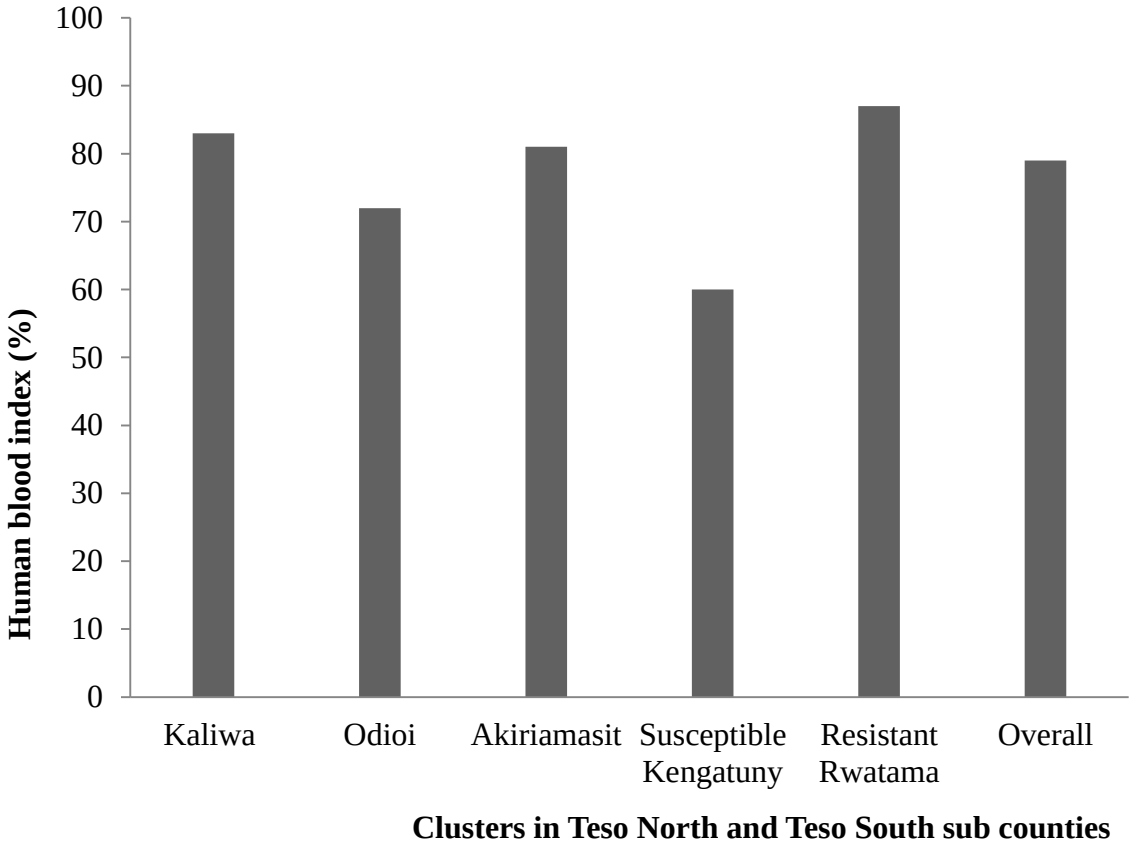


Figure 4.4: Proportion of blood fed female mosquito samples containing human blood per cluster

Among the homozygous resistant vectors, 80% of them were human blood feeders (Figure 4.5 & Appendix 12). All heterozygous resistant female mosquitoes had imbibed human blood while proportion of human blood feeders and non-human blood feeding homozygous susceptible mosquitoes were 50:50. Resistant mosquitoes are more of human blood feeders than susceptible ones.

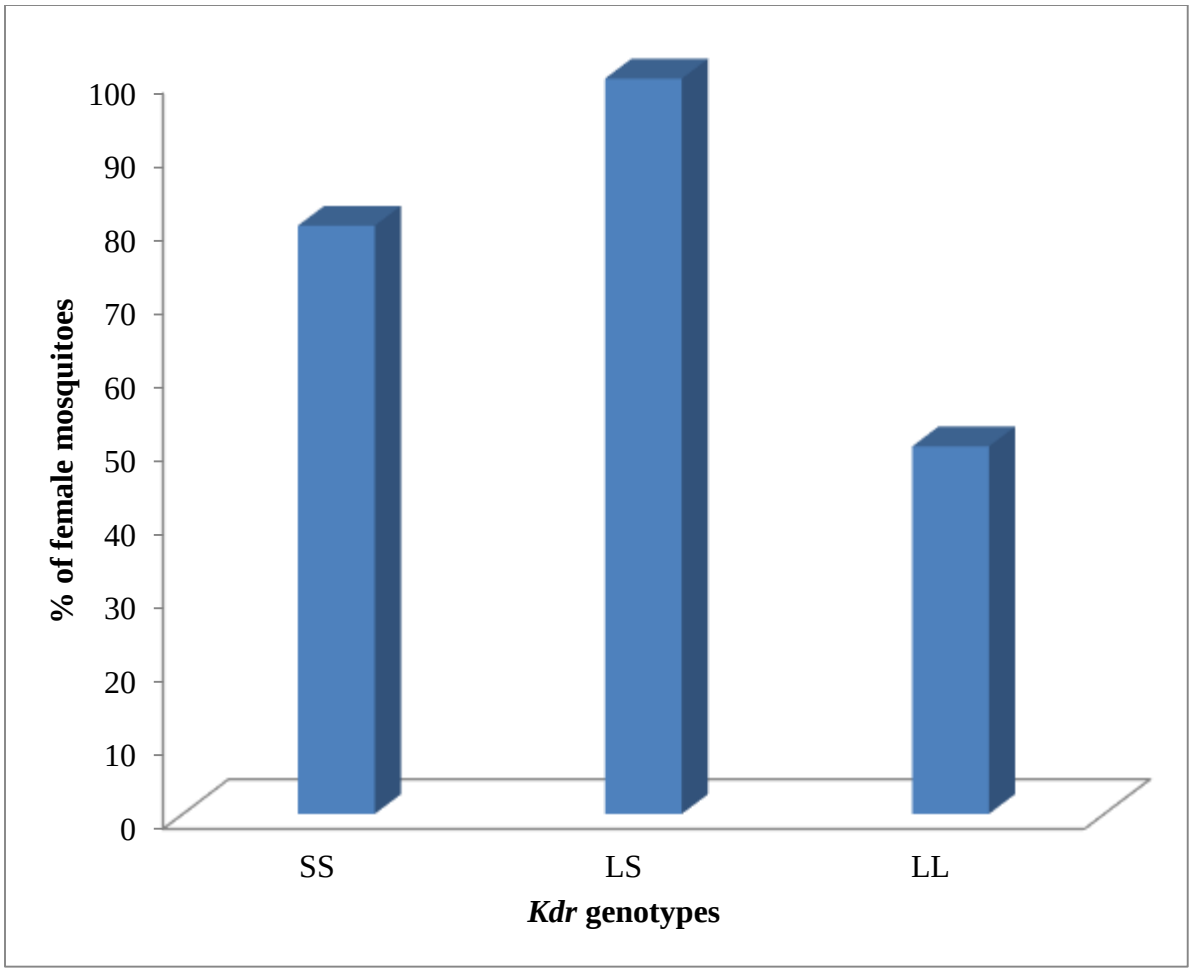


Figure 4.5: Human blood indices in each group of *Kdr* genotype in mosquito samples collected in Teso North and Teso South sub counties

Among the female mosquitoes found to have fed on human blood, 83% were homozygous resistant while 17% comprised of both heterozygous resistant and homozygous susceptible (Figure 4.6). Therefore successful human blood meals were significantly higher among resistant vectors and significantly lower among susceptible female group $F[(1,10) = 4828$, $p<0.05$). Heterozygous allele carrying mosquitoes were more effective in acquiring a human blood meal than the homozygous susceptible ones.

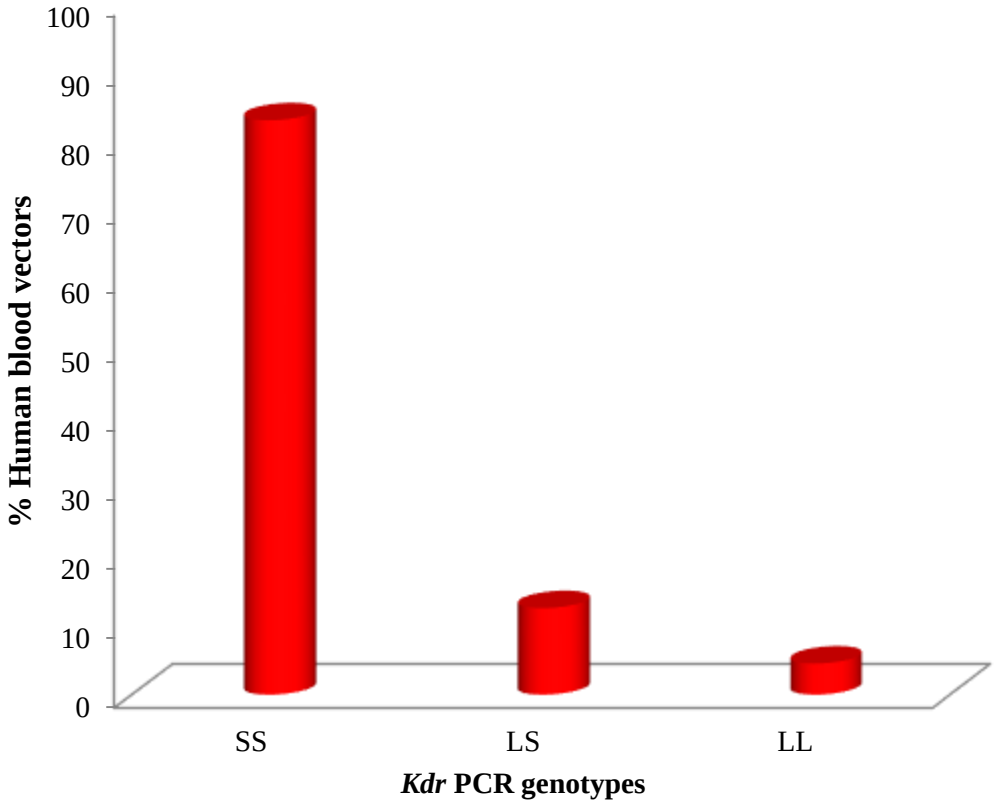


Figure 4.6: Comparison between human blood fed mosquito proportions in various allelic groups

4.3.4 Entomological inoculation rates

Resistant Kaliwa and Rwatama clusters had the highest number of infectious bites per individual per night (Table 4.13 & Appendix 12). Monthly and annual EIR were also extrapolated from the daily entomological inoculation rates. Susceptible Akiriamasit and Kengatunyi clusters had the lowest entomological inoculation rates.

Table 4.13: Entomological inoculation rates (EIR) in different clusters in Teso North and Teso South sub counties in Busia County, Western Kenya

Cluster	Daily EIR	Monthly EIR	Annual EIR
Kaliwa	0.4	12	146
Odioi	0.06	1.8	21.9
Akiriamasit	0.02	0.6	7.3
Susceptible Kengatuny	0.03	0.9	11.0
Resistant Rwatama	0.4	12	146

4.4 Impact of insecticide resistance on vector feeding and resting behaviour

Both vector feeding and resting behaviour were investigated in regions with varying intensities of insecticide resistance. Biting patterns and host preference at different insecticide resistance levels were used to determine mosquito feeding behaviour.

4.4.1 Biting pattern

Both indoor and outdoor vectors exhibited a unimodal biting pattern with heightened biting attempts at around 3am to 5am (Figure 4.7). Dawn biting rate at 6am (11.1 bites per hour) was higher than at dusk biting rate at 6pm (0.5 bites per hour). Outdoor collections were higher (331 female mosquitoes per night) than indoor collections (237 female mosquitoes per night). Indoor biting rate (10.8 bites per hour) was higher before midnight than outdoor biting rate (8.7 bites per hour) while outdoor biting rate (38.5 bites per hour) was higher after midnight than indoor biting rate (23 bites per hour). Indoor or outdoor biting rate had significant difference to both phenotypic resistance levels [$F(1) = 128.44$, $p < .05$] and geotypic *kdr* status [$F(1) = 5.86$, $p < .05$].

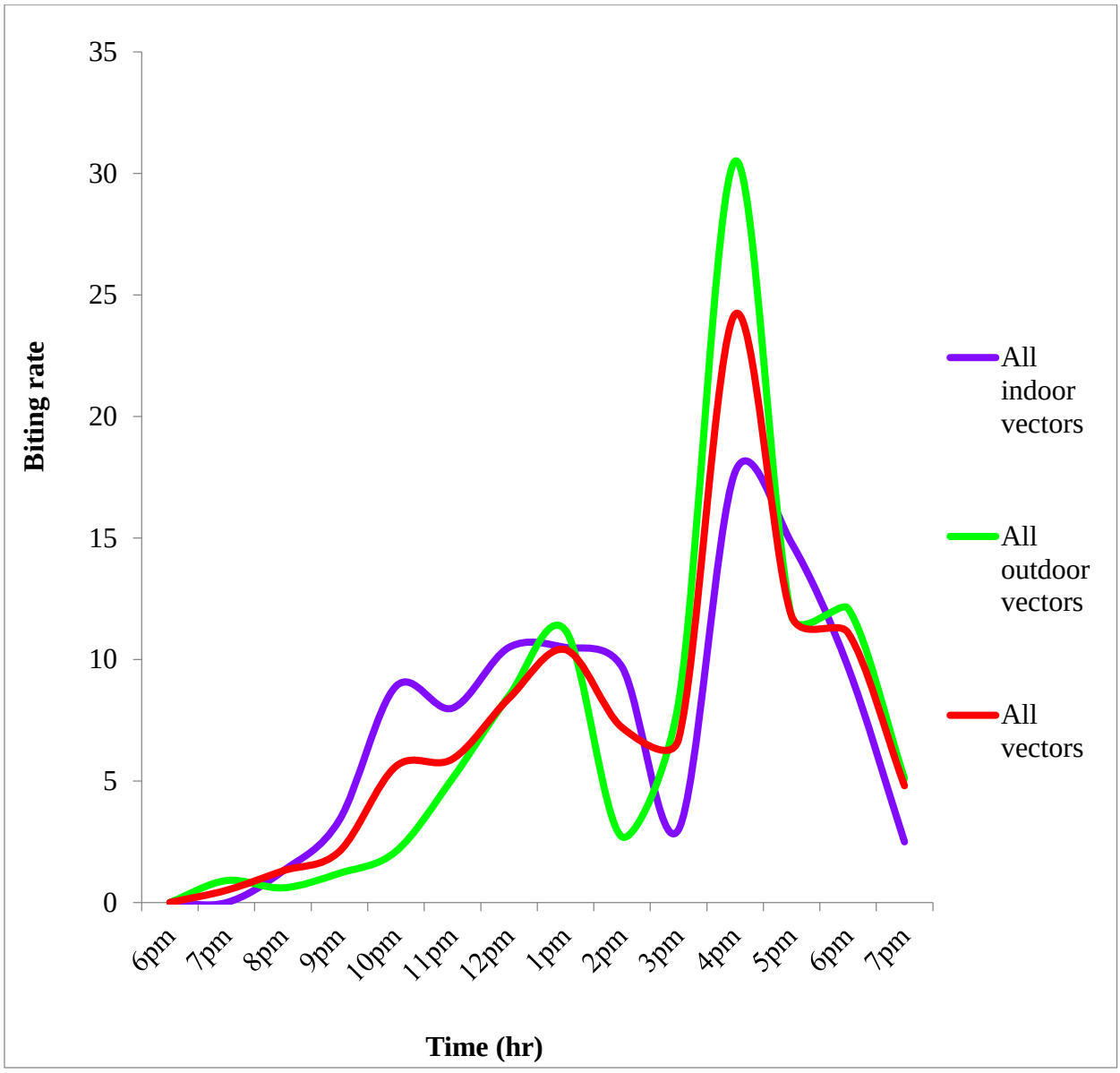


Figure 4.7: Biting patterns of both outdoor and indoor malaria vectors in Teso North and Teso South subcounties in Busia county, western Kenya.

Both the resistant Rwatama & Kaliwa and susceptible Kengatunyi & Akiriamasit mosquitoes had a bimodal biting patterns (Figure 4.8). Clusters with lower levels of resistance were more active in biting before mid night than after midnight while clusters with higher levels of insecticide resistance were more active in biting after midnight than before midnight. Biting rates for both resistant and susceptible were higher at dawn than at dusk.

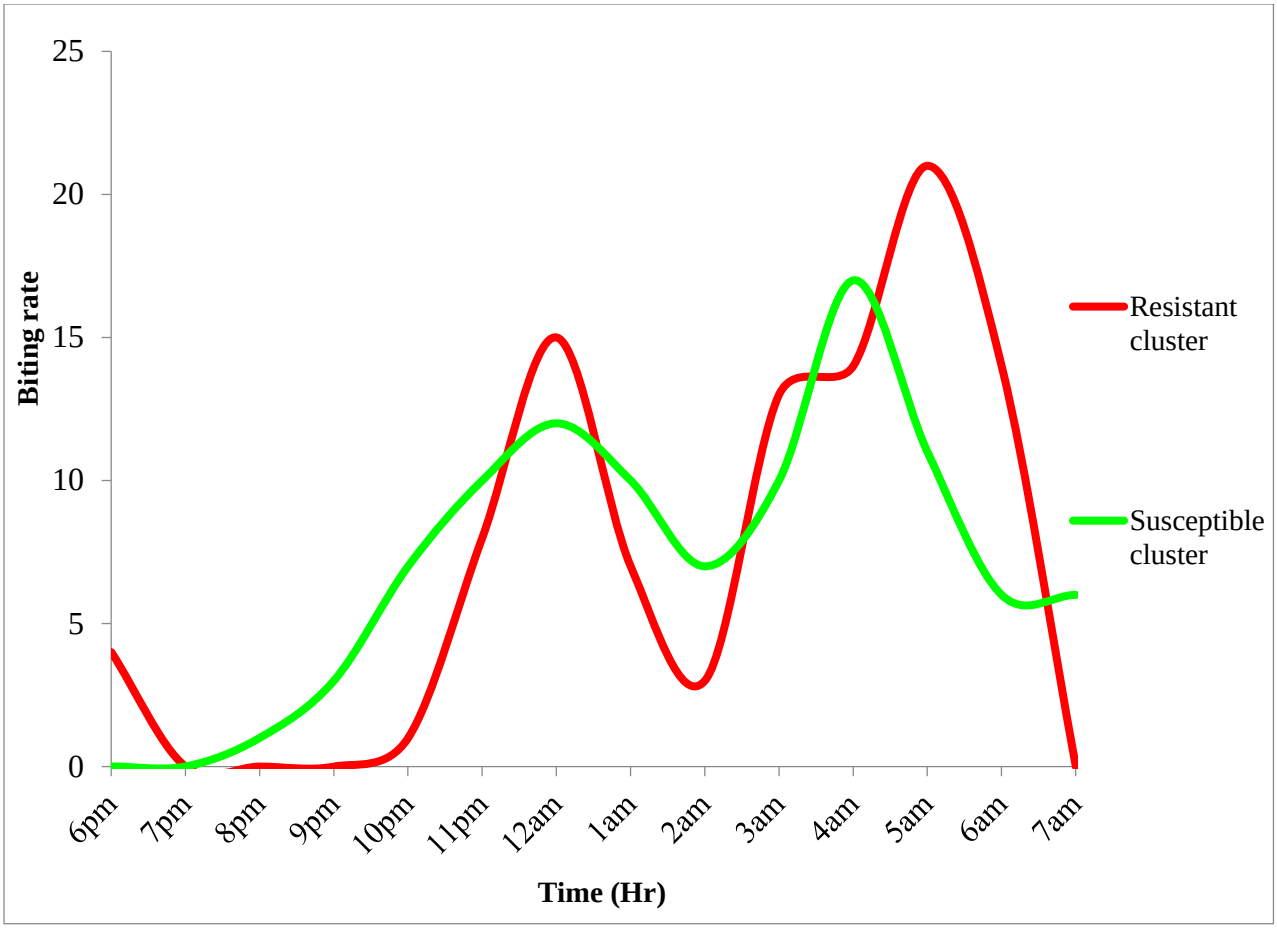


Figure 4.8: Biting trends among *Anopheles* mosquitoes from resistant and susceptible clusters in Teso North and Teso South sub counties, Busia County, western Kenya

Homozygous SS allele carrying vectors had an unimodal 5pm to 7am biting pattern, heterozgous LS mosquitoes had a bimodal biting pattern while recessive LL *Anopheles spp* had a multimodal biting aptterns with sharp flactuations from hour to hour (Figure 4.9). Susceptible vectors were earlier biters than the heterozygous LS and homozygous SS biters. Heterozygous LS female mosqitoes maintained the all time 10pm peak biting hour. All SS, LS and LL carriers were biting at dawn.

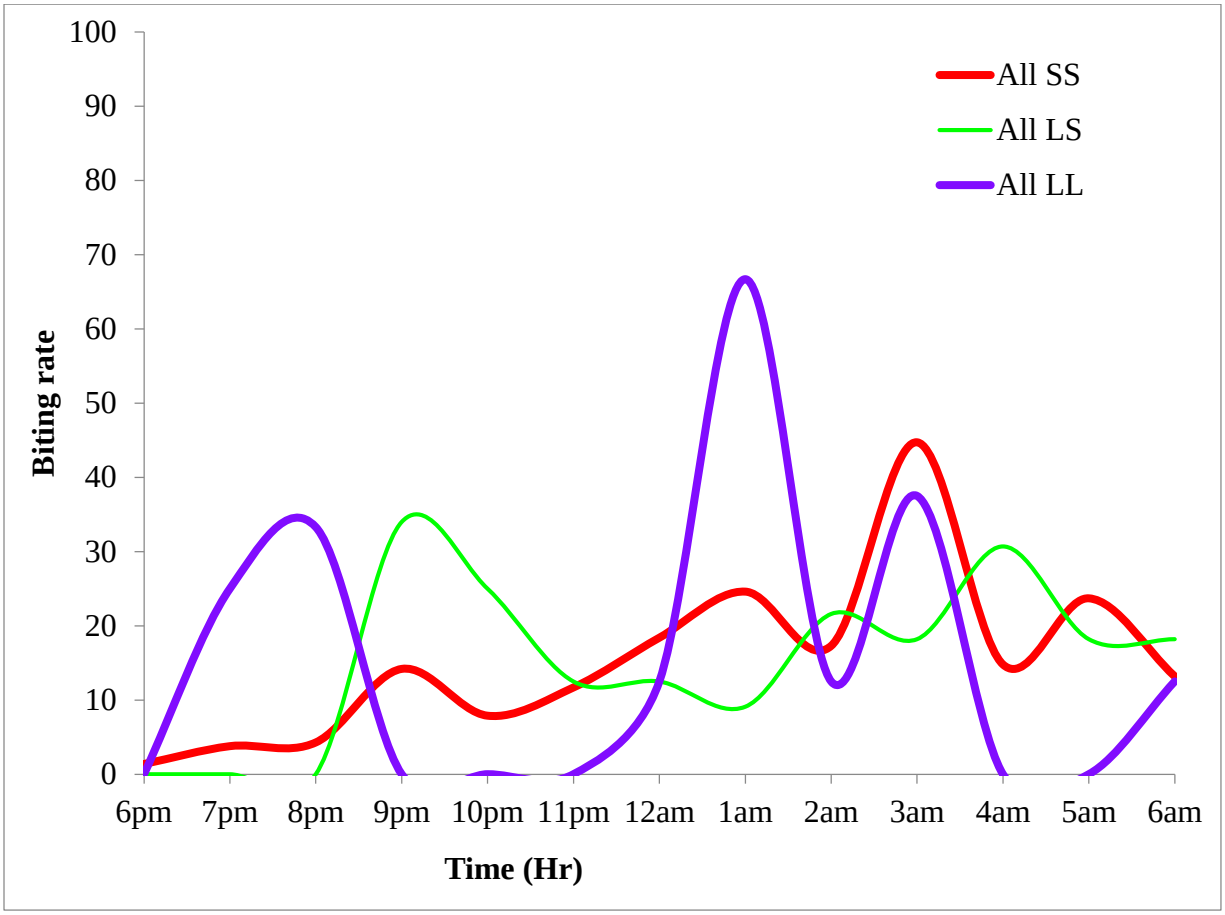


Figure 4.9: Biting trends in different *Kdr* genotypic frequencies of *Anopheles* mosquito in Teso North and Teso South sub counties in Busia County, western Kenya

4.4.2 Host preference for a blood meal source per study site cluster

Host preference in the study site differed in terms of clusters, species and frequency of resistance alleles.

Human blood meal remained the most preferred (Figure 4.10) by female Anopheles mosquitoes. The highest numbers of human blood fed Anopheles mosquitoes were caught in the cluster with highest insecticide resistance while lowest proportion of human blood fed mosquitoes were caught in the cluster with lowest resistance to the insecticides. None of the analysed blood meals had been sourced by the mosquitoes from dog, cat, donkey and chicken. The low resistance cluster had highest number of mosquitoes that had fed on bovine blood. Pig's blood was also a preference among female mosquitoes collected in another low resistance cluster by the name Akiriamasit.

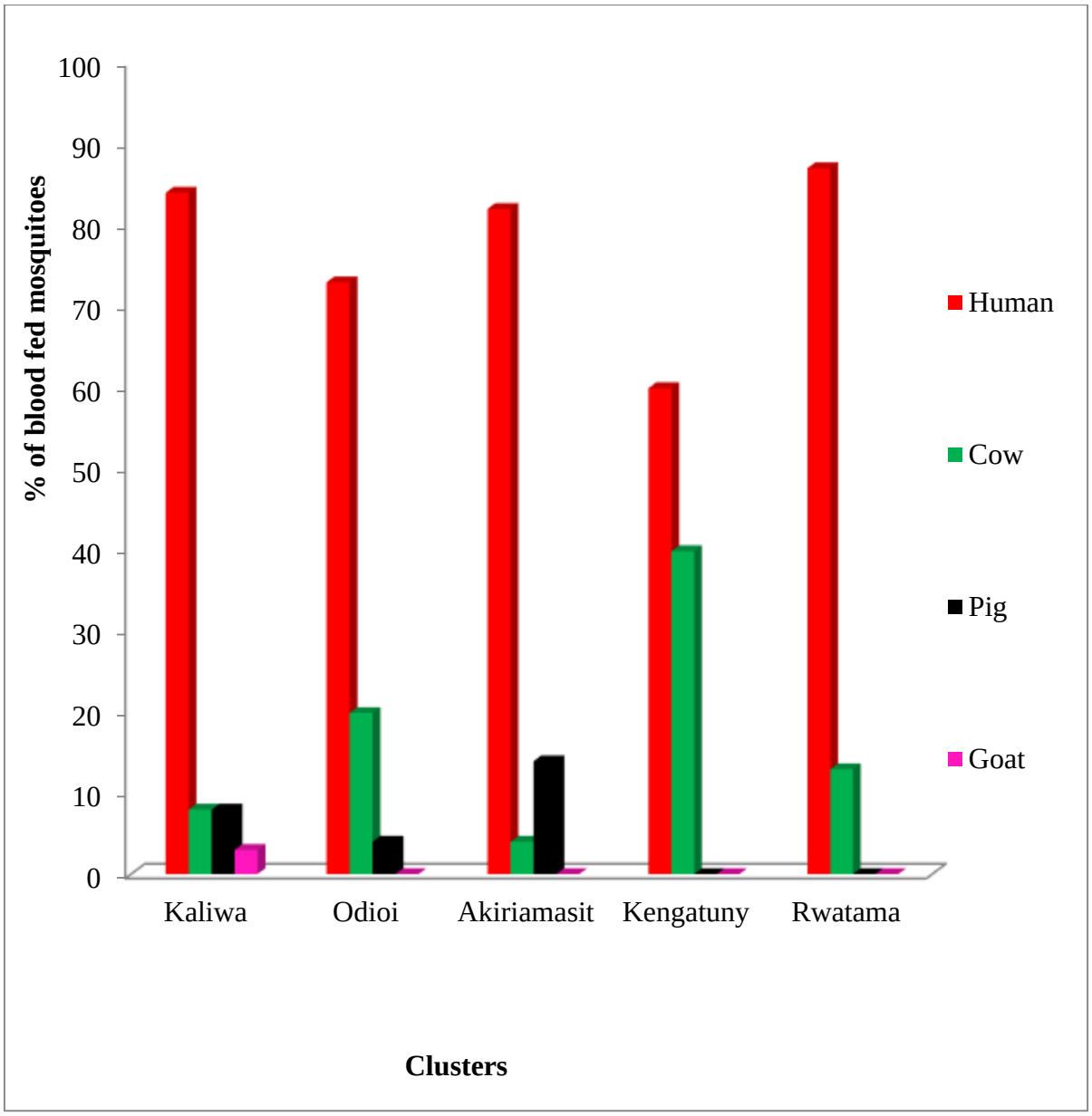


Figure 4.10: Host blood preferences in different clusters in Teso North and Teso South sub counties, western Kenya

4.4.3 Specific mosquito species host preference for a blood meal source

An. gambiae had the highest preference for human blood (71.9%) (Figure 4.11). An almost equal preference for human, cow and pig's blood was found out among *An. Arabiensis* [F (2, 12) = 28.2222, p < .05]. The cow's blood meal source was the most preferred among *An. funestus* [F (2, 12) = 5.1429, p < .05]. All the identified species had zero preference for dog, cat, donkey and chicken blood meal sources. The blood meal and phenotypic resistance model is significant at F-Value = 4.41, DF=3, Pr > F= 0.0015 at 0.05 level of significance.

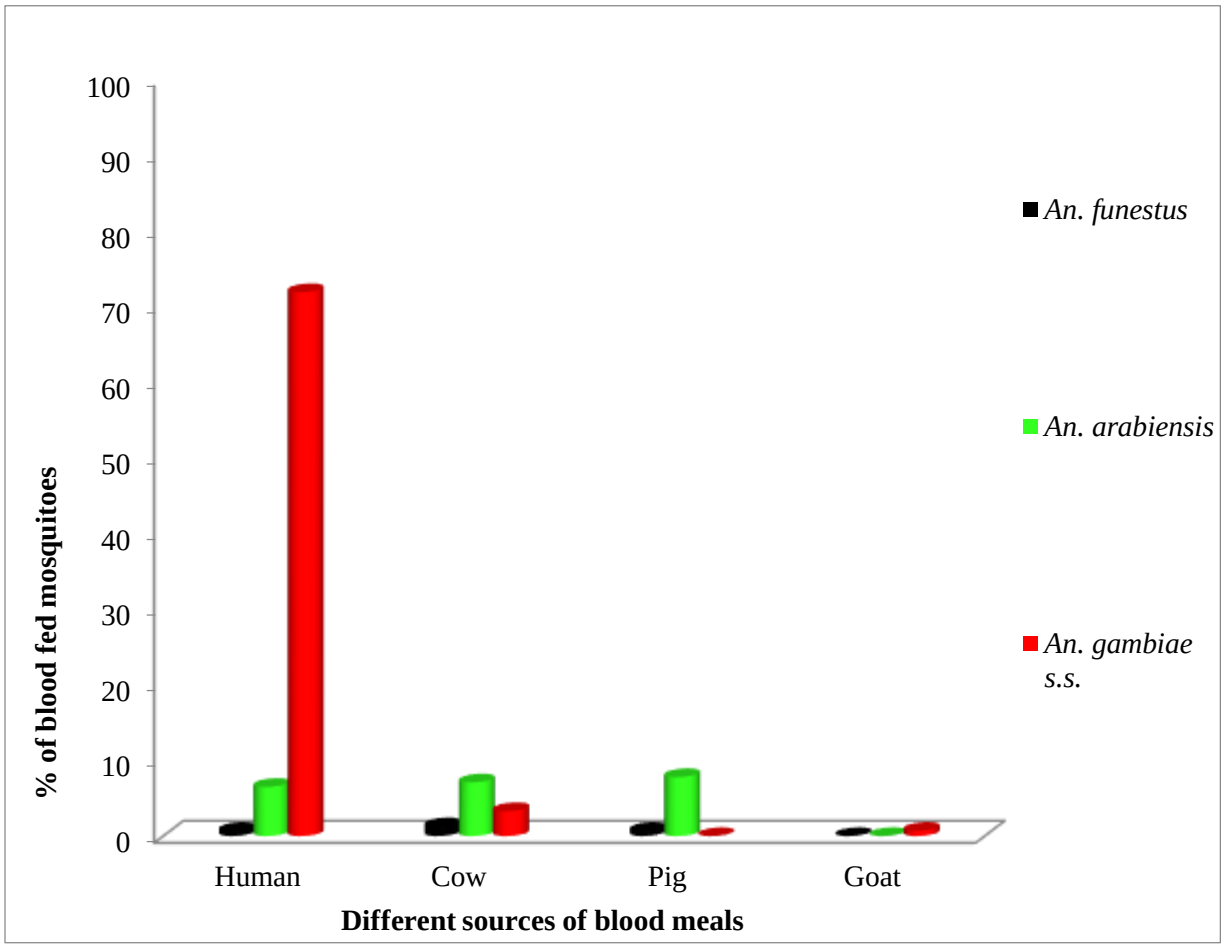


Figure 4.11: Species preference for different blood meal sources in mosquitoes collected in Teso North and Teso South sub counties, western Kenya

4.4.4 Frequency of resistance alleles compared with blood meal source

Susceptible LL mosquito vectors had equal preference for both human (3%) and bovine blood meals (3%) (Figure 4.12). Heterozygous LS allelic female mosquitoes had a greater attraction for human blood meal (10%) than cow's (0%). Mosquito carrying resistance SS alleles had a greater attraction for human blood meal (67%) rather than bovine blood (17%).

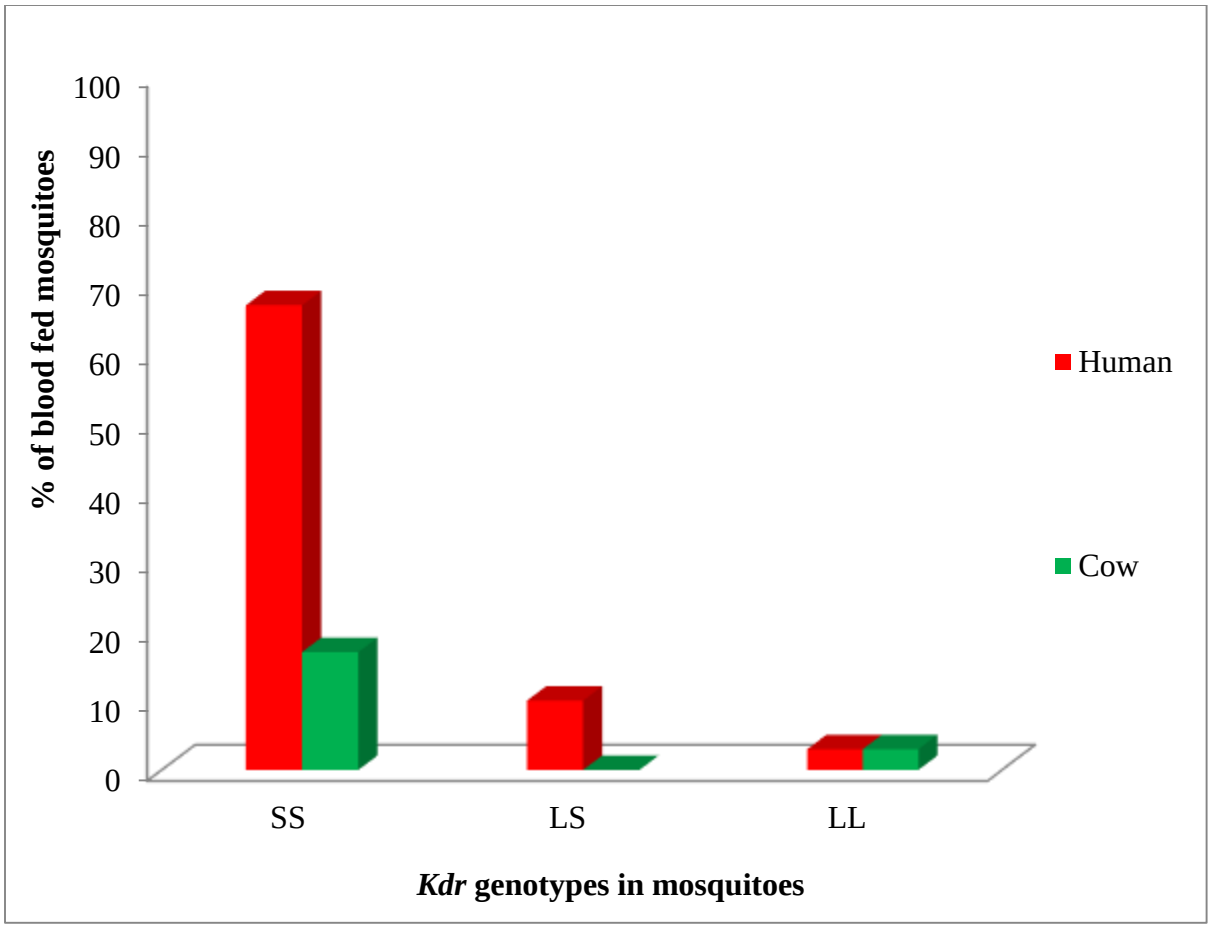


Figure 4.12: *Kdr* genotypic frequencies compared to host preference for *Anopheles gambiae* collected in Teso North and Teso South sub counties, Busia county, western Kenya

4.4.5 *Kdr* frequency and female mosquito indoor - outdoor feeding behaviour

The homozygous and heterozygous resistant allele carrying female mosquitoes were more exophagic than endophagic while resistant allele carrying female mosquitoes were more endophagic than exophagic (Figure 4.13). Resistant mosquitoes had the highest proportions both indoors (86.4%) and outdoors (78.3%).

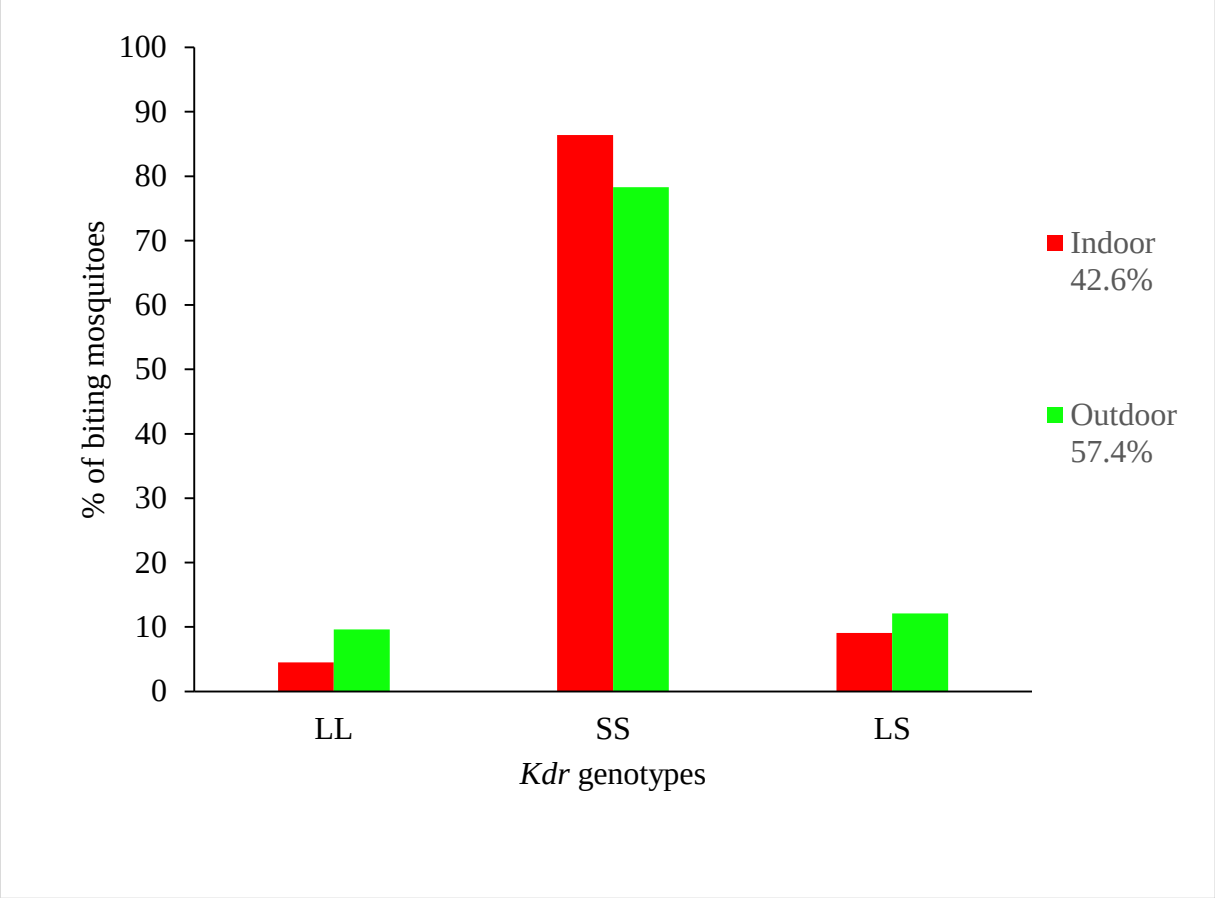


Figure 4.13: Impact of insecticide resistance on indoor and outdoor biting patterns of human blood meal seeking *Anopheles* mosquitoes in Teso North and Teso South sub counties, western Kenya; Human Landing Catch (HLC) (n = 605)

During dusk and dawn, there were lower proportions of homozygous resistant gene carrying female *Anopheles* mosquitoes (83.4%) as compared to 87.5% indoor human biters (Table 4.14). There were also more resistant exophagic *Anopheles* vectors (82.8%) at bed time than during non-bed times (66.7%). The percentage of homozygous susceptible and heterozygous resistant vectors seeking for a human blood meal was the same indoors (13.3%) during out of bed times of the day and outdoor (8.6%) during bed time hours of the night. There was a significantly higher proportion of outdoor female mosquito seeking human blood meal during bed time hours (82.8%) than during dusk dawn times (66.7%) [$t(4,4) = 4.4011$, $p < .05$]. The vectors were more endophagic than exophagic during both 1700-2200/0500-0700hrs (83.4%) as well as 2200-0500hrs (87.5%).

Table 4.14: Impact of insecticide resistance on bed and non-bed time hours' human biting patterns in indoor and outdoor environments in Teso North and Teso South sub counties, Busia county, Western Kenya

		Proportion of *kdr* Genotypes (%)		
Time (hrs)	Moquitoes collected Inside or Outside the house	LL	SS	LS
1700-2200/0500-0700hrs (7hrs of non-bed time)	Indoor	13.3	83.4	13.3
	Outdoor	12.5	66.7	20.8
2200-0500hrs (7hrs of bed time)	Indoor	5.4	87.5	7.1
	Indoor	8.6	82.8	8.6

*HLC (n = 605)

4.4.6 Insecticide resistance and Anopheles resting behavior

Pyrethrum spray catch which targets mosquitoes resting inside the houses showed that 100% of female mosquitoes caught in higher resistance cluster were *Anopheles gambiae* s.s while no vector species were found indoors in lower resistance cluster (Table 4.15). In the higher resistance cluster, 85.2% female *Anopheles gambiae* s.s. were exiting during the night either after feeding, flying and / or resting indoors. Higher proportions of *Anopheles arabiensis* were exiting in lower resistance cluster than in higher resistance one. *Anopheles funestus* were found only in higher resistance area resting outdoors during the day either in cowsheds or outdoor pots in higher proportions than the two *Anopheles arabiensis* and *Anopheles gambiae* s.s.

Table 4.15: Percentages per method of collection of *Anopheles spp* in higher resistant cluster as compared to more susceptible cluster in in Teso North and Teso South sub counties, Busia county, Western Kenya

Cluster		Higher resistance area			Lower resistance area		
Collection method	n	*Anopheles gambiae* (%)	*Anopheles arabiensis* (%)	*Anopheles funestus* (%)	*Anopheles gambiae* (%)	*Anopheles arabiensis* (%)	*Anopheles funestus* (%)
PSC	338	100	0	0	0	0	0
WET	722	85.2	14.8	0	75	25	0
OPC	36	25	25	50	0	0	0
HLC	605	87.3	10.6	2.1	79.1	20.9	0

Proportion of resistant female *Anopheles* mosquitoes which were resting inside the houses was the highest while homozygous susceptible allele carrying mosquitoes made up 11.1 % (Figure 4.14 & Appendix 11). There were no heterozygous resistant female mosquitoes feeding and resting indoors.

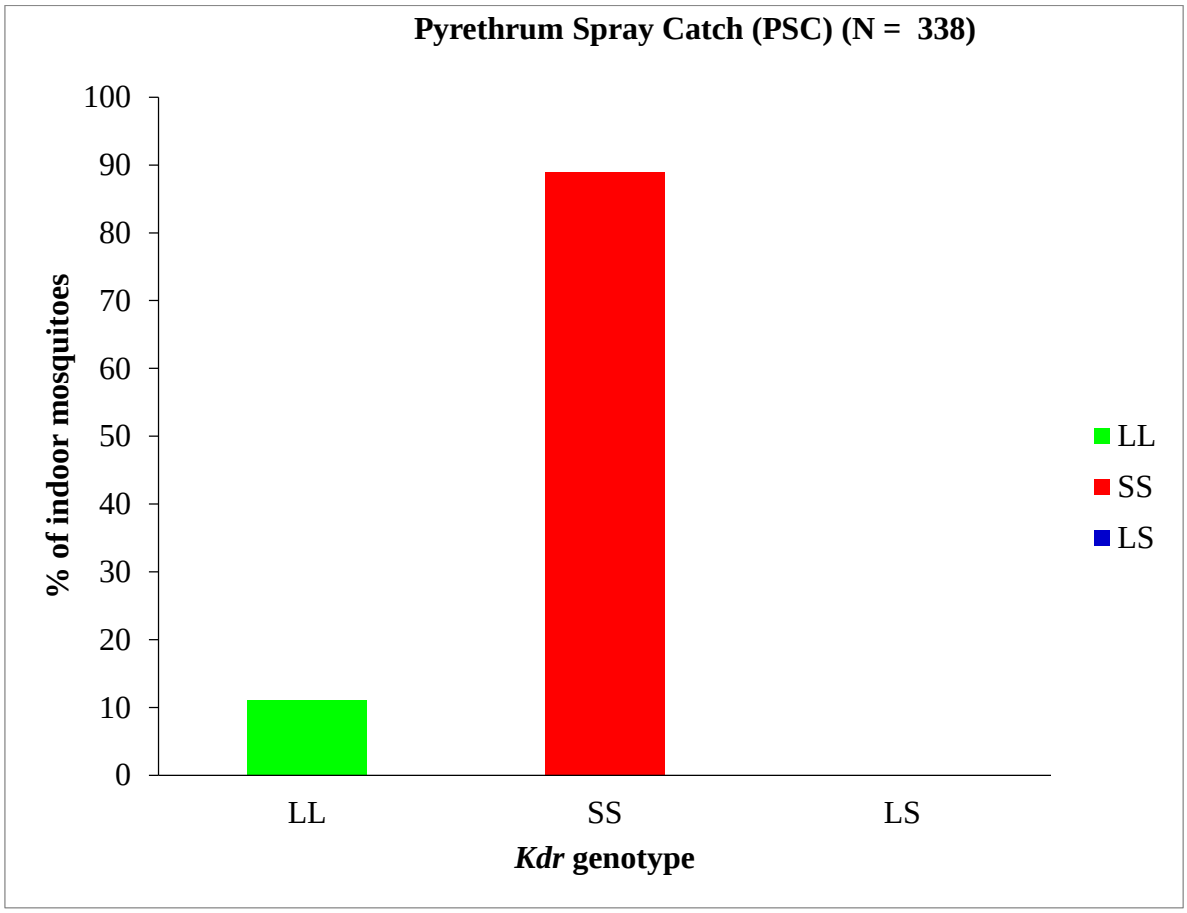


Figure 4.14: Impact of insecticide resistance on endophagic and endophilic behavior in female *Anopheles* mosquitoes in Teso North and Teso South sub counties, Western Kenya

Homozygous resistant malaria vectors feeding indoors and exiting through the widow were the highest in proportion followed by the homozygous susceptible mosquitoes while the least were heterozygous resistant (Figure 4.15 and Appendix 11).

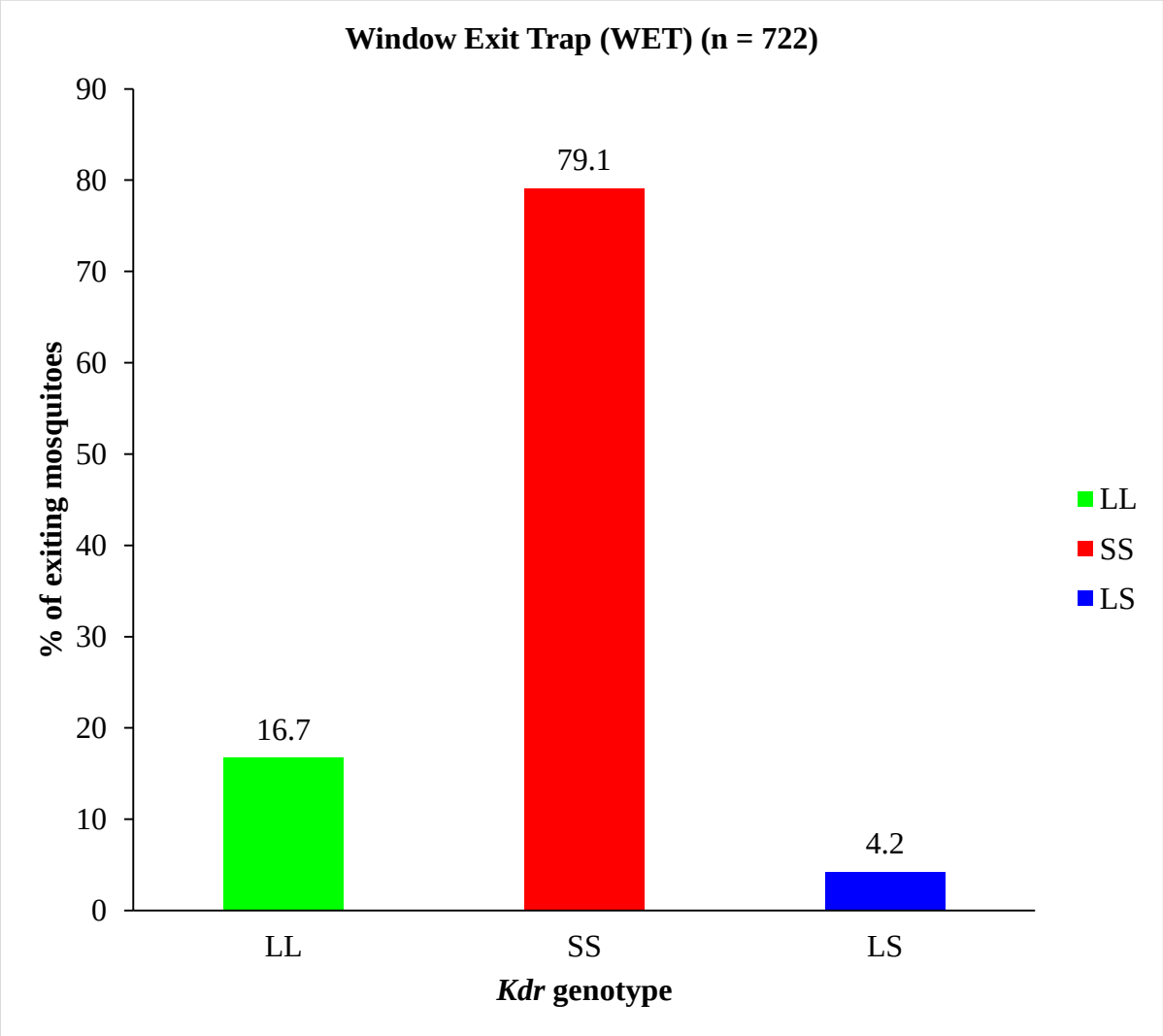


Figure 4.15: Impact of insecticide resistance on female *Anopheles* mosquitoes feeding indoors and resting outdoors in Teso North and Teso South sub counties, western Kenya

CHAPTER FIVE

5.0 DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

5.1 Discussion

5.1.1 Species composition and resistance levels

Anopheles gambiae being the main malaria vector in Teso north as well as south sub counties is very unique as several studies indicate that *Anopheles arabiensis* is the major malaria mosquito inside the Lake Victoria basin (Thongwat & Bunchu, 2015). These malaria vectors breed in various environments stretching from short-term rain ponds to water bodies that are permanent. *Anopheles gambiae* breeds in temporary fresh shallow recently ploughed water pools and hoof prints with clean water. The study showed that *Anopheles funestus* and *Anopheles arabiensis* are the minor *P. falciparum* vectors in Teso subcounties.

Apart from susceptibility to insecticidal agents in the environment, a number of factors such as anthropogenic undertakings, for example development projects also govern distribution of vector species in East Africa. Further, climate-related atmospheric conditions, predominantly rainfall as well as temperature, has been considered as the dependent of habitations which cause varying vector distribution and abundance between high and low attitude areas. Distribution of vectors and parasites is influenced by the movement and migration of people from high land to low land (Ebenezer *et al*., 2014). Furthermore, density and distribution of vector in all regions has largely been influenced by topography. Therefore, the density and distribution of proficient vectors have caused the ardent use of intensive interventions and control tools through out the sub-region hence subsequent selection pressure.

A higher bulk of resistance alleles came from *An. gambiae* as opposed to *An. arabiensis* and *An. funestus* since the susceptible Kengatunyi cluster had higher proportions of *An. arabiensis*

than the resistant Rwatama cluster. The rate of genes selecting for non-vulnerability to insecticidal chemicals depends on the species incriminated in a given area. *Anopheles gambiae* was resting and feeding indoors hence greater exposure to longlasting insecticidal nets having pyrethroids on them. Most *An. arabiensis* and *An. funestus* were resting outdoor thus minimal exposure to insecticidal nets. But still exophillic *An. arabiensis* and *An. funestus* had traces of resistance genes possibly due to the residual effect of insecticidal air emanating from air flow obstructing pyrethroid treated nets or from agropesticides and domestic chemicals which contains insecticides as active ingradients (Bradley *et al*., 2015). Because of its pressure of the vapor (1.5×10^{-8} mmHg at 25 °C), deltamethrin has a low capability to volatilize hence possibly lower selection pressure as compared to permethrin (2.5×10^{-8} mmHg) (Appendix 9). This may tend to explain why female mosquitoes were more vulnerable to deltamethrin than permethrin. (Strode *et al*., 2014b) reported forced exophily, that is, premature escape of mosquitoes from the hut, deterrence, and knock-down by pyrethroid treated bed nets. Depending on the type of insecticide used on nets and mosquito species, effects range from reduced house entry, reduced blood feeding success to greater likelihood of house exit (Mathenge *et al*., 2001).

Deltamethrin was more lethal and quicker in knocking down female mosquitoes than permethrin. Both Deltamethrin and permethrin may be used in longlasting insecticidal nets while only deltamethrin can be exploited inside the house residual jetting for it has a longer indoor half life. Pyrethroids are man-made biotic composites made from *Chrysanthemum* flowers that are widely utilized as commercial and household insecticidal agents. Since the pyrethroic as well as keto-alocoholic esters of chrysanthemic acid are lipophilic, they account for the insecticidal components through easy penetration into the insect body and immediate

induction of toxicosis (Rehman *et al*., 2014). Pyrethrins contain active insecticidal extracts and dusts, with an active component of approximately 30%. Pyrethrin-I and pyrethrin-II are the most known types of pyrethrins. The pyrethrins further possess four dissimilar active elements, jasmolin I and II and cinerin I and II (Appendix 9). The primary use of pyrethrin elements is to kill cockroaches, human lice, beetles, mosquitoes, as well as flies. Several "pyrethrin dusts", used to regulate insects in horticultural farming, are merely 0.3% - 0.5% pyrethrins, and are utilized at the ratio of up to 22.7 kg / acre (Rehman et al., 2014). Usage of other pyrethrin chemical agents aims at killing fleas and rice in poultry pens, on cats and dogs and warehouse weevils and beetles during storage of grains.

Natural pyrethrins are toxins which speedily infiltrates the insect's nerve system whenever it comes into contact (Rehman *et al*., 2014). Some minutes after the insecticide has been applied, the insect cannot fly away or move hence the knockdown dose. However, a "knockdown dose" may not necessarily kill as insect enzymes rapidly detoxify the natural pyrethrins. Some pests recover as a result. Carbamates, organophosphates, or synergists such as piperonyl butoxide (PBO) are combined with the pyrethrins as way of delaying the enyme action, and thus a lethal dose is ascertained (Aïzoun *et al*., 2013).

Results indicated that knock down rates in permethrin were different from deltamethrin. Knock down rates in samples collected during dry season were also significantly different from the ones collected during wet season. Pyrethroids take effect on nerve cell membranes by deferring the closing of the stimulation gate for the sodium ion channel. Type II pyrethroids, as well as deltamethrin; have α-cyano groups that prompt a "long-lasting" obstruction of the sodium channel activation gate. This leads to extended penetrability of the neurone to sodium and generate a chain of recurring nerve signals in sensory nerves, sensory organs and muscles.

According to studies, researchers found out that deltamethrin and other Type II pyrethroids may also affect ions passages in the nervous system apart from sodium passages, probably because of their phosphorylation nature (Antonio-Nkondjio *et al*., 2015; WHO, 2012). Deltamethrin is potent against insects through direct contact and ingestion. Deltamethrin's working principle is considered to be primarily central in action, or at least initiated from the brain. Once poisons lasts for a few hours in the insects body, the damage that the nervous system suffers is irreversible, this consequently leads to death of insects.

Physiological dysfunction or cellular injury is the immediate effect of amplified free radical concentration in the insect's body. The two sources of reactive oxygen species (ROS) and reactive nitrogen species (RNS) free radicles includes endogenous sources (endoplasmic reticulum, phagocytic cells, mitochondria, peroxisomes, etc.) and exogenous sources (heavy metals, pollution, industrial solvents, alcohol, tobacco smoke, transition metals, pesticides, certain drugs like paracetamol, halothane, and radiation). Free radicals can negatively affect a number of vital groups of biotic molecules for example, proteins, nucleic acids and lipids hence changing the standard redox rank resulting to enhanced oxidative stress. Endogenous is the primary source of reactive oxygen species (ROS) as well as organic free radicals, predominantly through bioactivation and metabolism of xenobiotics (such as a carcinogen, drug or pesticide) or the reactivity of the existing parent compound.

Numerous compounds including insecticides and pesticide have been identified to cause free radical production and have the capability to facilitate these kinds of injuries. The destruction to protein, membrane lipids, and DNA is the termination point biomarker of oxidative stress-inducing consequences of insecticides and pesticides. Therefore, the amounts of membrane lipids and proteins which in turn may be determined by climatic patterns and or edaphic factors,

affect the knock down rates and patterns by any given insecticidal agents. Among pyrethrins, allethrin is the least poisonous; deltamethrin is the most toxic to aquatic and terrestrial organisms; fenvalerate, permethrin and cypermethrin are intermediarily lethal pyrethroids (Awolola *et al*., 2007; IRAC, 2012; Rehman *et al*., 2014). Exposure to cessation products is not a problem since deltamethrin is chemically steady (Appendix 9). It has a low vapour pressure (2.0×10^{-6} Pa or 1.5×10^{-8} mm Hg) and is regarded to be substantially non-volatile; breathing hazards are therefore expected to be minimal. It is lipophilic (log Po/w 5.43) and insoluble in water but it is readily soluble in organic solvents hence more portent during rainy seasons when lipid and protein contents in insects may be higher than during dry spells.

Chemical formula for permethrin is 3-phenoxybenzyl (1RS,3RS;1RS,3SR)-3-(2,2-dichlorovinyl)-2,2-dimethyl-) (Appendix 8). Permethrin bonds firmly to soil and is decomposed mainly by not only microorganisms, but also by photolysis. Sixty percent of the permethrin continued to be on an indoor surface alongside a window and open to daylight, after 20 days of exposure (Okumu *et al*., 2012). In water column, usual half-life range for permethrin is approximately 19-27 hours. Nevertheless, permethrin can last for more than a year when adsorbed to sediments. Permethrin affects the nervous system of insects making it hypersensitized to excitations from sense organs as nerves denuded to permethrin send series of impulses. This stimulation develops as a result of sodium ions being blocked by permethrin to move from outer to inner parts of the nerve cells. It obstructs sodium channels to disorder the role of neurons, and induce muscles to contract, ending in paralysis and demise. Permethrin's mechanism of action is through ingestion or contact, and can still function as a slight repellent due to its higher vapour pressure (Rehman *et al*., 2014).

Bendicarb, a carbamate caused 100% mortality rates even before the one hour of exposure to WHO insecticidal papers was over (Appendix 10). It works against an extensive variety of irritating insects and vectors of diseases. It kills wasps, mosquitoes, silverfish, fleas, flies, cockroaches, ants, ticks and the rest of the pests in households, commercial firms, and food stores. In agriculture it is effective against diverse insects, particularly those that inhabit the soil. Bendiocarb is as well used to treat maize seeds and sugar beets and against slugs and snails. Agrochemicals having bendiocarb are prepared as ultra- low volume sprays, dusts, wettable powders, and as granules. Bendiocarb is extremely poisonous if it penetrates the skin or if it is absorbed in the body. Industrial-grade bendiocarb is a non-corrosive, odorless, white crystalline solid matter (Appendix 10). Most at risk are persons exposed under environmental condtions of high humidity and temperature since such circumstances enhance speedy penetration of bendiocarb through the skin and thus a higher insect killing and selection pressure in hot times.

Bendiocarb interrupts the usual operational mode of the nervous system in an insect and may lead to poisoning after ingestion or contact (CNPI, 2002). The chemical neurotransmitter acetylcholine is discharged to transmit nervous system alerts across the nerve synaptic junction. Once the neurotransmitter is released into the junction, it is disassembled by the acetylcholinesterase enzyme, which is essential for nerve to function properly (CNPI, 2002). Acetylcholine builds up whenever there is suppression of the enzyme, leading to hypersensitization of the nervous system (CNPI, 2002). It is through addition of carbamyl moiety to the active spot of the acetylcholinesterase enzyme that bendiocarb is able to interupt the nervous system. As a result, it hinders acetylcholine from arriving at the active spot, hence inactivation of the enzyme. However, spontaneous hydrolysis is responsible for releasing the

carbamyl group, which consequently reverses the interruption and restores nervous coordination activity back to normal (CNPI, 2002). Cholinesterase, a crucial nervous system enzyme is normally prohibited by bendiocarb, a reaction which can be reversed.

Among all insecticides, pyrethroids are the only ones being utilizedto treat LLINs, due to their low toxic nature to humans. The other types of chemicals such as organochlorides, carbamates, organophosphates and pyrethroids are often effective during IRS. Due to environmental concerns and a significant number of malaria vectors having developed resistance to organochlorides especially DDT, a majority of East African nations have banned this group of insecticide (Kweka, 2016). Non-vulnerabilty of mosquitoes to different groups of insecticides can be attributed to several determinants (Kar *et al*., 2014; Kweka, 2016; Strode *et al*., 2014b). Firstly, hereditary determinants include the frequency and number of resistance alleles in the insect population, fitness costs and relative dominance of the characters. Secondly, biotic determinants such as life history parameters of the insect, initial population size and the fitness cost of the homozygous and heterozygous resistant phenotypes. Thirdly, reproductive considerations for example, rate of increase and fluctuations in population size of the mosquitoes. Fourthly, preceeding assortment with other insecticides, application techniques of the chemical, insecticide composition, proportion of the population exposed to selective dose, dosage of insecticide absorbed by insects under experiment and the age of the sampled mosquitoes as the operational considerations (Kweka, 2016).

Proper legislation and enactment of appropriate laws and regulations is needed for a country to switch from one agrochemical or public health chemicals to another. Non-vulnerability to insecticide by insect vectors is not a strange occurrence. It is a hereditary characteristic that can escalate in the vector community due to the heighteined non-vulnerability selection

pressure; an attribute that can swiftly proliferate. Permethrin mortality levels were higher during draught while deltamethrin mortality levels were lower during the same period. This means that meterological drivers such as wind, rainfall, temperatures, atmospheric pressure and humidity and edaphic factors namely drainage, parental rock material, soil type, soil texture and topography affects retension capacity of active compounds, insecticidal activities and eventually intensity of selection pressure forces on mosquitoes (Rehman *et al*., 2014; Roe, 2005).

Anatomical pliability and adjustments could have made adult female mosquitoes aged between 3 – 5 days (from reared field larvae) sampled during the dry season non-vulnerable, more so against deltamethrin in which main cause of damaging biotic free radicals as well as reactive oxygen species (ROS) is autogenous and not allocable to any extraneous or ecologically factor. Diminished rate of permeation of toughened exoskeleton could have caused decelerated knock down by deltamethrin. Deltamethrin has a higher capacity to vaporize from water due to its Henry's law constant of 1.2×10^{-4} atm·m^3/mol at 25 °C, compared with other pyrethroids (Appendix 9).

But permethrin excitation and hypersensitisation of nervous system by stimuli from sense organs was not affected by physiological plasticity or exoskeleton thickening. Cyano group in deltamethrin enables its prolonged endurance in the membrane, while permethrin can penetrate easily from the lipid bilayer with its lipophilic quality to easily access cellular subcompartments such as endoplasmic reticulum (ER) membranes which accommodate CYP450s, the iron source useful during DNA oxidizing hydroxyl radicles formation. Therefore the hydrophobic hydrophilic tendancies in the voltage-gated sodium channel and plasma membrane as well as in deltamethrin and permethrin chemicals may have had a bearing to

levels of selective pressure action, knock down rates and resistance levels in female mosquitoes sampled during dry or wet seasons of the year. Chemicals which are stable to climatic and ecological conditions and intra- extracellular environs may have reduced selection pressure in insects. Choice of insecticides by insecticide resistance management programmes should also take into consideration the appropriate insecticide in respect to prevailing climatic and ecological conditions because knockdown rate varied in different insecticides and different rainfall seasons.

Different clusters showed different insecticide reistance allele frequencies. These variations may be related to the flow of mutant genes from mosquitoes in the selection pressure foci. Types of soils, terrain, drainage patterns, topography and climatic factors come into play by reducing or increasing half lives of the active ingredients in insecticides and agrochemicals hence reduced or increased selection pressure, respectively. Type of species and species resting and feeding behavior determined the intensities of exposure to the insecticidal agents to be genetically selected for. The number of deletion or insertion repeats in a given genome in a mosquito population may also explain the geographical variations in resistance levels. The fact that resistance had been detected earlier in neighbouring Tororo district in Uganda may not necessarily mean that mutant genes originated from there. Only phylogenic studies can fix the question on origin of resistant gene frequencies. Collection of mosquito sample as well as preparation techniques has been found to affect insecticide vulnerability bioassay results. Death rate of female adults collected from the field was between 10-15% greater than in F1 adults nurtured in the insectary from eggs laid by field collected blood-fed females (Githinji *et al*., 2020; Spillings *et al*., 2008).

5.1.2 Impact of SS frequencies on age structure

Individuals in populations from resistant clusters had a higher survival rate and longer life span than individuals in populations from susceptible clusters. Mosquito lay eggs in water. Larval and pupal stages are lived in aquatic environments while adults lead a terrestrial life flying to feeding, resting, mating and ovipostion sites. Early or late hatch characteristic encompass ecological and hereditary factors (Ayele, 2016; Blackwell & Johnson, 2000; Koenraadt *et al*., 2003). Sluggish emergence in *An. gambiae* is probably an alteration to increase procreation yield regardless of the high risk of dehydration in an unbalanced aquatic condition. Based on a study on eggs' embryonic developmental duration, fertility, hatchability, larvae death and adult sex-ratio, it was found that deltamethrin resistance had a negative effect on the developmental duration with the medial range of 70 hrs, 7 folds hatchability and death rate in resistant population which are lower than in the vulnerable population but no major variances were noticed in fertility and adult sex-ratio of the two populations under investigation.

Moulting and cuticle synthesis are modulated by three kinds of growth regulators; the juvenile hormones, the ecdysones and the brain hormones. Growth modulators in standard concentrations control other developmental functions in insects including reproduction, colour differentiation and sexual maturation. In reproduction and development of mosquitoes, Juvenile hormone (JH) acts as a vital regulator. The key role of JH is to delay molting till the larvae realize a suitable mature phase and size. A decline in JH titre at that moment allows a metamorphic moult. The late pupa turns out to be mature enough to make JH, when antimoulting function of JH ends. It plays an important part in enhancing reproductive maturation. JH formation is regulated by the degree of flux of isoprenoid precursors, with a complicated interaction of variations in precursor pools, enzyme concentrations as well as

developmental and nutritional regulators, like insulin, 20-hydroxyecdysone, allatostatin-C, and ecdysis-triggering hormone. JH uses numerous molecular tactics to apply its pleiotropic purposes at various phases in the life cycle of a mosquito. JH operates through an intracellular receptor, methoprene-tolerant protein and unnamed membrane receptor (Machani *et al*., 2019; Noriega, 2014).

Methoprene-tolerant protein acts as a transcription feature and it controls the expression of JH target genes directly. Through gene expression regulation, hormones have powerful implications on reproductive physiology in mosquitoes. Gene expression is not confined to their adjustments of the frequency of gene transcription; instead, hormonal impacts on the constancy of a particular mRNA can completely vary its unfluctuating-state intensity. Autoregulatory feedback loops are formed as mRNAs encoding hormone reseptors are controlled by their own chemical messangers. Positve and negative autoregulatory feedback nooses serve to expand or restrict hormonal responses, respectively. The likelihood that insecticide resistance might impact susceptibility to insect growth regulators such as juvenile hormone analogues adds another twist to resistance to exogenously applied hormonal based insecticides. Through these posttranscriptional inducements, juvenile hormones affect the manifestation of a great population of genes. Demonstration of the molecular mechanisms of hormonally controlled mRNA consistency proceed to designate critical mRNA array of elements and their operative connection with proteins (Ffrench-Constant, 2013; Weedall *et al*., 2019; Zhu & Noriega, 2016).

What could be the trade off between JH target genes and resistance genes expression and its impacts on fertility, hatching of eggs, fecundity, pupal, and adult emergence? According to a study by Zhang *et al*., 2015, costs of fitness as a result of an organophosphate, chlorpyrifos

resistance in cabbage moth may partially have caused excessive energy consumption triggered by detoxification enzymes as well as *hsp*s being over produced. Only a small quantity of proteins is required at favorable conditions but decreased protein expressions when they are extremely required by the insects to fight ecological strains. Surplus expressions of apoptotic genes under heat pressure causes apoptosis to increase.

Longevity of the anophelines spanning between 13 and 23 days is adequate for finishing the fundamental *Plasmodium* parasite incubation cycle and for malaria transmission thus a bigger threat of malaria transmission whereas life span of less than 5 days is not enough for parasite brooding as well as transmission. (Kar *et al*., 2014; Mayagaya *et al*., 2009) proposed that insecticides selectively killing adult mosquitoes, and preferably adult mosquitoes infected with *Plasmodium*, can offer useful malaria control while poorly selecting for non-vulnerability. This approach would significantly improve the operational life of any given insecticide. Nevertheless, linkage of non-vulnerability to fitness costs in mosquitoes would render poor selection for resistance by the chemical compound unprofitable. As such, insecticides that selectively kill aged infected mosquitoes would certainly not be challenged by evolving mosquitoes.

A hereditary alteration of population energetics of the resilient versus vulnerable populations in the regions where resistance to insecticide has been encountered is therefore recommended. Age and infection dependence of chemicals in relation to selection pressure levels is momentous to the implementation of monitoring tests of resistances as well as success in insecticide resistance management (Alout *et al*., 2017; Machani *et al*., 2019; Thiévent *et al*., 2018; Xu et *al*., 2014)

5.1.3 Impact of SS frequencies on vector densities and infectivity effectiveness

SS frequency levels were directly proportional to female mosquito densities. Survival for the fittest principle ensured that individuals with potential to overcome knock down effect and or killing effects by insecticides oviposited and passed on their outstanding genes to their subsequent generations. Individuals from susceptible clusters fed and rested outdoors more than indoor hence induced exophagy and exophilly behaviours inorder to avoid insecticidal surfaces and barriers which is a form of behavioural resistance.

Survival frequency was subject to the mean size of males assembled in copula (Mayagaya *et al*., 2009; Menze *et al*., 2016a). The most optimal male age for effective fertilization of females was between 4 and 8 days; signifying that size of a male is an essential attribute in defining male mating competitive capability. Even though age was not identified as a major aspect in mating competitiveness, it was considerably interrelated with swarming characteristics in the field as well as success of fertilization in the laboratory. Size of males and the effect on mating competitiveness in turn determines vector densities, sporozoite rates, infectivity rates, thus malaria transmission.

As found out, mutant genes not only reduced knock down and death rate in the mutant female mosquitoes but also possibly reduced both fitness cost in lack of an insecticidal agent and or energy cost in the presence of the lethal compounds (WHO, 2012). Competitive fitness tests of the population being considered or the growing rate of any given strain or species can be determined by the rate of duplication under prevalent ecological settings. Normally, resistant strains at a rate relative to the size of the cost enforced by resistance should be replaced by sensitive genotypes that cannot pay a cost of resistance (Melnyk *et al*., 2014). For instance,

pseudomonads have damaged motility, whenever there is resistance to fluoroquinolones. Further, changes can occur on the formation of the ribosome, and so interrupt primary cellular activities even protein synthesis when there is non-vulnerability to aminoglycosides (Drlica, 2004).

Individuals from resistant clusters fed and rested indoor more than outdoor. Exiting densities were higher than indoor feeding and resting densities in all the clusters. Higher biting and sporozoite rates in clusters with higher resistance levels indicates that resistant individuals had stronger cues to seek a blood source irrespective of prevailing chemical or physical barriers of longlasting insecticidal nets. The guts of resistant female mosquitoes may be more receptive to gametocytes innocultion and development than guts of susceptible vectors hence higher sporozoite rates in the former (Ayele, 2016). Successful blood meals may have provided the vector with more nutrients for eggs and parasites developmet while capacity to survive longer may have given *Plasmodium* infected females enough time to incubate and transmit parasites to many human hosts hence possible higher malaria incidences and prevalence in such areas. Resistant fed and gravid vectors were more in proportion than susceptible fed and gravid. Susceptible unfed were higher in proportions that resistant fed. Therefore resistant female mosquitoes are more successful feeders and better survivours than the susceptible (Msami, 2013; Pimenta *et al*., 2015).

Higher preference for the highly nutritive human blood by the resistant female mosquitoes increased their *Plasmodium* transmission capacity. Higher human blood indices among heterozygous females may be a case of heterozygous superiority where the heterozygote genotype has a greater proportional fitness than either the homozygote recessive or

homozygote dominant genotype. The particular case of heterozygote advantage because of overdominance or a single locus happens once the phenotype of the heterozygote get located outside the phenotypical scale of both heterozygous individuals, and homozygote parents thus gains a greater fitness compared to homozygous parental individuals (Alout *et al*., 2014; Brooke & Koekemoer, 2010; Sogoba *et al*., 2008; WHO, 2012).

Selecting the favoured heterozygote can help mainatain polymorphism. This mechanism is used to elucidate incidences of variability in genes. The gene in sickle cell anaemia is a case of an entrenched heterozygote advantage. Superior genes often have multitudinous impact (pleiotropism), which can concurrently bear distinct advantageous characteristics as well as detrimental peculiarities on the same individual. In this case, organism's insecticidal surroundings will offer selection, with a net outcome either supporting or functioning in contrary to the gene, till an ecologically unwavering stability is attained. Heterozygote superiority is a significant inherent mechanism for hybrid vigor (heterosis), which is the enhanced or elevated function of any biotic grade in a hybrid offspring. Preceding study, comparing overdominance, measures of dominance, and epistasis (commonly in plants), indicated that most of the instances of heterozygote advantage were as a result of complementational domination and the concealing of harmful receding alleles by wild-type alleles. However, there werer other results of excess dominance, particularly in rice (Bell & Winstanley, 2004; Ter Kuile *et al*., 2003; Terlouw *et al*., 2003)

Lower entomological inoculation rate among susceptible female mosquitoes underscores the function of anophelines in spreading malaria which primarily relies on their dispersion, affintiy to suck humans as well as their receptibility to *Plasmodium* gametocytes, all of which are

influenced by local ecological circumstances. The bovine blood index (BBI) of *An. arabiensis* was same to that of its human blood index (HBI) demonstrating its opportunistic blood meal acquiring trait in Teso sub counties. *Anopheles gambiae* ss was the most abundant, most human dwellings dweller, most anthropophagic and the greatest sporozoite overloaded species confirming the crucial part it plays in the sub counties as the key malaria vector. However, the disparities in the HBI of the vector could occur due to variations in the comparative distance and ease of access of hosts in either human or bovine dwellings or both, exophagic and or endophagic inclinations, and to a larger extent insecticidal and or physical net barrier (Awolola *et al*., 2007; Sinka *et al*., 2011). Even though LLINs does not kill resistant mosquitoes immediately, it curtails their transmission and survival potential due to its long-term fitness costs that persevere past the initial 24 hr after contact with the insecticidal net (Alout *et al*., 2014; Basil D Brooke & Koekemoer, 2010; Brown *et al*., 2013; Menze *et al*., 2016a).

5.1.4 Impact of SS frequencies on malaria vector biting, feeding and resting behaviour

A shift in resistant *Anopheles gambiae* sl highest peak hour of aggressiveness from 9-10pm to 3-4am midnight is a key change in its biting pattern. Due to development of resistance, mosquitoes no longer have to compete against the time the human host enters into the formerly lethal chemical and or physical barrier (Githinji *et al*., 2020b). Instead the vector keeps away from the hours the human host is awake and hostile to its bites to the hours when the human host is immobilized and most vulnerable to bites due to sleep. Towards dawn when the human host is awake and active again, the biting rate went down but not as low as during early hours of night. Out door biting exceeded indoor biting mosquitoes perhaps due to repellent effect of permethrin in the insecticidal nets and also behavoural resistance fomentations. This indeed confirms that insecticidal nets are still effective as physical, chemical and repellent barriers to malaria vectors. Residual malaria transmission may be taking place more at dawn than at dusk. Cross border early in the morning activities as goods and services are smuggled across the border may have lead the vector to change from its former bitting patterns.

The number of bitings in highly resistant clusters exceeded those of lower susceptibility clusters. Susceptible mosquitoes were more of early biters than late biters while susceptible females were more of late biters than early biters. Smoke has been known to repel mosquitoes questing for a human blood meal hence a deep in biting volumes during cooking hours of the night (Ayele, 2016). More hungry resistant mosquitoes were active in seeking a blood meal as early as 5pm while unfed susceptible mosquitoes could be seen attempting a successful bite as late as 7.00am in the morning. Furguson and Read, 2004 reported that infected mosquitoes were not only excessively attracted to hosts that are infected with *Plasmodium* parasites but also more likely to consume their second blood meal than their uninfected counterparts (Kelly-

Hope & McKenzie, 2009; Mayagaya *et al*., 2009; Olanga *et al*., 2015; Takken & Verhulst, 2013).

This study found out that resitant vectors had higher sporozoite rate than susceptible ones and unfed females were higher in proportion among susceptibles than among mutants. Resistant strains of mosquitoes preferred human blood, fed and rested indoors. Different biting rates of mosquito are required by both the mosquito and its malaria parasite to guarantee mosquito breeding successful achievement and effective transmission of the parasite, respectively. Efforts by both associates to maximize their achievements restrain the trade-off prevailing between mosquito survival and biting rate (Adedolapo & Olajumoke, 2008). Changes witnessed in knock down, bitting and resting patterns may have led to enhanced transmission and defense system hence abundant survival of both host and parasite (Adedotun A Adedolapo1, 2008).

Mosquito vectors are not harmed by the malaria parasites in their gut. Due to the sturdy positive connection that prevails between the number of eggs laid and the amount of blood drank by the mosquito, the fecundity of the mosquito rises with the intensified blood feeding rate (Koenraadt *et al*., 2003; Lindh *et al*., 2015). However, the rate of blood feeding increases as the likelihood of mosquito decimation increases. Likelihood to die may be due to the high probability of the mosquito being maimed or killed by the irritated vertebrate host while trying to feed or when feeding, slowered flight as a result of the gained blood mass hence prone to predation or insecticidal barriers. (Billingsley *et al*., 2005) characterized *Plasmodium* transmission success and mosquito reproductive success as a function of mosquito feeding rate. The conclusion was that the parasite's success is greatest at a feeding rate that is higher

compared to the necessary optimal mosquito success, provided that transmission escalates more quickly with fecundity and feeding rate.

Multimodal bitting pattern with acute flactuations in susceptible LL mosquitoes could have been as a result of knock down effects and repellency by insecticidal nets. LS curve was smoother as an intermediary but the smoothest curve was shown by SS carrying female mosquitoes whose more definite biting rythyms seem undeterred and undisturbed through out the night. Resistant *Anopheles gambiae* ss are still highly anthropophagic. Opportunistic *An. arabiensis* was almost as zoophagic as anthropophagic. Resistant vectors were more endophagic and endophilly than exophagic and exophilly while LS and LL female mosquitoes were more exophagic and exophilly than endophagic and endophilly. No LS vector rested indoors possibly due to disadvantages of heterozygosity which could have increased their fitness cost as well as energy cost in the presence of the insecticidal agents in permethrin treated nets. SS mosquitoes were biting more during bed time while LS and LL vectors were seeking a human blood meal more during out of bed hours of the dusk to dawn period than sleeping hours of the night.

But, affinity for a given host is normally affected by numerous intrinsic and extrinsic factors. Genetic selection largely determines the inherent factors which seem to be regulated by adaptational benefits that come from suckling on a particular host species. Even though the mosquito preference for a given host species has a genetic basis, heightened pliability mediated by the accessibility and density of host species acts as the major determinant. The behavior of mosquitoes in selecting their host is not a rule but more of an exception. Species that demonstrate inherent as well as strong host-selection trait belong to the vectors of utmost

importance as far as transmissible diesases are concerned, which propose that this behavioral characteristic may have genetically transformed concurrently with that of parasite-host evolution (Takken & Verhulst, 2013). Energy costs during metabolism and downregulation of insecticidal active ingradients through enzyme activities may have increased the aggressiveness in seeking a host blood meal long before dusk (Mwanziva *et al*., 2011). Fitness costs may considerably reduce the lifetime of a female mosquito; prohibit fertilization and egg laying, and substantialy block development of *Plasmodium falciparum,* three constituents that are vital to transmission of malaria.

5.2 CONCLUSIONS

i. Both phenotypic and genotypic insecticide resistance has been confirmed in Teso sub counties in western Kenya. Insecticide resistant levels significally differed in different clusters at different climatic seasons and types of insecticides.

ii. Resistant mosquitoes live significantly longer than the susceptible ones.

iii. Susceptible malaria vectors have significantly lower entomological inoculation rates.

iv. Insecticide resistant levels have impacted on early and late feeding by resistant and susceptible vectors respectively. Most resistant vectors rest in doors while susceptible ones feed and rest outdoors. Biting rate is higher at dawn than at dusk hence a plausible cause of residual malaria taking place more at dawn than at dusk. Higher selection pressure for resistance may be more present indoors than outdoor.

Insecticide resistance has impacted significantly on vector species composition, age structure, density and infectivity, along with feeding and reposing characteristic traits in vectors of *Plasmodium falciparum* wich cause malaria disease in Teso sub counties, Busia County, Western Kenya.

5.3 RECOMMENDATIONS

I therefore recommend the following:

1. The implication of such high resistance levels in mosquitoes collected in Teso sub counties is that resistance is likely to persist and or even increase if mono-molecules of permethrin and deltamethrin or both continue to be used in all net and non-net based mosquito control purposes. Usage of mutually reinforcing Piperonyl butoxide (PBO) that prohibit particular enzymes vital in metabolic activities inside mosquito systems and has been integrated into pyrethroid-LLINs to create pyrethroid-PBO nets is an extremely viable option.
2. Insecticide resistance management practices in Kenya to be fastracked and harmonised with agricultural sector agrochemical based activities and possibly switch to carbamate use in order to ease selection pressure on pyrethroids which are useable in insecticidal nets due to their low human toxicity.
3. Further entomological surveillance and monitoring to be done regularly in a predictable schedule.
4. Further studies to be done on alternative sources of selection pressure on malaria vectors.
5. Additional studies on the evolvement of *Plasmodium falciparum* virulency in mutant mosquito populations would generate profound knowledge to forecast parasite transmission responsiveness to vector acclimation.
6. Further studies be done on fitness costs and energy costs in mutant malaria vectors in the presence and or absence of insecticidal active ingradients.

CHAPTER SIX

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CHAPTER SEVEN

APPENDICES

Appendix 1: Extraction Protocol: Chelex method

1. This is a fast, cheap, and effective method of DNA extraction. Because this is the first step towards PCR and amplifying your template DNA, you must maintain excellent sterile technique to prevent the contamination of your DNA extractions. Always use a negative chelex control (below) to evaluate your technique during the extraction phase. Work in LOW DNA part of lab. 1. Remove premade tubes filled with 300uL 10% Chelex from refridgerator. You will need for each sample, plus 1 extra for a control. Handle the container with gloves and shake out the number you need into your gloved hand. Do not put extra tubes back into jar.

2. Label each Chelex tube to correspond to your sample listed on your Chelex worksheet. Label the tubes on the cap. Put as much information as you can manage, but keep labels legible. Adding initials and a date is always a good idea. Avoid labeling on the side of tube as this writing can be washed off during the incubation stage.

3. Turn on heating block. Set to 95°C. Fill holes with ddH_2O water.

4. Dip forceps into ethanol, then wave forceps through flame of an alcohol burner to ignite. When you are certain that the flame on the forceps has extinguished, repeat 2 times.

5. Using the sterile forceps, remove a small piece of tissue from your sample, uncap the tube of chelex, place sample in the appropriately labeled tube and close lid. The piece of tissue should be big enough to be visible, but not so big as to be easily visible. Imagine cutting a

0.2mm section of a standard staple. This is plenty big. Too much tissue may inhibit your reactions. The piece of tissue should be about as big as a period.

6. Repeat (step 5) with each sample in a new Chelex tube, being sure to sterilize forceps 3 times between samples (step 4). When finished, make a negative Chelex control by dipping your sterilized forceps into a tube of Chelex slurry. (It may be necessary to wipe excess tissue from forceps with a kimwipe prior to flame sterilization)

7. When finished with all tubes, vortex samples in chelex slurry for 10-15 seconds. Be sure lids are snapped on tightly before begining

8. Spin samples briefly (10-15 sec) at high speed in a microcentrifuge. This step is to ensure that the sample is inside the slurry of Chelex.

9. Incubate samples for 20 minutes at 95°C. The block temperature may drop slightly when doing this step. This drop is normal. Check tubes while incubating to ensure that lids have not popped off.

10. Vortex samples again for 10-15 seconds (Be careful as steam may pop lid off of centrifuge tube. Hold lids down).

11. Spin tubes again at high speed in microcentrifuge to ensure that all contents are in the bottom of the microcentrifuge tube.

12. Samples are ready to use (or not, see below). ONLY USE SUPERNATE FOR PCR REACTIONS. CHELEX BEAD WILL INACTIVATE TAQ!

13. Chelex is notorious for being as fickle as it is cheap and easy. Here are some tips for good amplifications:

    i. Sometimes samples work best if used immediately. Sometimes it is better to wait overnight before using them. Experiment and find what works for your species. Results can vary by taxa and by individuals.

    ii. When doing initial PCRs, do a serial dilution of template. The amount of a Chelex DNA extraction used in a PCR can be as high as half of the volume of the PCR or as low as 1 microliter of a 1:10,000 dilution. I find that 1 microliter of a 1:1 is good for most applications, but if your PCR doesn't work initially, vary template concentration. [Supernate] Chelex

    iii. If you don't get amplifications from your PCR the first time with a Chelex extract, repeat the vortex, spin, incubate, vortex, spin, sit overnight procedure described above. Often this will make a negative PCR work.

Appendix 2: Species identification PCR

1. Mosquito samples from $^{-}80^{o}C$ freezers were first sorted randomly to make up for the representative sample size. Numbers of samples were recorded on PCR chart taking into account the format of the gel to be run so that there are *A. gambiae* and *A. arabienses* control in each row of the gel. A blank was left at the end of the rest to be run which contained master mix kept on ice. Taq enzyme was added last, hence left in the freezer until one was ready to add it. Plates used for PCR were costar® serocluster™ 'u' Vinyl plate cat. No 2797 96 well plate non sterile not treated Costar 205, Broad way Cambridge MA 02139 – also used for ELISA).
2. 14ul of Master Mix were added to all wells including controls and blank. 0.8 ul of DNA template was added and vortexed. Vial was opened while positioned below (near the floars) to avoid DNA "flying" about and possibly contaminating other wells. The whole plate was softly tapped on the bench so as to settle and mix master mix with the DNA template samples. Four to five drops of mineral oil were added into each well. No pipette was dipped twice into the mineral oil to avoid contamination. PCR – Taq plate was then left to settle. All DNA templates were again frozen at $^{-}80^{0}C$ in case there was need to repeat the PCR again. Failure to load the plate directly to the gene machine, prompted storage at $^{-}4^{o}C$. Some oil was added in the PCR machine slots while avoiding the overflow. Plate was loaded into the machine; lid closed and machine switched it on.
3. PCR Cycle Conditions were $95^{o}C$ for 5 minutes X 1 cycle, [$95\,^{o}C$/30 sec, $50\,^{o}C$ /30 sec, $72\,^{o}C$] x 30 cycles, $72\,^{o}C$/5min x 1 cycle at $4\,^{o}C$ hold. Final proportions of

PCR ingredients were 100ul of each of the four NTP (Nucleotides), 0.67 ng/ul of Specific mosquito primers, 0.02 units/ul Taq enzymes, 1Mm $MgCl_2$, and 10x Buffer = 1x (if diluted with H_2O in the Master mix)

4. Making the Master Mix. Volume of each ingredient was multiplied by the number of samples to be run (Table 2.1). An extra sample was allowed when assembling a master mix, for example calculating a master mix for 33 samples when running 32 samples. H_2O was added first and Taq last; master mix was vortexed and dispensed into micro plate. 1ul less per well than the final volume was dispensed (for 15ul reaction, 14ul master mix was used). DNA was then added to each well (0.8ul – 1ul). Reaction mixture was finally overlaid with 4 drops of heavy mineral oil, covered with plate sealer, and then placed in the gene machine.

Table 2.1: Proportions in the master mix for efficient PCR amplification of DNA templates during species identification

	Microliters needed for each reaction of:-		
INGREDIENTS	**15ul/well sample**	**20ul/well sample**	**25ul/well sample**
Water (H_2O) USB TAQ	10.36	14.15	17.91
Perkin – Elmer Taq	(10.34)	(14.12)	(17.87)
Buffer 10x	1.5	2.0	2.5
dNTP (Total)	1.2	1.6	2.0
Universal Primers: GA, AR	0.25	0.33	0.42
MgCl2	0.15	0.21	0.27
Taq (USB)	0.036	0.048	0.06
(Perkin – Elmer)	(0.58)	(0.077)	0.10

5. Making the agarose gel and setting it on the electrophoresis tank: Twenty litres of TBE (Tris Boric Acid EDTA) electrophoresis buffer were made using Tris base 216g, Boric acid 110g, 0.5 M EDTA (Ph 8.0), 80ml (186g) and distilled water. Ethidium bromide was made up into a gel. Carcinogenic Ethidium bromide was kept at 4^{o}c covered with aluminium foil. Gloves were won when handling all gel containing Ethidium bromide. Agarose and TBE were mixed on a hot plate while stirring with magnetic stirrer. It took $1\text{-}1^{1/2}$ hrs for the gel to solidify. Tank edge support blocks were removed and edge slaps pushed down to leave gel edges free. TBE buffer (201 – 220 ml) was added, covering the gel completely. Solidified gel was not allowed to sit for long without adding the buffer to avoid drying.

6. Adding the loading dye, gel loading and electrophoresis: After PCR time was over, the plate were removed, oil blotted out of the bottom and plate sealer removed carefully avoiding the spill. 3ul of the loading dye (Bromophenol blue) was added into the bottom of the mineral oil using fresh tips (one for each well); making sure that the tip goes right below the mineral oil, otherwise the dye would float. The gel was loaded with 15ul of the mixture/loading dye (dipping the tip to the bottom of the well to avoid picking mineral oil). The lid of the electrophoresis tank was replaced and run from negative to positive. The gel was run for 15 – 30 minutes but was constantly checked and stopped when the dye was almost at the end. The power was switched off, gel scooped and placed on a plastic tray awaiting darkroom visualization.

7. Band visualization by ultra violet source, photography or scoring: After removing the gel from the electrophoresis chamber, it was kept at 4^{o}C for a few hours before visualization. Film cartridges for the camera at were kept at 4^{o}C and loaded almost an hour before use in order to attain room temperature. Eye covers were used from this point onwards. Gel was placed on to the UV source slab; main light switched off UV source was switched on for scanning. After visualization, camera was placed squarely on the gel and photo taken. The lower paper was then pulled. Gradually and uniformly, the upper bigger paper was pulled out to contact and develop the film as it comes out. The film was let to rest for 30 – 60 seconds. The photograph was then peeled out and visualized. The gel was put back onto the tray and UV source wiped with paper towel. The read gel was discarded for incineration. Electrophoresis equipment was rinsed and placed aside to dry. The buffer was usable one more time. The data was recorded and filed.

Appendix 3: Measuring susceptibility to insecticides in adult mosquitoes: WHO bioassay test procedure

1. Six exposure tubes were placed on the working bench and each of the 4 red dotted exposure tubes were lined with a sheet of insecticide-impregnated paper, while the 2 yellow-dotted control exposure tubes were lined with oil-impregnated papers; each was fastened into position with a copper spring-wire clip.

2. The empty exposure tubes were attached to the vacant position on the slides and with the slide unit open the mosquitoes were blown gently into the exposure tubes.

3. Once all the mosquitoes were in the exposure tubes, the slide unit was closed and the holding tubes could be detached and set to one side.

4. 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 (60 minutes).
5. At the end of the 1-hour exposure period, the mosquitoes were transferred back to the holding tubes by reversing the previous procedure.

6. The exposure tubes were detached from the slide units.

7. A pad of a cotton-wool soaked in sugar water was placed on the mesh-screen end of the holding tubes.

8. Mosquitoes were maintained in the holding tubes for 24 hours (the recovery period). During this time, it was important to keep the holding tubes in a shady, sheltered place free from extremes of temperature (an insectary was ideal). Temperature and humidity was recorded during the recovery period.

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

10. An adult mosquito was considered to be alive if it is able to fly, regardless of the number of legs remaining. Any knocked down mosquitoes, whether or not they have lost legs or wings, were considered moribund and were counted as dead.

11. On completion of the susceptibility test, mosquitoes were transferred to individual, clearly labeled 0.5 ml Eppendorf tubes (separating dead and live mosquitoes into separate tubes) for storage until time for species identification and *kdr* gene analysis.

Appendix 4: Circumsporozoite enzyme-linked immunosorbent assay

1. The head and thorax of individual female Anopheles mosquitoes were homogenized in 250 ul of grinding buffer (PBS, pH 7.4 containing 0.5% NP-40 and 0.5% casein) using a glass pestle.

2. CS protein micro-plate ELISA using 50 ul/well of the homogenate was done in 96-well microtitre plates coated with anti-*P. falciparum* monoclonal antibodies at 22–25 ^{o}C for 30 min (Wirtz et al. 1987).

3. Captured CS antigen was revealed by monoclonal antibody (MoAb) horseradish peroxidase conjugate incubated for 1 hr.

4. Addition of ABTS [2,2¢-azino-di-(3-ethylbenzthiazoline- 6-sulphonate)] substrate gave a green colour reaction for positive results which were read by visual assessment of the colour reactions, and OD measured within 30 min using spectrophotometer (Multiskan Ascent, Model 354; ThermoLabsystems, Finland) at 414 nm.

5. Sample positivity was determined by titration of PfCSP-positive control antigen using cut-off OD values equivalent to 12pg of PfCSP or 50 sporozoites (Collins *et a.,* 1988). The PfCSP concentrations of test samples were determined by extrapolation from a graph of PfCSP OD versus protein concentration in the controls, and the corresponding sporozoite loads estimated.

Appendix 5: *Kdr* polymerase chain reaction

Mosquitoes from Teso were pyro sequenced for East-kdr (L1014S) mutations. The pyro sequencer unambiguously scored the homozygote resistant (SS), heterozygote resistant (LS) and homozygote susceptible (LL) genotypes for L1014S-kdr genotypes (Figure 7.1).

Sample preparation for 96-well plate

1. A sample template was created first making sure that a FAM control, heterozygote control, HEX control and no template control was in each run. Reagents were thawed in ice.
2. PCR tubes were loaded onto a tube holder, allocating one tube per sample and four tubes for control. Master Mix (MM) for 105 samples was prepared containing 406.9ul of water, 525ul for sensimix and 13.1 of probe/primer.
3. Nine microliters of MM were aliquoted into each sample, controls and no template tubes.
4. One microliter of DNA was pipetted into tubes and 1 ul of water for negative control.
5. Lid strips were fitted onto tubes after each column in the rack was completed.
6. Gently rocking of lids onto the tube strips made each lid secure.
7. The 96 well plate was vortexed and then quickly centrifuged at 3000 rpm for 30 seconds.
8. The instrument was loaded and data interpreted using the MxPro Software

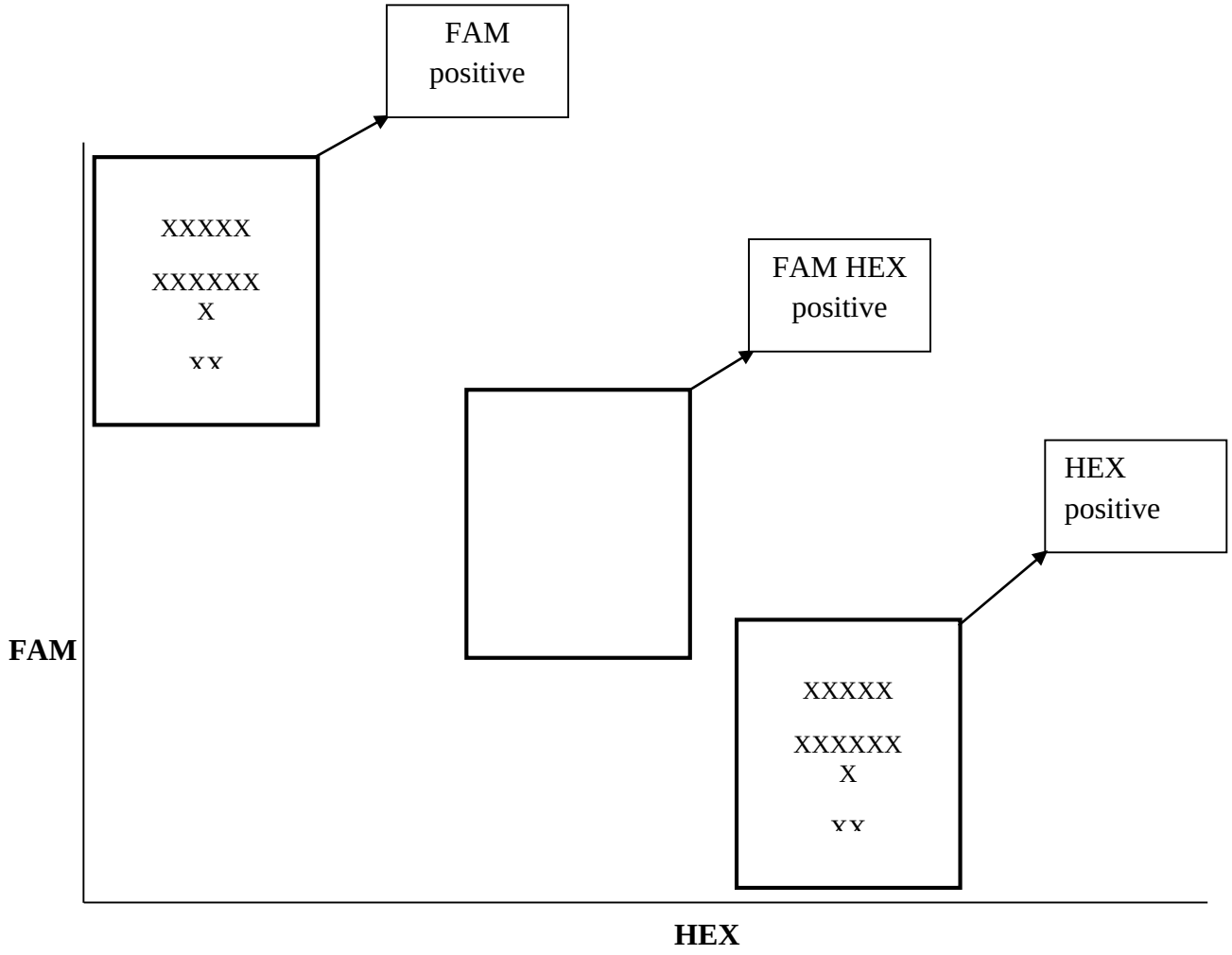


Figure 7.1: Graphic illustration of positions of the FAM/HEX fluorescent dyes during *Kdr* gene lab analysis (Source: This Study)

Appendix 6: Blood meal PCR procedure

1. A sample template was created, making sure to include positive controls for human, pig, cow, goat, dog and a 'no-template control' in each run.

2. Reagents on ice/freezer block were thawed. A 96 – well PCR plate was set out and the required number of strip tubes placed in a rack.

3. Primer stock (100um) was diluted by 1:10 in nuclease –free water. Master Mix (MM) with 5-10% extra was prepared for the samples and controls.

4. 22ul of MM was put in each well using a filtered pipette tip.

5. Starting with the controls, 3ul of DNA was put into the wells while 3ul of water was put into the no-template control.

6. Strip cups or sticky seals were hard pressed on top of tubes to ensure that the tubes were well sealed.

7. The plate was then placed in PCR thermo cycler set at 95^{O}C for 5 minutes, 95^{O}C for one minute, 56^{O}C for one minute, 72^{O}C for one minute, 72^{O}C for 7 minutes and held at 4^{O}C.

8. The programme was saved as 'blood meal' and run for 2-3 hours.

9. In the meantime 2% TAE-agarose gel was made by adding 4g of agarose to 200ml1xTAE in a conical flask.

10. The conical flask was placed in a microwave and timer set to 3 minutes.

11. The conical flask was checked in 20-30 seconds intervals at the beginning but 10-20 seconds interval at the end. The liquid turned transparent when heated enough. Protective gloves were worn when handling the heated liquid.

12. The conical flask was then cooled under running cold water until the flask cooled to approximately $40^{O}C$.

13. Seven microlitres of Ethidium bromide was added using a pipette and suitable tips. Extra caution was taken when handling Ethidium bromide. The liquid was poured carefully into a gel electrophoresis tray containing appropriate combs and the liquid left to set for 20-30 minutes.

14. Once the liquid set, the platform was placed in a gel electrophoresis tank filled with 1x TAE buffer and the combs were removed. Once the PCR was complete, 7-10ul of DNA ladder was loaded into the first and the last well in the row.

15. Seven to ten microlitres of PCR product from each well was placed in a new tube. A suitable loading buffer such as Orange G was added to each sample.

16. The contents of the tube were mixed by slowly pipetting the liquid in and out of the tip. The liquid was drawn up and pipetted into one of the wells in the gel.

17. Once all the samples were loaded into the gel, the lid was put on the tank and the red wires connected to the red connection and the black wires to the black connection. With the lid properly placed, the other end of the wire was plugged into the voltmeter, again red wire to red connection and black wire to black connection. Once it was properly connected and the current switched on, bubbles appeared at

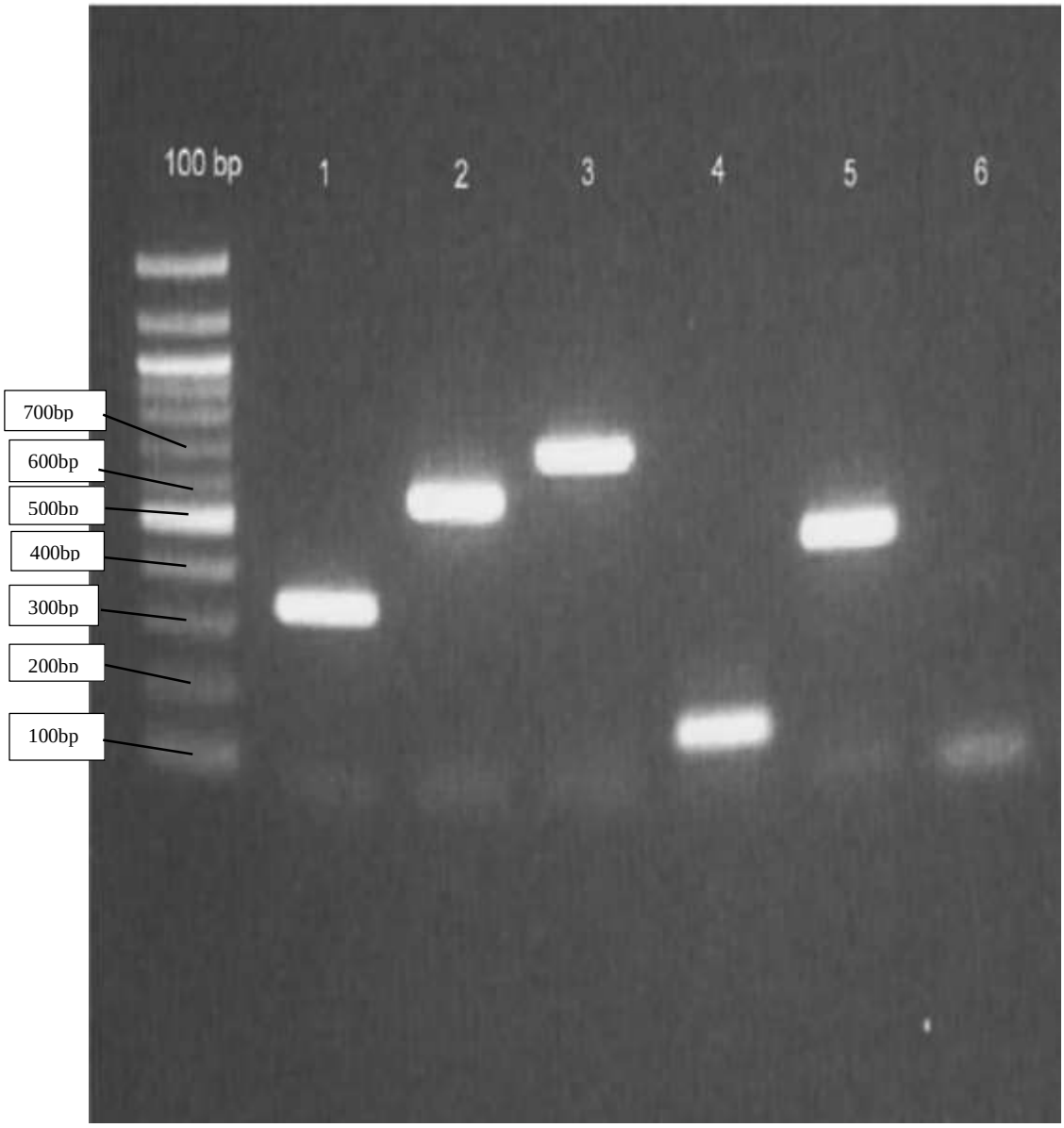
either end. The black connection as the negative terminal was orientated to the back and the DNA migrated to the front of the tank. The gel was ran for approximately 30-40 minutes at 100-120V.

18. After 30-40 minutes, the power pack was turned off, lid removed and the tray also removed from the tank. The door of the UV gel-documentation system was slid open and a snap shot of the gel taken and the picture saved to a relevant file for easy recall for printing purpose.

19. When analyzing the PCR results, negative/no-template controls were first confirmed to be negative. By comparing with size markers, positive controls showed bands of correct size at 334basepairs (bp) for Human, 453bp for pig, 132bp for goat, 680bp for dog and 561bp for cow (Figure 7.2). Primer dimer bands visible in all lane and running at 100bp or lower were ignored.

20. If markers and PCR products were not well separated, it was then necessary to return the gel to the gel tank and ran for further 10-15 minutes.

21. Once a satisfactory gel picture was obtained, blood meal in each of the mosquito sample was identified by comparing PCR fragment sizes with the sizes of the positive control samples of human, pig, goat, dog and cow.



*Lane 1 = human; lane 2 = cow; lane 3 = dog; lane 4 = goat; lane 5 = pig; lane 6 = negative control.

Figure 7.2: PCR assay for blood meal identification using the mtDNA cytochrome-b region (Source: This Study).

Appendix 7: Blood meal ELISA procedure

1. FF anophelines, from all catches, were assayed for human and bovine blood antigens simultaneously by ELISA. Abdomen of each FF mosquito was ground in 50 µL phosphate-buffered saline (PBS) and final volume brought to 200 µL with PBS buffer.

2. 50 µL of the triturate was coated in duplicate wells on two separate U-bottomed 96-well microtitre plates simultaneously: one plate for human blood meal identification and the other for bovine. Plates were incubated overnight at room temperature and washed twice with PBS-Tween 20. 50 µL peroxidase-conjugated anti-human IgG was added in the first plate and the same volume of peroxidase-conjugated anti-bovine IgG in the second plate incubated for one hour at room temperature and washed thrice with PBS-Tween 20.

3. Finally 100 µL ABTS peroxidase substrate was added, incubated at room temperature for 30 min and observed for green colour reaction visually and absorbance read at 405 nm (by MRX Microplate Reader, Dynex Technologies, 14340 Sullyfield Circle, Chantilly, VA. 20151–1683, USA).

4. Positive control (either human or bovine blood meal) and negative controls (abdomen of laboratory-bred UF *An. arabiensis*) were included in each plate. Human blood index (HBI) and bovine blood index (BBI) of each anopheline species was determined by dividing human fed and cattle fed anophelines respectively to the total tested.

Appendix 8: Permethrin

Chemical Class and Type:

- Permethrin is an insecticide in the pyrethroid chemical family. The International Union of Pure and Applied Chemistry (IUPAC) name for permethrin is 3-phenoxybenzyl (1RS,3RS;1RS,3SR)-3-(2,2-dichlorovinyl)-2,2-dimethyl-cyclopropanecarboxylate and the Chemical Abstracts Service (CAS) registry number is 52645-53-1. Permethrin is considered a type I pyrethroid.
- Permethrin was originally registered for use by the United States Environmental Protection Agency (U.S. EPA) in 1979, and it was re-registered in 2006.
- Permethrin is a blend of two stereoisomers. For the remainder of this fact sheet, note that permethrin refers to an isomer blend and not one isomer alone.

Physical / Chemical Properties:

- Technical permethrin ranges from a colorless crystal to a yellow or brown viscous liquid. No information on the odor of permethrin was found.
- Vapor pressure: 2.15×10^{-8} mmHg
- Octanol-Water Partition Coefficient (K_{ow})[1]: 6.1 at 20 °C
- Henry's constant: 1.4×10^{-6} atm·m^3/mol
- Molecular weight: 391.3 g/mol
- Solubility (water): 5.5×10^{-3} mg/L, 6×10^{-3} mg/L
- Soil Sorption Coefficient (K_{oc}): 1.00×10^{5}

Uses:

- Permethrin can be used in public health mosquito abatement programs and on a variety of food or feed crops and livestock; or in structures and buildings, including

livestock housing and food-handling establishments. Permethrin can also be used in numerous residential sites, both indoor and outdoor, and on pets and clothing. When permethrin is used on large areas like crops, nurseries, and sod farms it is considered a restricted use pesticide. For other applications, it is considered a general use pesiticide. Formulations of permethrin used for treatment of head lice and scabies on humans are available, but these are considered pharmaceuticals.

- Signal words for products containing permethrin may range from Caution to Danger. The signal word reflects the combined toxicity of the active ingredient and other ingredients in the product.

Mode of Action: Target Organisms

- Permethrin acts on the nervous system of insects. It interferes with sodium channels to disrupt the function of neurons, and causes muscles to spasm, culminating in paralysis and death.
- Permethrin can be effective by contact or ingestion and also acts as a mild repellent.

Mode of Action: Non-target Organisms

- Permethrin is highly toxic to honeybees, fish, and aquatic invertebrates due to disruption of sodium channels.
- In general, mammals are less susceptible to permethrin compared to insects because their sodium channels are less sensitive to pyrethroids and recover more rapidly. Also, because of their larger body size and greater metabolic capabilities, mammals are more likely to metabolize pyrethroids, such as permethrin, before they can affect the nervous system. Conversely, cats can be sensitive to products with high

concentrations of permethrin, possibly due to insufficient glucoronide conjugation capability, which hinders the metabolism of permethrin.

Environmental Fate:

Soil

- The average half-life of permethrin in aerobic soils is 39.5 days, with a range from 11.6 to 113 days.
- Permethrin binds tightly to soil and is broken down primarily by microorganisms, but also by photolysis.

Water

- When permethrin enters an aquatic system, some is degraded by sunlight while in the water column but the majority binds tightly to the sediment.
- In water, permethrin is broken down by photolysis into 3-phenoxybenxzyl alcohol (PBA) and dichlorovinyl acid (DCVA). The average half-life range for permethrin in the water column is about 19-27 hours, however permethrin adsorbed to sediments can persist more than a year.
- Permethrin is not likely to contaminate groundwater due to its low water solubility and strong adsorption to soil.

Air

- Permethrin has the potential to drift depending on application technique, however it has a very low vapor pressure and is not expected to volatilize.

Plants

- The half-life of permethrin on plant foliage varies depending on the species. The approximate range is from one to three weeks.

Indoor

- Permethrin was applied in a thin layer to an indoor surface beside a window and exposed to daylight. After 20 days, 60% of the permethrin remained on the surface.

Food Residue

- In the FDA's Total Diet Study of 2003, permethrin residues were detected in 3% of the 1039 food samples tested. The range of permethrin levels found was 0.0008-4.7130 ppm.
- When testing for the cis- and trans-isomers separately, the USDA examined several additional crops, including broccoli, cranberries, peaches, and spinach. Of the 8948 samples tested, 6.7% had detectable residues ranging from 0.004 to 5.30 ppm. Spinach samples alone accounted for 97% of the detected levels of permethrin.

Appendix 9: Deltamethrin

Chemical Class and Type:

- Deltamethrin is in the chemical class of pyrethroids. Pyrethroids are synthetic chemicals modeled after the pyrethrin components of pyrethrum. Unlike other pyrethroids, deltamethrin consists of one pure compound.
- Other names for deltamethrin include (S)-α-cyano-3-phenoxybenzyl (1*R*,3*R*)-3-(2,2-dibromovinyl)-2,2-dimethylcyclopropanecarboxylate and the former, rejected name decamethrin.

Physical / Chemical Properties:

- Technical grade deltamethrin (≥98% pure) consists of odorless crystals that are non-corrosive and colorless or white to light beige.
- Vapor pressure: 1.5×10^{-8} mmHg at 25 °C
- Octanol-Water Partition Coefficient (log K_{ow}): 6.1
- Henry's constant: Henry's constant may be determined by estimation or experimentally derived. Reported values include 1.2×10^{-4} atm·m^3/mol at 25 °C and 5.0×10^{-5} atm·m^3/mol, depending on the technique used.
- Molecular weight: 505.2 g/mol
- Solubility (water): ranges from 0.002-0.0002 mg/L
- Soil Sorption Coefficient (K_{oc}): adsorption ranges from 7.05×10^5 to 3.14×10^6; desorption ranges from 1.14×10^6 to 4.54×10^6

Uses:

- Deltamethrin is a broad-spectrum insecticide. Deltamethrin has been registered for use on areas such as golf courses, ornamental gardens, lawns, outdoor perimeter treatments, indoors as spot and crack and crevice treatments, and pet collars. Uses for individual deltamethrin products vary widely. Always read and follow the label when applying pesticide products.
- Deltamethrin is registered for use on various crops including cotton, corn, cereals, soybeans, and vegetables for pests such as mites, ants, weevils, and beetles.

Mode of Action:

Target Organisms

- Deltamethrin is effective against insects via ingestion and direct contact.
- Pyrethroids, in general, interfere with normal production and conduction of nerve signals in the nervous system. Pyrethroids act on nerve membranes by delaying the closing of the activation gate for the sodium ion channel.
- Researchers distinguish between two classes of pyrethroids based on electrophysiological studies with nerves and symptoms of toxicity. Type II pyrethroids, including deltamethrin, have an α-cyano group that induces "long-lasting" inhibition of the sodium channel activation gate. This results in prolonged permeability of the nerve to sodium and produces a series of repetitive nerve signals in sensory organs, sensory nerves, and muscles.
- Researchers observed that deltamethrin and other Type II pyrethroids may also affect ion channels in the nervous system other than sodium channels, possibly due to their phosphorylation state.

Non-target Organisms

- The mechanism of action of pyrethroids, including deltamethrin, is the same for target and non-target organisms.
- Pyrethroids are less toxic to mammals compared to insects due to mammals' higher body temperature, larger body size, and decreased sensitivity of the ion channel sites.

Environmental Fate:

Soil

- Reported half-lives under aerobic laboratory conditions for deltamethrin in sandy loam or silt loam soil ranges from 11-72 days. Half-lives can vary widely based on environmental factors.
- In anaerobic soil conditions, the half-life of deltamethrin ranges from 31-36 days.

- The half-life of deltamethrin ranged from 5.7-209.0 days terrestrial field dissipation studies.
- Deltamethrin degrades via hydrolysis, photolysis, and microbial action. It is not susceptible to photo-oxidation, and is more persistent in soils with a high clay or organic matter content.
- Hydrolysis of deltamethrin results in the formation of Br_2CA and PBA.
- Deltamethrin is considered relatively immobile in soils, while its two major degradation products, Br_2CA and PBA are more mobile. Deltamethrin adsorbs to soil organic matter so strongly that biodegradation can be stalled.
- Deltamethrin has little potential to leach into groundwater due to its strong tendency to bind to soil organic matter.
- In a field study, approximately 24% of deltamethrin volatilized from the soil surface within 24 hours of application.

Water

- In one study, the aquatic half-life of deltamethrin ranged from 8-48 hours, where the primary metabolite was Br_2CA. Variations of the half-life were due to the method of application.
- Deltamethrin was stable to hydrolysis in solutions of pH 5 and 7. In a pH 9 solution, the average half-life was 2.5 days. Deltamethrin was stable to direct aqueous photolysis in a 30 day study.
- Due to its Henry's law constant (1.2×10^{-4} atm·m^3/mol at 25 °C), deltamethrin has a higher potential to volatilize from water compared with other pyrethroids. In a field study, researchers detected maximum levels in air 14 hours after a pond was sprayed with deltamethrin.
- Maximum levels in sediment were reached at 48 hours after treatment
- Pyrethroids, including deltamethrin, have been found in aquatic sediment. Reported concentrations were rarely greater than 20 ng/g for any pyrethroid.
- In the same study, 12 creeks in Tennessee were also sampled to detect pyrethroids in the sediment. Of the 14 sites sampled, five contained no pyrethroids at

concentrations above 1 ng/g. Seven other sites detected one pyrethroids present per sample, with reported concentrations just above detection levels.

- In another study, 30 creeks in California were sampled for pyrethroids in sediment. Deltamethrin was reported to be detected infrequently from 90 samples tested.

Air

- Due to its vapor pressure (1.5×10^{-8} mmHg at 25 °C), deltamethrin has a low potential to volatilize.

Plants

- The half-life of deltamethrin on vegetative surfaces ranges from 5.9-17.0 days, depending on the plant species.
- Deltamethrin is not likely to adsorb as strongly to leafy components of vegetation, so volatilization from these surfaces may be higher compared to soil. In one field study, 12-72% of deltamethrin volatilized from plant leaves 24 hours after application, depending on the plant species.
- Deltamethrin is unlikely to be taken up by terrestrial plants due to its tendency to bind to soils and rapid degradation. Uptake was not observed through leaves or roots of most plants and therefore it is considered a non-systemic compound.

Indoor

- Under indoor laboratory conditions, soil treated with deltamethrin had a half-life of 4.8 weeks.
- Pyrethroids have a higher rate of volatilization from floor or glass surfaces than from soils since they are not as likely to adsorb to these surfaces.

Food Residue

- In 2006, the United States Department of Agriculture (USDA) Pesticide Data Program (PDP) analyzed 9030 samples of fruits and vegetables for deltamethrin and its parent compound, tralomethrin. Of the samples tested, only one sample had detectable residues and the amount detected was eight times less than the U.S. EPA tolerance level.
- In the same study, 133 finished water samples, 133 untreated water samples, 734 peanut butter samples, and 655 samples of poultry breast and thigh were analyzed for deltamethrin and tralomethrin. No samples had detectable residues.

Terrestrial Invertebrates

- Deltamethrin is highly toxic to honeybees (*Apis sp.*) under laboratory conditions. One study reported an oral LD_{50} of 51 ng/bee and a contact LD_{50} of 51 ng/bee. In field studies, deltamethrin did not harm bees at rates up to 12.5 g a.i./ha and formulated products had a repellant effect lasting for 2-3 hours.
- Researchers observed no effects on earthworms when the soil was treated with 12.5 g/ha of deltamethrin for 28 days.

Regulatory Guidelines:

- The reference dose (RfD) for deltamethrin is 0.01 mg/kg/day. Reference Dose (RfD) is an estimate of the quantity of chemical that a person could be exposed to every day for the rest of their life with no appreciable risk of adverse health effects. The reference dose is typically measured in milligrams (mg) of chemical per kilogram (kg) of body weight per day

Appendix 10: Lambdacyhalothrin

Lambda cyhalothrin is a synthetic pyrethroid insecticide and acaricide used to control a wide range of pests in a variety of applications. Pests controlled include aphids, Colorado beetles and butterfly larvae. Crops on which it may be applied include cotton, cereals, hops, ornamentals, potatoes, vegetables or others. It may also be used for structural pest management or in public health applications to control insects such as cockroaches, mosquitoes, ticks and flies which may act as disease vectors.

Lambda cyhalothrin is available as an emulsifiable concentrate, wettable powder or ULV liquid, and is commonly mixed with buprofezin, pirimicarb, dimethoate or tetramethrin. It is compatible with most other insecticides and fungicides.

Lambda cyhalothrin is moderately toxic in the technical form, but may be highly toxic via some routes in formulation (e.g., as Karate). Available data indicate that lambda cyhalothrin is moderately toxic via the oral route in test animals. Reported oral LD50 values are 79 mg/kg and 56 mg/kg for male and female rats, respectively. The vehicle used was corn oil. The rat oral LD50 has also been reported as 144 mg/kg. The reported rat LD50 for the technical product is similar, 64 mg/kg. These indicate moderate acute toxicity via the oral route of exposure.

Effects on Other Animals (Non-target species)

Lambda cyhalothrin is highly toxic to bees, with a reported oral LD50 of 38 ng/bee and reported contact LD50 of 909 ng/bee (0.9 ug/bee).

Environmental Fate

Breakdown of Chemical in Soil and Groundwater

Lambda cyhalothrin is moderately persistent in the soil environment. Reported field half-lives range from four to 12 weeks. Its field half-life is probably close to 30 days in most soils. It shows a high affinity for soil; the reported Koc is 180,000. Lambda cyhalothrin is not expected to be appreciably mobile in most soils. There is little potential for groundwater contamination. Soils with high sand content or with very low organic matter content may tend to retain the compound to a lesser degree. In field studies of Karate, leaching of lambda cyhalothrin and its degradates from the soil were minimal.

Breakdown products formed in the soil environment are similar to those formed in mammalian systems, via the hydrolysis of the central ester bond and oxidation. Breakdown rates of both the technical product and Karate were similar under aerobic and anaerobic conditions.

Breakdown of Chemical in Surface Water

Lambda cyhalothrin has extremely low water solubility and is tightly bound to soil, it is therefore not expected to be prevalent in surface waters. One possible source of infiltration into surface waters would be surface runoff. In this event, the compound would most probably remain bound to the solid particle and settle to the bottom.

Breakdown of Chemical in Vegetation

No data were available regarding the breakdown of lambda cyhalothrin in vegetation.

PHYSICAL PROPERTIES AND GUIDELINES

Lambda cyhalothrin is a colorless solid at room temperature, but may appear yellowish in solution.

Exposure Guidelines:

RfD:	0.005 mg/kg/day
Chemical Name:	(RS)-alpha-cyano-3-phenoxybenzyl 3-(2-chloro-3,3,3-trifluoropropenyl)-2,2,-dimethylcyclopropanecarboxylate
CAS:	91465-08-6
Molecular Weight:	449.9
Water solubility:	0.005 mg/L @ pH 6.5 and 20 degrees C
Solubility in other solvents:	acetone v.s, methanol v.s., toluene v.s., hexane v.s.
Melting Point:	49.2 degrees C
Vapor Pressure:	negligible at 20 degrees C
Partition Coefficient (octanol/water):	10,000,000
Adsorption Coefficient:	180,000

Appendix 11: Bendiocarb

- Bendiocarb belongs to a class of insecticides (chemicals that kill or control insects) known as carbamates.
- Bendiocarb was first registered in the United States in 1980. Its registration was voluntarily canceled in September 1999, and all products containing bendiocarb lost registration in December 2001.
- Products with canceled registrations cannot be purchased after that date, but existing stocks can still be used according to label directions.
- Products containing bendiocarb may be labeled for such outdoor uses as applications on turf, soil, shrubs, trees and ornamental plants.
- They are also used against various types of pests, such as beetles, aphids, mites, and caterpillars.
- Labels may indicate that a product is designed to be used in various indoor applications such as houses, hotels, restaurants, warehouses, hospitals, railroad boxcars, and aircraft, where it may be used to control spiders, wasps, ants, flies, and stored- product pests.
- Products with bendiocarb as an active ingredient are formulated as granules, dusts, or sprays. What are some products that contain bendiocarb? Ficam ™ Turcam ™ Tattoo ™ Multamat ™

- The Pesticide Label: Labels provide directions for the proper use of a pesticide product. Be sure to read the entire label before using any product.

- Signal words, listed below, are found on the front of each product label and indicate the product's potential hazard. CAUTION - low toxicity WARNING - moderate toxicity DANGER - high toxicity Laboratory Testing: Before pesticides are registered by the U.S. EPA, they must undergo laboratory testing for short-term (acute) and long-term (chronic) health effects.

- Laboratory animals are purposely fed high enough doses to cause toxic effects.

- These tests help scientists judge how these chemicals might affect humans, domestic animals, and wildlife in cases of overexposure.

- When pesticide products are used according to the label directions, toxic effects are not likely to occur because the amount of pesticide that people and pets may be exposed to is low compared to the doses fed to laboratory animals.

- NPTN General Fact Sheets are designed to answer questions that are commonly asked by the general public about pesticides that are regulated by the U.S. Environmental Protection Agency (U.S. EPA).

- This document is intended to be helpful to professionals and to the general public for making decisions about pesticide use.

- This fact sheet was created in 2002; some of the information may be out-of-date. NPIC is not planning to update this fact sheet.

- More pesticide fact sheets are available here. Please call NPIC with any questions you have about pesticides at 800-858-7378, Monday through Friday, 8:00 am to 12:00 pm PST.

- How does bendiocarb work?

    - On Pests Bendiocarb disrupts the normal functioning of an insect's nervous system and may kill by either contact or ingestion

    - Bendiocarb disrupts the nervous system by interfering with an enzyme necessary for normal nerve transmission

    - The inhibition caused by bendiocarb is temporary because the enzyme it affects can regenerate

- How toxic is bendiocarb?

    - Bendiocarb-containing products are low to moderate in toxicity. The pure chemical is highly toxic. The formulated products are typically lower in toxicity because of the reduced concentration of bendiocarb.

- Bendiocarb is moderately to highly toxic to both male and female rats. Bendiocarb is highly toxic to guinea pigs and rabbits

- When researchers applied bendiocarb to the skin of rats, they found it to be moderately toxic.

- Signs of Toxicity – Animals: The signs of bendiocarb poisoning may include behavioral changes, excessive tearing and salivation, muscle tremors, twitching, vomiting, and diarrhea. Severe intoxications can result in paralysis. Dogs given very high doses of bendiocarb recovered completely within 24-25 hours. Cats are typically more sensitive to the effects of bendiocarb than dogs.

- Signs of Toxicity – Humans: Early symptoms associated with bendiocarb exposure may include headache, malaise, muscle weakness, nausea, gastrointestinal cramps, sweating, and restlessness.

- Greater exposures to bendiocarb may lead to pin-point pupils, tearing, excessive salivation, nasal discharge, vomiting, diarrhea, muscle twitching, and problems with coordination. Severe poisonings can result in convulsions, coma, and death

- The red blood cell cholinesterase test may be able to document an acute bendiocarb poisoning if administered immediately after exposure, but it may be misleading due to the rapid regeneration of the affected enzyme.

- Precautionary Labeling Toxictiy High Toxicity (Danger) Moderate Toxicity (Warning) Low Toxicity (Caution) Very Low Toxicity (Caution) Oral considered highly toxic when the LD_{50}/LC_{50} is small and practically non-toxic when the value is large.

- Exposure: Effects of bendiocarb on human health and the environment depend on how much bendiocarb is present and the length and frequency of exposure. Effects also depend on the health of a person and/or certain environmental factors.

- Could bendiocarb cause reproductive problems or birth defects? Animals- when scientists fed pregnant animals increasing quantities of bendiocarb, most offspring did not have any birth defects. However, some animals that were given the highest dose levels had offspring with increased eye abnormalities and underdeveloped pubic bones. In some cases, rats given daily various doses during pregnancy had reduced offspring survival rates or lowered birth weights in their offspring. This occurred only with rats fed the highest amounts of bendiocarb. Humans No human data was found on the ability of bendiocarb to cause reproductive problems or birth defects.

- What happens to bendiocarb outdoors? Soil depending upon local conditions, bendiocarb has a half-life in soil ranging from 3-21 days, with an average of 5 days. Plants Bendiocarb residues on plant surfaces usually have a half-life of 3 to 18.3 days. Water Bendiocarb does not dissolve well in water and binds to soil, so it is unlikely to move through the soil to groundwater.

- What happens to bendiocarb indoors? Indoor applications of bendiocarb do not last long. Residues are greatly reduced after only a couple of days

Appendix 12: Summary results of t-tests on means of various malaria transmission potential indicators as impacted by insecticide resistance on *Anopheles gambiae* s.l.

	SS/LS/LL genetic resistance groups				High/Low phenotypic resistance groups		
Resistance status Entomological parameter	High resistance SS	Low resistance LS	No resistance LL	ρ-value	Resistant Rwatama cluster	Susceptible Kengatunyi cluster	ρ-value
Sporozoite rate	27.5%	5.3%	4.2%	<0.001	28.1%	7.8%	<0.001
Human blood index	80%	100%	50%	<0.001	87%	60%	<0.001
Parity Nulliparous:	82%	5%	13%	<0.001	12%	74%	<0.001
Parous	76%	3%	21%	<0.001	88%	26%	<0.001
Longivity	14.5 days	14.9 days	8.0 days	<0.001	16 days	5 days	<0.001
Outside biting densities/Exophagic	78%	12%	10%	<0.001	44.4%	83.3%	<0.001
Inside biting densities/Endophagic	86%	9%	5%	<0.001	55.6%	16.7%	<0.001
Outside resting densities/Exophilic	79%	4%	17%	<0.001	100%	0%	<0.001
Inside the house resting densities/Endophilic	89%	0%	11%	<0.001	100%	0%	<0.001
Abdominal status: Unfed	75.2%	6.1%	15.7%	<0.001	21.2%	63.2%	<0.001
Fed	84.8%	9.1%	6.1%	<0.001	8.8%	6.6%	>0.05
Gravid	86%	6%	8%	<0.001	59.5%	30.2%	<0.001
Trap method PSC	100%	0%	0%	<0.001	100%	0%	<0.001
WET	79.1%	4.2%	16.7%	<0.001	100%	0%	<0.001
OPC	0%	0%	0%	-	0%	0%	-
ALL HLC	79.4%	11.6%	14.2%	<0.001	30.6%	69.4%	<0.001
INDOOR HLC	86.6%	11.9%	1.5%	<0.001	77%	23%	<0.001
OUTDOOR HLC	75.9%	13.9%	10.1%	<0.001	34.8%	65.2%	<0.001
Eir	-	-	-	-	146	11	<0.001
Mortality levels: Permethrin	-	-	-	-	66%	86%	<0.001
Deltamethrin	-	-	-	-	79%	86%	<0.001
Kdr levels	79.4%	6.9%	13.7%	<0.001	93.4%	57.8%	<0.001