STUDIES OF *ANOPHELES* MOSQUITOES TRANSMITTING MALARIA IN PARTS OF JIGAWA STATE, NIGERIA

BY

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AHMADU BELLO UNIVERSITY, ZARIA, NIGERIA

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DECLARATION

I declare that the work in this Thesis entitled "Studies on *Anopheles* mosquitoes transmitting malaria in parts of Jigawa State, Nigeria" was carried out by me in the Department of Biological Sciences under the supervision of Prof. I.S. Ndams, Prof. I.H. Nock and Dr. (Mrs) S.A. Luka.

	_____	_____
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CERTIFICATION

This Thesis entitled "Studies on *Anopheles* mosquitoes transmitting malaria in parts of Jigawa State, Nigeria" by Umar Adamu Ahmed meets the regulations governing the award of the degree of Doctor of Philosophy of Ahmadu Bello University, Zaria and is approved for its contribution to knowledge and literary presentation.

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DEDICATION

This work is dedicated to my parents, teachers and well wishers.

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ABBREVIATIONS

An**.**	*Anopheles*
AIDS	Acquired Immune Deficiency Syndrome
Bp	Base pair
BB	Blocking Buffer
CSP	Circum sporozoite protein
DNA	Deoxyribonucleic acid
DDT	Dichlorodiphenyltrichobroethane
ELISA	Enzyme-Linked Immunosorbent Assay
EtBr	Ethidium bromide
FMH	Federal Ministry of Health
Gr.	Greek
HIV	Human Immunodeficiency Virus
IRS	Indoor Residual Spray
ITN	Insecticide Treated Net
M-form	Mopti-form
MM	Millimeter
M	Metre
MAb	Monoclonal Antibodies
n	Number
PCR	Polymerase Chain Reaction
Pfcsp	*Plasmodium falciparum* circumsporozoite protein
PBS	Phosphate Buffer Saline

RNA	Ribonucleic Acid
rDNA	Ribosomal deoxyribonucleic acid
rpm	Revolutions per minute
S.l	*Sensu lato*
S.S	*Sensu stricto*
TBE	Trisboric ethidium
vec test	Vector test
W.H.O.	World Health Organization

ABSTRACT

Studies of *Anopheles* mosquitoes transmitting malaria in some villages of Jigawa State were conducted to establish morpho-species and highliting the members transmitting malaria. A total of 1,863 adult *Anopheles* were collected, 591 (31.72%) by indoor residual spray collection while 1,272 (68.28%) were reared *Anopheles* larvae/pupae. Morphological characters and Polymerase Chain Reaction (PCR) were employed in the identification of *Anopheles* species while Enzyme-linked Immunosorbent Assay (ELISA) was employed to determine their infection by *Plasmodium.* Morphologically, two species, *An. gambiae s.l.* and *An. funestus* were identified, these comprised of 1,362 (73.11%) and 501 (26.89%) respectively. Molecular studies by Polymerase Chain Reaction (PCR) separated *An. gambiae s.l.* into 1149 (84.36%) *An. gambiae s.s* and 213 (15.64%) *An. arabiensis.* Molecular study of *An. gambiae s.s.* yielded 222 (19.32%) M-form and 927 (80.68%) S-form. The amplification of the 16s rDNA has revealed occurrence of a M/S hybrid in Auyo indoor anopheline population collected. There were 1,373 (73.70%) *Anopheles* during the rainy season and 490(26.30%) in dry season. The highest population of mosquitoes caught was in September 311 (16.70%). The distribution of *Anopheles* according to study locations showed that Kirikasamma had the highest 162 (8.70%) mosquito population while Jahun had the lowest 16 (0.85%). Of 591 *Anopheles* examined by ELISA, 43 (7.28%) were infected with *Plasmodium.* Of the 43 infected mosquitoes, 10 (23.25%) were *An. arabiensis*, 5 (11.63%) M-form and 28 (65.12%) S-form. *Plasmodium falciparum* 32 (74.42%) was more prevalent than *P. malariae* 11 (25.58%). In conclusion, *Anopheles funestus, An. Arabiensis*, M-and S-form of *Anopheles gambae s.s* were the abundant species in the study area. *Anopheles* mosquitoes were more abundant in rainy season than in dry season. Transmission was more

in rainy season than in dry season. *Anopheles* species M-and S-forms and *An. arabiensis* were transmitting *Plasmodium malariae* and *P. falciparum.*

CHAPTER ONE

INTRODUCTION

Mosquito is a common insect in the family Culicidae. It has mouthparts that are adapted for piercing the skin of animals. While males typically feed on nectar and plant juices, the female needs to obtain nutrients from a blood meal (Jordan and Verma, 2011).

There are about 3,500 species of mosquitoes found throughout the world. In some species, the females feed on human blood, and are therefore vectors of a number of infectious diseases affecting millions of people per year. Species of *Anopheles, Culex, Culiseta* and *Mansonia* are common in warm climate and species of *Anopheles* are responsible for transmission of malaria (Jordan and Verma, 2011).

In a general way, the life cycles of all mosquitoes are much alike, but they differ in the details. The eggs of mosquitoes are usually oval with various surface markings and in *Anopheles,* with peculiar "floats" of air cells. *Anopheles* lays eggs singly in loose clusters on water; the larva lies horizontally in water and has no trumpet shaped siphon or breathing tube (Toply, 2008).

Members of the *An. gambiae* complex are the most important vectors of malaria in sub-Saharan Africa. The complex consists of about seven species that vary in their ability to transmit malaria. Two species of the complex, *An. gambiae s.s* and *An. arabiensis* are both the most broadly distributed and the most efficient vectors of malaria (Coetzee *et al.,* 2000).

Singh *et al.* (1996) observed that the same species of *Anopheles* can behave differently in different ecological settings. They observed that *An. culicifacies* in India was mainly

exophilic in forest villages, whereas in villages away from the forest, *An. culicifacies* was predominantly endophilic.

Due to their variable breeding habits, choice of breeding places, taste in blood, extent of travels and willingness to come indoors to bite or to rest, different species of *Anopheles* relate differently with humans (Chandler and Read,1961). A few domestic species that feed in or near houses readily enter them and feed on human blood and are regarded as house pests include *An. quadrimaculatus* in the United States, *An. maculipennis* in Europe, *An. darling* in South America and *An. gambiae* in Africa.

Species that regularly come in contact with humans outdoors may come into homes to feed but not to rest. They breed in rice fields, seepage, overflow, in swamps, temporary rain pools, in tree holes, axile of banana leaves, coconut shells, near villages or cultivated areas. Important species in this group are *An. scutedaris* in the Pacific Islands and many species of *Anopheles* in various parts of the world (Jordan and Verma, 2011).

Gilles *et al.* (1968) described morphological characters of *Anopheles* and this had aided their classification. Gilles and Coetzee (1987) used the same identification features to report on the distribution of various anopheline species in Nigeria. *Anopheles gambiae s.l.* was considered a single species (Coluzzi, 1978) although Davidson (1964) distinguished the following species based on chromosomal studies: *An. arabiensis, An. bwambe, An. gambiae s.s, An. melas, An. merus,* and *An. quadriannulatus* (Hunt *et al.,* 1998). Scott *et al.* (1993) reported two sibling species, namely M-and S-forms from *An. gambiae s. s.* (the M-form is presently known as *An. coluzzi* using molecular polymerase chain reaction technique).

Service (1980) described *An. funestus, An. moucheti, An. nilli, An. hancocki* and *An. hagreavesi. Anopheles funestus* belongs to a group of five sibling species, *An. funestus, An. leesoni, An. parensis, An. rivulorum* and *An. vaneedenip.* These species have been identified in North Africa, Western Africa and Central Africa (Frederic *et al.*, 2003). Molineaux and Grammicia (1980) reported eleven species of *Anopheles* in the Sudan Savannah of West Africa, namely *An. gambiae sensu stricto, An. arabiensis, An. funestus, An. rufipes, An. pharoensis, An. wellcomei, An. squamosus, An. coustani, An. maculipalpis, An. nilli* and *An. pretoriensis.*

Onyabe *et al.* (2003) reported broad distribution pattern of the M- and S-molecular forms in Nigeria and, also the occurrence of *An. arabiensis* in the Southern Guinea savanna of Nigeria. Ndams (2004) reported eight taxa in Northern Guinea Savanna of Nigeria, namely *An. funestus, An. rufipes, An. maculipalpis, An. gambiae s.l, An. gambiae s.s, An. arabiensis,* M-and S-forms. The abundance of *An. gambiae s.l, An. arabiensis, An. gambiae s.s,* M-and S-forms from Guinea, Sahel and Sudan Savanna of northern Nigeria were reported (Tukur, 2010).

There are five species of *Plasmodium* responsible for human malaria these are *Plasmodium falciparum, P. knowlesi, P. vivax, P. ovale* and *P. malariae. Plasmodium* has a complex life-cycle, which includes stages in the female *Anopheles* mosquito. The infection starts with the bite of an infected mosquito, the sporozoite rapidly makes its way to the liver, where it invades a hepatocyte (Toply, 2008). Different species of *Anopheles* have been found to inoculate *Plasmodium* into humans. For instance, *An. gambiae s.l* and *An. funestus* transmit malaria in the Sudan savanna of West Africa, *An. gambiae* M-form is the most important and

widespread malaria vector in Angola and the sporozoite rate of *Anopheles*, determined by ELISA, was 1.9% for *An. gambiae* (n=580) and 0.7% for *An. funestus* (n=140) (Molineuax and Gramiccia, 1980; Martinez – Torres *et al*, 2002). Ndams (2004) reported the prevalence of *Plasmodium* circumsporozoite, protein antigens in *An. gambiae s.s* molecular forms in which 19 (2.84%) (n=668) were positive.

Malaria is a major public health problem and the cause of much suffering and premature death in tropical Africa, Asia and Latin America. In many endemic areas, it is becoming increasingly difficult to control the disease because of the resistance of the parasite to anti-malarial drugs and lack of adequate vector control measures (Cheesbrough, 2005).

In Garki, Jigawa State, there was a WHO intervention programme in 1969, which sought to establish a link between entomological and parasitological variables, especially vectoral capacity and the prevalence of *Plasmodium falciparum.* The project also measured the effect of specified interventions, such as spraying with residual insecticides, (propoxur alone and in combination) with mass drug administration, using a combination of sulfaline and pyremethamine (Molineaux and Grammicia, 1980). Two species of *Anopheles gambiae s.l,* namely *An.arabiensis* and *An. gambiae s.s* were found transmitting the malaria parasite in Garki (Molineaux and Grammicia, 1980).

Statistics on malaria in Jigawa State shows that it kills more people than HIV/AIDS, tuberculosis, measles and whooping cough (Taiwo, 2010). One out of every three children in Jigawa State that dies before his or her fifth birthday is killed by malaria and 11% of maternal mortality is also due to malaria (Taiwo, 2010). Entomological studies are an essential foundation for malaria control; *Anopheles* species and *Plasmodium* transmission

status in Jigawa should therefore contribute to a more accurate design of vector control, and by extension, malaria.

The Federal Ministry of Health (FMH) is currently undertaking malaria control activities in Jigawa State, which involve distribution of insecticide treated bed nets (ITNs), indoor spray of pyrethroid and free distribution of antimalaria drug (coartem). With all these, malaria still remains a problem in the state and this makes the study of *Anopheles* mosquitoes in Jigawa State important.

The older techniques that were used for the identification of *Anopheles* and presence of *Plasmodium* were light microscopy and dissection. Dissection involved first examining the ovaries of each mosquito for parity. Midguts and salivary glands of parous mosquitoes were then examined for oocysts and sporozoites respectively. Oocysts were counted, graded according to relative size, and differentiated oocysts and free sporozoites were noted. Salivary gland sporozoite infections were graded either 1+ (1 – 10 sporozoites), 2+ (11 – 100 sporozoites), 3+ (101 – 1,000 sporozoites), or 4+ (> 1,000 sporozoites) (Bangs *et al*., 2002). However, availability of skilled microscopists, for processing large number of samples to detect the sporozoites and identification of sporozoite species are the main limitations of this method (Mahapatra *et al*., 2006). Hence a method with greater sensitivity and specificity has been looked for. The polymerase chain reaction (PCR) and enzyme linked immunosorbent assay (ELISA) are highly sensitive and specific (Mahapatra *et al*., 2006).

Polymerase is an enzyme that participates in making a copy of each Deoxyribonucleic acid (DNA) strand using the original template. Polymerase Chain Reaction (Scott *et al*., 1993) is a quick technique for determining *Anopheles* species complex. It involves denaturation in

which the original DNA is heated to make individual strands to separate; annealing in which oligonucleotide primers bind to the separated DNA strands and polymerization in which the polymerase copies the DNA rapidly. Enzyme linked immunosorbent assay also provides immediate result for detection of *Plasmodium* in *Anopheles* mosquito. Molineaux and Grammicia (1980) employed the dissection of *Anopheles* salivary glands technique which is difficult and time consuming.

Therefore, there is need to update the abundance of *Anopheles* and transmission status in Jigawa State using PCR and ELISA techniques which are more reliable than the techniques initially employed. The outcome of this study will be an essential tool for fighting malaria in the study area.

1.1 Statement of the Problem

Malaria remains one of the greatest human health burdens in Jigawa State. Jigawa has a long history in the research of malaria such as ‘’the Garki project’’ and recently W.H.O malaria control interventions such as free distribution of antimalaria drugs, insecticide treated bed nets and vector control in some Local Government Areas such as Auyo, Ringim, among others. There are also significant gaps in the existing knowledge on the transmission of malaria. These compound the difficulty of developing targeted and effective control measures that would be evaluated. The sheer complexity and number of malaria vectors in the state are daunting and such receive detailed studies. The difficult task of summarizing the available information for each species and/or species complex is compounded by patchiness of the data: while relatively plentiful in one area or region, it can also be completely lacking in others. Compared to many other states in Nigeria, only scant information on vector

bionomics and response to chemical measures is available in Jigawa State. It is to the light of the complexities highlighted above that this was designed.

1.2 Justification

Published work on malaria vectors in Jigawa State is patchy or scanty. Determination of risk of malaria transmission requires quick, frequent and accurate methods of assessing transmission intensity, which is the product of human-biting rates and infectivity of vectors (WHO, 1975).

There is need to update information on *Anopheles* species and malaria transmission to assist in developing effective intervention in the study area. The behaviour of *Anopheles* species largely determines their vector status, and insights into their distribution are essential to evaluate the appropriateness of vector control measures. Therefore, the study will shed lights in greater detail, the species, composition and abundance, the species that are actively involved in transmission of *falciparum malaria* during the period of the study.

1.3 Aim of the Research

The aim of this research is to evaluate the species composition of malaria vectors and their invovlement in the transmission *falciparum* malaria in Jigawa State.

1.4 Objectives of the Research

i. To determine the species composition and distribution of *Anopheles* mosquitoes
ii. To determine the seasonal variation within the *Anopheles* population and the relationship between seasons and transmission.
iii. To determine *Anopheles* infection with *Plasmodium* and *Plasmodium falciparum* species causing malaria in the study area.

iv. To determine whether *Plasmodium* transmission is associated with *Anopheles* species.

1.5 Research Hypotheses

i. *Anopheles* species are not abundant and not well distributed in the study area.

ii. *Anopheles* species are not infected with *Plasmodium* and only *Plasmodium falciparum* is responsible for malaria in the study area.

iii. There is no seasonal variation among the *Anopheles* population and *Plasmodium* transmission is independent of season.

iv. *Plasmodium* transmission is not associated with *Anopheles* species.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 *Anopheles* Mosquito

Anopheles (Greek anofelis: "useless") is a genus of mosquito first described and named by J. W. Meigan in 1818. About 460 species were recognized; while over100 can transmit human malaria, only 30 – 40 commonly transmit parasites of the genus *Plasmodium*, which cause malaria in humans in endemic areas. *Anopheles gambiae* is one of the best known, because of its predominant role in transmission of the most dangerous malaria parasite species (to humans) – *Plasmodium falciparum* (Angus, 2010).

Some species of *Anopheles* also can serve as the vectors for canine heartworm *Dinoflaria immitis*, the filariasis-causing species *Wucheraria bancrofti*, *Brugia malayi*, and viruses such as one that causes "O'nyong'nyong'' fever. An association of brain tumor incidence and malaria suggests the *Anopheles* might transmit a virus or other agent that could cause a brain tumor (Steven, 2010). Mosquitoes in other genera (*Aedes*, *Culex*, *Culiseta*, *Haemagogus* and *Ochlerotatus*) can also serve as vectors of disease agents, but not malaria.

Correct species identification is the starting point for the epidemiological role of vectors identification mostly achieved by using morphologicaland molecular criteria (Van Bortel *et al*., 2000). Recently, David (2008) reported that most part of Jigawa State lie within the Sudan Savannah and that the total vegetation cover in the state is very much below the national average of 14.8% due to both natural and human factors, as the vegetation cover is being depleted, making it vulnerable to desert encroachement. Members of the *An. gambiae* complex are most important vectors of malaria in sub-Saharan Africa. The complex consists

of about seven species that vary in their ability to transmit malaria (White, 1974; Hunt *et al*., 1998). Two species of the complex, *An. gambiae* and *An. arabiensis*, are the most broadly distributed and the efficient vectors of malaria (Coetzee *et al*., 2000). The primary malaria vectors in Africa, *An. gambiae s. s* and *An. funestus*, are strongly anthropophilic and consequently, are two of the most efficient malaria vectors in the world (Brogdon and McAllister, 1998). The range and the relative abundance of *An. arabiensis* and *An. gambiae* appear to be strongly influenced by climatological factors (Lindsay *et al*., 1998).

Onyabe *et al*. (2003) indicated *An. gambiae s. l* complex is dominant in Nigeria, while Ndams (2004) reported eight species of *Anopheles* from the northern Guinea Savanna namely *An. funestus, An. maculipalpis, An. gambiae s. s, An. arabiensis, An. gambiae s*.s (M-and-S forms) and more recently, Tukur (2010) reported the abundance of *An. gambiae s. l, An. arabiensis*, M-form and S-form from Guinea, Sahel and Sudan Savanna of northern Nigeria.

2.2 Evolution

The ancestors of *Drosophila* and the mosquitoes diverged 260 million years ago. The *Culicine* and *Anopheles* clades of mosquitoes diverged between 120 million years ago and 150 million years ago. The Old and New World *Anopheles* species subsequently diverged between 80 million years ago and 95 million years ago (Mereno, 2010). *Anopheles darling* diverged from the African and Asian malaria vectors 100 million years ago (Eric, 2009). The *Anopheles gambiae* and *Anopheles funestus* clades diverged between 80 years ago and 36 million years ago. A molecular study of several genes in seven species as provided additional support for an expansion of this genus during the Cretaceous period (Jyotsana, *et al.,* 2010).

The *Anopheles* genome, at 230 – 284 million base pairs, is comparable in size to that of *Drosophila*, but considerably smaller than those found in other *culicine* species the genome is diploid with six chromosomes. The only known fossil of this genus are those of *Anopheles* (*Nyssorhynchus*) contained in Dominican amber from the Late Eocene (40.4 million years ago). (Edward, 1932).

2.3 Systematics

The genus *Anopheles* belongs to a subfamily Anophelinae with three genera: *Anopheles* Meigen (nearly worldwide distribition), *Bironella* Theobald (Australia only) and Chagasia Cruz (Neotrophics) with *Bironella* Theobald emerged as a genus in 1917. Despite the passage of time, the taxonomy remains completely settled. Classification into species is based on morphological characteristics – wing spots, head anatomy, larval and pupal anatomy, chromosome structure, and more recently, on DNA sequences.

The genus has been subdivided into seven subgenera based primarily on the number and positions of specilised setae on the gonocoxites of the male genitalia.The system of subgenera originated with the work of Christophers, who in 1915 described three subgenera: *Anopheles* (widely distributed), *Myzomyia* (later renamed *Cellia*) (Old World) and *Nyssorhynchus* (Neotropical) which was first described as *Lavernia* by Theobald (1917). Edwards in 1932 added the sub genus *Stethomyia* (Neotropical distribution). *Kerteszia* was also described by Edwards (1932), but then recognized as a sub-grouping of *Nyssorhynchus*. It was elevated to sub-genus status by Komp (1937), and it is also found in the Neotropics. Two additional sub-genera have since been recognized: *Baimaia* (Southeast Asia only)

(Jyotsana *et al*., 2010). All species known to carry human malaria lie within either the *Myzorhynchus* or the *Anopheles* series.

Two main groupings within the genus *Anopheles* are used: one formed by *Cellia* and *Anopheles* subgenera and asecond by *Kerteszia*, *Lophopodomyia* and *Nyssorchyncus*. Sub-genus *Stethomyia* is an outlier with respect to these two taxa. Within the second group, *Kerteszia* and *Nyssorhynchus* appear to be sister taxa.

The number of species currently recognized within the subgenera is given here in parentheses: *Anopheles* (206 species), *Baimaia* (1 species), *Cellia* (216 species), *Kerteszia* (12 species), *Lophopodomyia* (6 species), *Nyssorhychus* (34 species) and *Stethomyia* (5 species).

Taxonomic units of between subgenus and species are not currently recognized as official zoological names. In practice, a number of taxonomic levels have been introduced. The larger subgenera (*Anopheles*, *Cellia* and *Nyssorhynchus*) have been subdivided into sections and series which in turn have divided into groups and subgroups. Below group but above species level is the species complex. Taxonomic levels above species complex can be distinguished on morphological grounds. Species within a species complex are either morphologically identical or extremely similar and can only be reliably separated by microscopic examination of the chromosomes or DNA sequencing. The classification continues to be revised.

Sub-genus *Nyssorhyhchus* has been divided in three sections: *Albimanus* (19 species), and *Myzorhynchella* (4 species). The *Argyritarsis* section has been subdivided into *Abitarsis* and *Argyritarsis* groups.

The *Anopheles* group was divided by Edwards into four series: *Anopheles* (worldwide), *Myzorhynchus* (Palearctic, Oriental, Australasian and Afrotropical), *Cyclolepppteron* (Neotropical) and *Lophescelomyia* (Oriental); and two groups, *Arribalzagia* (Neotropical) and *Cristya* (Afrotropical). Reid and Knight (1961) modified this classification and consequently subdivided the sub-genus, *Anopheles*, into two sections, *Angusticorn* and *Laticorn* and six species. The *Arribalzagia* and *Christya* Groups were considered to be series. The *Angusticorn* section includes members of the *Anopheles*, *Cyclolepppteron* and *Lophoscelomyia* series.

2.4 Life Cycle of *Anopheles*

The life cycles of all mosquitoes are very much alike, but they differ in details (Toply, 2008). *Anopheles* mosquitoes go through four stages, egg, larva, pupa and adult in their life-cycle. Adult females lay their eggs in standing water, which can be a salt–marsh, a lake, a puddle etc.

The first three stages are aquatic and last for 5-14 days, depending on the species and the ambient temperature; eggs hatch to become larvae which developed into pupae. The adult emerges from the pupa as it floats on the water surface. Adults live for 4–8 weeks (Jordan and Verma, 2011). The adult females can live up to a month (or more in captivity), but most probably do not live more than two weeks in nature.

Several species of *Anopheles* are the carriers of *Plasmodium* which cause malaria fever. It transmits the parasite from one human to another. *Plasmodium* also cause malaria in monkeys, hence monkeys act as reservoir host of the malaria parasite. The female *Anopheles*,

when feeding on the blood of a malarial patient it takes in the gametocyte stages of *Plasmodium* which develop and undergo sexual reproduction in the *Anopheles* to form thousands of sporozoites. When it bites a person, it transfers the infective sporozoites into host's blood along with saliva.

2.4.1 Eggs

Adult female lays 50–200 eggs per oviposition. The eggs are quite small (about 0.5 x 0.2 mm). They are laid singly and directly on water. They are not resistant to drying and hatch within 2 – 3 days, although hatching may take up to 2 – 3 weeks in colder climates.

The eggs are usually oval with various surface markings and, in *Anopheles,* with a peculiar "floats" of air cells. The number of eggs laid by one female varies between 40 to several hundreds and lays them singly in loose clusters on water (Toply, 2008). The eggs are pointed at both ends and have a pair of lateral air floats. They lie horizontally on water, and owing to surface tension, they form geometrical patterns lying in loose clusters (Jordan and Verma, 2011).

Species of *Anopheles,* which are common in warm climates, lay their eggs on the open surface of water or attach them to some partially submerged object. When eggs are laid on water they may hatch in few days, or even within 24 hours, but those laid out of water lie unhatch until submerged which may be weeks or months.

2.4.2 Larvae

The larvae occur in a wide range of habitats, but most species prefer clean, unpolluted water. They have been found in freshwater or salt water marshes, mangrove swamps, rice-fields, grassy ditches the edges of streams and rivers, and small temporary rain pools. Many species

prefer habitats with vegetation. Others prefer habitats with none. Some breed in open, sun lit pools, while others are found only in shaded breeding sites in forests. A few species breed in tree holes or the leaf axils of some plants.

The larvae of *Anopheles,* which are aquatic and are well known as wrigglers or wiggle–tails and when first hatch, are almost microscopic, but they grow rapidly to a length of 8 to 15 or 20mm (Toply, 2008). The larvae have well-developed head with mouth brushes used for feeding, a large thorax with no legs and a segmented abdomen. Larvae breathe through spiracles located on the eighth abdominal segment, or through a siphon and therefore must come to the surface frequently. The larvae spend most of their time feeding on algae, bacteria and other micro-organisms in the surface microlayer. Unlike the larvae of other genus, the *Anopheles* larvae rest horizontally and live below the surface only when disturbed. They swim either through propulsion with the mouth brushes, or by Jerky movements of the entire body, giving them the common name of "Wrigglers" or "Wigglers". The larvae develop through four stages, or instars, after which they metamorphose into pupae. At the end of each instar, the larvae molt, shedding their skin to allow for further growth.

Jordan and Verma (2011) had added that there is no respiratory siphon in the larva of *Anopheles* but on the eighth segment it has a raised chitinous quadrilateral plate with two spiracles. The spiracles are surrounded by fine small leaf-like flaps. There are small bristles near the spiracles which form a pair of pectin. The comb is formed by rows of bristles on thesegments. The larva has palmate bristles or hairs forming a pair of tufts on the thoracic and most of the abdominal segment. Chandler and Read (1961) and Jordan and Verma (2011) reported that the larva of *Anopheles* hang horizontally from the surface of water by

means of the palmate bristles and the quadrilateral plate of the eighth segment. The development is slow and the larval life lasts for two to four weeks.

2.4.3 Pupae

Anopheles pupae are comma–shaped and are called "tumblers". The head and the thorax are merged into a cephalothorax with the abdomen underneath. As with the larvae, pupae must come to the surface frequently to breathe, which they do through a pair of respiratory trumpets on the cephalothorax (Toply, 2008).

The respiratory trumpets are short and broad with a large terminal opening; from this opening a split or cleft runs down on one side. The abdomen is strongly curved and the abdominal paddles at their tips have one large and one small bristle (Jordan and Verma, 2011). The pupae have pair of peg-like spines at the posterior ends of all but the last abdominal segments. The pupal period lasts from two to seven days. After a few days, the pupa rises to the water surface, the dorsal surface of the cephalothorax splits and the adult emerges.

2.4.4 Adults

The adult *Anopheles* emerges from the cephalothorax, after its exit, it rests a few moments on the old pupal skin, dry its wings and then take flight, (Sevice, 1980).

The compound eyes of adults develop in a separate region of the head (Jordan and Verma, 2011). New ommatidia are added in semi circular rows at the rear of the eye; during the first phase of growth, this leads to individual ommatidia being square, but later in development they become hexagonal. The hexagonal pattern will only become visible when the carapace of the stage with square eyes are molted. The head also has an elongated, forward–projecting "stinger – like" proboscis used for feeding, and two sensory palps. The maxillary palps of the

males are longer than their proboscis. As with many members of the mosquito family, the female is equipped with an elongated proboscis that she uses to collect blood for development of eggs (Jordan and Verma, 2011).

The thorax is specialized for locomotion. Three pairs of legs and a pair of wings are attached to the thorax. The wings are outgrowth of the exoskeleton. The wings allow themosquito to fly for up to four hours continuously at 1 to 2 km/hour travelling up to 12km in one night. The abdomen is specialized for food digestion and egg development. This segmented body part expands considerably when a female takes blood meal. The blood is digested over time serving as a source of protein for the production of eggs which gradually fill the abdomen.

The adult *Anopheles* has been described by Chandler and Reed (1961), Gilles *et al.* (1965), Gilles and Coetzee (1987), Toply (2008) and Jordan and Verma. (2011). It has a slender body except when the abdomen is filled with blood. In males, the maxillary palps are longer than the proboscis and are five-jointed; the last two joints are flat and broad so that the palps appear club – shaped. In the female the maxillary palps are always more than half as long as the proboscis, but usually as long as the proboscis. On the thorax, the scutellum is crescentric with bristle on its posterior margin. The wings are marked with dark spots and the sterna have no scales. The adult rests with its body parallel to the surface and its proboscis is not in straight line with its body. Some *Anopheles* such as *An. culicifaces* rests parallel to the surface it rests on (Toply, 2008).

The duration from egg to adult varies considerably among species, and it is strongly influenced by ambient temperature. They can develop from egg to adult in as little as five days, but it can take 10-14 days in tropical conditions (Jordan and Verma, 2011).

Adult *Anopheles* can be distinguished from other mosquitoes by the palps, which are as long as the proboscis, and by the presence of discrete blocks of black and white scales on the wings. Adults can also be identified by their typical resting position: males and females rest with their abdomens sticking in air rather than parallel to the surface on which they are resting (Toply, 2008).

Adults usually mate within a few days after emerging from the pupal stage. In most species, the males form large swarms, usually around dusk, and the females fly into the swarms to mate. Males usually live for about a week, feeding on nectar and other sources of sugar. Females also feed on sugar sources for energy, but usually require a blood meal for development of eggs (Toply, 2008; Jordan and Verma, 2011).

The cycle repeats itself until the female dies. Females can live longer than one to two weeks in nature. Their life spans depend on temperature, humidity, and their ability to successfully obtain blood meal while avoiding host defenses. Females carrying malaria parasites aresignificantly more attracted to human breath and odours than uninfected (Science, 2013).

2.5 Habitat of *Anopheles*

Anopheles dwell in marshy places, but some species like *An. stevensi, An. quadrimaculatus*, invade human dwellings in suburban and rural areas. They breed mostly in natural waters such as ponds, swamps, rice fields and grassy ditches, but some species breed in flowing streams, like *An. listoni* in the Himalayan streams. *An. rossi,* in temporary rain filled pools, and *An. stevensi* breeds in wells (Jordan andVerma, 2011).

The breeding places of *Anopheles* include practically any kind of water except open sea; some species show very little preference whereas others seem to be unreasonably choosy (Chandler and Read, 1961). Some species breed only in pure, clear water, others prefer filthy water; some breed only in sunlit water, only in shade; some demand quiet water, others breed only in flowing streams.

Although malaria is nowadays limited to tropical areas, most notoriously the regions of sub-Saharan Africa, many *Anopheles* species live in colder latitudes. Indeed, malaria outbreaks have in the past, occurred in colder climates, for example during the construction of Rideau Canal in Canada during the 1820s (William, 1983).

2.6 Habits of *Anopheles*

2.6.1 Food Habits

Heretically, mosquitoes feed mainly on plant juice. Most *Anopheles* larvae, however, are able to use a considerable variety of food though human blood is usually preferred. Most hibernating larvae return to the bottom of their breeding pools and most survive the cold either as adults or as larvae but not as eggs. *Anopheline* mosquitoes generally hibernate as adults, hiding in trees, rocks and caves (Chandler and Read, 1961).

Most *Anopheles* are crepuscular (active at dusk or dawn) or at nocturnal (active at night). Some feed indoors (endophagic), while others feed indoors (exophagic). After feeding, some *Anopheles* prefer to rest indoors (endophilic), while others prefer to rest outdoors (exophilic), though this can differ regionally based on local ecotype, and vector chromosomal makeup, as well as housing type and local microclimatic conditions. Biting by nocturnal, endophagic *Anopheles* can be markedly reduced through the use of insecticide-treated bed nets. Because

transmission of disease requires ingestion of blood, the gut flora may have abearing on the success of infection of the host (Wang, 2011). The larval and pupal gut is largely colonized by photosynthetic cynobacteria, while in the adult, proteobacteria and bacteroidetes predominate. Blood meals drastically reduce the diversity of organisms and favour enteric bacteria (Chandler and Read, 1961) and (Jordan and Verma, 2011).

2.6.2 Length of Life

The length of life of *Anopheles* varies with the species and the sex. The male seldom live more than 1 to 3 weeks; their duty in life is done when they have fertilized the females. They die soon after all their eggs are laid; with plenty of blood meals and readily available breeding places this may be only 3 or 4 weeks, whereas under less favorable conditions it may be several months (Chandler and Read,1961) and (Jordan and Verma, 2011).

2.7 Biological Races of *Anopheles*

A number of species of *Anopheles* species show only very slight morphological differences but which may differ sufficiently in their biological characters to make them very good or very unimportant malaria vectors (Chandler and Read, 1961).

In the Northern hemisphere *An. maculipennis* is such a species. It has races which differ in such characters as colour of the eggs and the number of teeth on the maxillae of the adults, and biologically in choice of breeding places, hibernation, and willingness to feed on man. In Europe *An. milabranchiae*, which breeds in both fresh and brackish water, has a wedge – shaped black spots on the eggs (Jordan and Verma, 2011).

Other species of *Anopheles* that have races that differ in food preferences or other characteristics that influence their importance as malaria vectors are *An. sinensis, An.*

maculates, An. aquasalis, An. pseudopunctuipennis, An. gambiae and *An. funestus* (Toply, 2008).

2.8 Species of *Anopheles*

There are approximately 3,500 species of mosquitoes grouped into 41 genera. Human malaria is transmitted only by females of the genus *Anopheles*. Of the approximately 430 *Anopheles* species, only 30-40 transmit malaria in nature. Anophelines are found worldwide even in areas where malaria has been eliminated except Antarctica. The latter areas are thus constantly at risk of re-introduction of the disease (Toply, 2008).

The genus *Anopheles* has many members that have been described (Jordan and Verma, 2011). *An. gambiae s.l* and *An. arabiensis* are the most broadly distributed and the most important vectors of malaria in Africa due to their highly anthropophilic nature. *An. gambiae* and *An. funestus* are *Plasmodium* transmitters in sub-Saharan Africa (Gilles *et al.,* 1968).

2.8.1 *Anopheles gambiae sensu lato*

Anopheles gambiae s.l is a complex of at least seven morphologically distinguishable species. This complex was recognized in the 1960s and includes the most important vectors of malaria in sub-Saharan Africa (Anara, 2011). This species complex consists of *An. arabiensis, An. bwambe, An. merus, An. melas, An.quadriannulatus* A, *An. quadriannulatus* B and *An. gambiae sensu stricto* (Besansky *et al.,* 1994). A review of *An. gambiae* complex (Molineaux and Gramiccia, 1980) gave the main differences between the two species with respect to transmission as follows:

a. *An. gambiae s. s* is strongly and uniformly anthropophilic and endophilic; *An. arabiensis* is less anthropophilic and endophilic but with considerable geographical variation (related to cytogenic variation);
b. Where the two species are, *An. arabiensis* is probably the longer-lived, but the evidence is indirect, limited and sometimes discordant;
c. Both the species have probably the same intrinsic susceptibility to infection with *P. falciparum*, but here are also the evidence is limited and sometimes contradictory;
d. Residual insecticides readily control populations of *An. gambiae s.s* but their effect on populations of *An. arabiensis* varies with degree of endophily.

Despite being morphologically indistinguishable, individual species of *An. gambiae* complex exhibit different behavioural traits. For example, the *An. quadriannulatus* is generally considered to be zoophilic whereas *An. gambiae s.s* is generally anthropophilic (taking blood meal from humans). A new cryptic subgroup – the Goundry subgroup of *Anopheles gambiae s.s* has been described (Anara, 2011).

An. gambiae s.s has been discovered to be currently in a state of diverging into two different strains – the Mopti (M) and Savannah (S) strains – (though as of 2007, the two strains were still considered as a single species). Currently, *An. gambiae* (M-form) is named as *An. coluzzi.* Reproduction isolation between the two forms is complete (Favia *et al*., 2001) and that the two forms represent distinct species (Coetzee, 2013). All current work assumes the two rarely hybridize (Talihun, 2013) and further explained that (i) there is a significant exchange of genes between the two forms, eventhough (ii) hybrids have reduced fitness and (iii) the gene exchange process is spatially and temporarily dynamic. The M-and-S forms have been the focus of intense study by malaria researchers and evolutionary biologists

interested in ecological speciation. The M-and-S forms have been the subject of intense study over the past decade. The focus has centred on models of the evolution and maintenance of genetic divergence between the two forms in relation to speciation (Lanzaro and Lee, 2013).

Divergence occurs at three discrete islands in genomes that are otherwise nearly identical. An ‘’island of speciation model’’ proposes that diverged regions contain genes that are maintained by selection in the face of gene flow. An alternative ‘’incedental island model’’ maintains that gene flow between M-and-S forms is effectively zero and that divergence islands are unrelated to speciation. A ‘’divergence island and single nucleotide polymorphism’’ explore the spatial and temporal distributions of hybrid genotypes. A temporal analysis revealed that assertive mating is unstable and periodically breaks down, resulting in extensive hybridization (Talihun, 2013). The first genome-wide comparison of the M-and-S forms by Turner *et al*. (2005) was consistent with earlier observations that divergence overall is low, but there are small, discrete regions of divergence representing about 3% of the genome. They identified three diverged regions: one near the centromere on the X chromosome, one on the left arm of chromosome 2 (2L), and one on the right arm of chromosome 2 (2R). A number of genome-wide scans comparing M-and-S forms have been conducted since. In 2008, several reports of much higher frequencies of M/S hybrid appeared. These were all observed in populations in coastal West Africa, an area where now thought to represent a zone of secondary contact (Caputo, 2011). These reports resulted in the emergence of this species as focus of research aimed at exploring the evolution and maintenance of genetic divergence within the gene flow.

The *An. gambiae s.s* genome has been sequenced, though there is controversy over the choice of strain used, which is considered a hybrid of two different strains (Externe, 2011).

Currently, 290 mRNA have been predicted (38 miRNA officially listed in miRBase) for *An. gambiae s.s* based upon conserved sequences top miRNA found in *Drosophila.* The mechanism of species recognition appears to be sounds emitted by the wings and identified by Johnston's organ (Pennetier *et al.,* 2009).

Anopheles gambiae invaded northeastern Brazil in 1930, which led to a malaria epidemic in 1938/1939 (Killeen, 2003). The Brazilian government, assisted by the Rockfeller foundation in a program spearheaded by Fredrick Super, eradicated these mosquitoes from the area. This effort was modeled on the earlier success in eradication of *Aedes aegypti* in yellow fever control program. The exact species involved in this epidemic was identified as *An. arabiensis* (Parmakelis *et al*., 2008).

2.8.1.1 *Anopheles arabiensis*

Of the seven species complex of *An. gambiae s.l, An. arabiensis* and *An. gambiae s.s* are the most important vectors of malaria in sub-Saharan Africa (Hunt *et al.,* 1998). The two species of the complex are distributed and the most efficient vectors of malaria (Coetzee *et al.,* 2000).

Anopheles arabiensis is everywhere; the abundance of this species increased markedly in the wet season in West Africa. The species is found to be less anthropophilic than *An. gambiae s.s* (Molineaux and Grammicia, 1980). The level of transmission by *An. arabiensis* is certainly high. It is more abundant in the Sudan Savannah of West Africa and this corresponds to what was known regarding the geographical distribution of its members (Molineaux and Grammicia, 1980).

In Nigeria *An. arabiensis* exists most in drier savanna, in semi – arid and arid areas (Coluzzi *et al.,* 1987). Onyabe and Conn (2001) and Ndams (2004) reported the abundance of *An. arabiensis* across Nigeria and in particular the Southern and Northern Guinea savanna, respectively. More recently Tukur (2010) reported that *An. arabiensis* was found predominantly in Sudan and Sahel Savannahs and it shared habitat with *An. gambiae s.s* in Guinea Savannah.

2.8.1.2 *Anopheles gambiae sensu stricto*

Anopheles gambiae sensu stricto is one of the *An. gambiae* complex identified in the 1960s and is one of the most important vectors of malaria in sub-Saharan Africa (Anara, 2011). *An. gambiae s.s* is generally anthropophilic.

More than 90% of annual malaria incidence is in Africa where the major vector is *An. gambiae* s.s (Martinez – Torres *et al.,* 2002). M-form is the most important and widespread malaria vector in Angola.

In Nigeria, *An. gambiae s.s* was found to be transmitting malaria than *An. arabiensis* and was found to be more anthropophilic than *An. arabiensis* in Garki, northern Nigeria (Molineaux and Grammicia, 1980). *An. gambiae s.s* was, however, found in nearly every single village from which at least 20 mosquitoes were identified and probably present everywhere; and there was some variation between villages with respect to the relative abundance, although no geographical pattern was detected in Garki, Jigawa State. The relative abundance of the species increased markedly in the wet season; this increase was more pronounced towards the middle of the main breeding season. The proportion of *An. gambiae s.s* in the total of *An.*

gambiae s.l varied according to method of collection from year to year (Molineaux and Grammicia, 1980).

2.8.2 *Anopheles funestus*

The possibility of exophilic transmission was considered and attention focused on a hitherto unrecognized outdoor biting member of the *Anopheles funestus* subgroup closely resembling *An. aruni*. This species called *An. aruni* was found to bite man readily out of doors and to be fully receptive to *Plasmodium falciparum.* In adult stage *An. aruni* can be distinguished from *An. funestus.* Workers in Zimbabwe have found that the polytene chromosomes and spermatogenesis of hybrids show *An. aruni* and *An. funestus* to be separate species (Bensaky, 2011).

An. funetsus is a major vector in Africa. It belongs to a group of siblings species that can be identified morphologically only at certain stages of their development. A diagnostic polymerase chain reaction (PCR) – based tool made it possible to differentiate five species of the group. The assay seems to be applicable over all their distribution area for four of these species: *An. funestus, An. leesoni, An. parensis* and *An. vaneedenip.* The fifth species, *An. rivolorum,* is the second most abundant species of the group and can be mistaken at its adult stage for the major vector of malaria *An. funestus* (Frederic *et al.,* 2003).

The molecular and morphological observations of specimens identified as *An. rivolorum* from Cameroon and South Africa showed that they belong to two different taxa. The species identified in Cameroon, and named here *An. rivolorum* – like, might extend to Western Africa and Central Africa. The species – specific PCR assay is supplemented by a primer specific to *An. rivolorum* – like might extend to Western Africa and Central Africa. The

species – like PCR assay is supplemented by a primer specific to *An. rivolorum* – liked and thus makes it possible to differentiate the five species of the *An. funestus* group and the newly defined taxon (Frederic *et al.*, 2003).

2.8.3 Control of *Anopheles*

Factor affecting a mosquito's ability to transmit malaria include its innate susceptibility to *Plasmodium*, its host choice and its longevity. Factors that should be taken into consideration when designing a control programme include the susceptibility of malaria vectors to insecticides and the preferred feeding and resting location of adult.

Jordan and Verma (2011) reviewed control of *Anopheles* and other mosquitoes in general. Control or protection against mosquitoes and mosquito – borne disease may be undertaken in the following ways:

i. In mosquito – infested area, protective clothing may be used such that will cover the exposed parts of the body, especially after sunset.
ii. Mosquito repellents are also useful, like mosquito cream, citronella, odomus and indalone which keep mosquitoes away.
iii. While sleeping in fine mesh mosquito nets to prevent them from biting; bed rooms or houses could be screened to prevent entry of mosquitoes.
iv. Painting walls with creosote repels mosquitoes.

The immediate destruction of adult *Anopheles* with residual sprays such as DDT or other chlorinated suspensions or emulsions at the rate of 200mg per square foot of the insecticide are needed. The surfaces sprayed are lethal for 3 to 8 months to *Anopheles* resting on them (Chandler and Read, 1961).

Residual spraying of houses has given sensational success against vectors that habitually enter houses and rest in them; *An. darling, An. quadrimaculatus, An. gambiae* and *An. funestus* are quickly reduced to a position of minor importance. Spraying chemicals of little or no use against the mosquitoes make them to develop three possibilities:

a. That some of the house – frequenting species may develop bite-and-run habits as a result of natural selection and survival of the fleets, just as they ones substituted domesticity for a sylvarian life before human houses were converted into lethal traps;
b. That some of the bite-and-run species, when their house-frequenting competitors are eliminated, may become of greater importance; and
c. Mosquitoes become resistant to DDT and allied chemicals. Up to the present time only a few species of *Anopheles* have developed any appreciable degree of resistance and only locally (Chandler and Read, 1961).

2.8.3.1 Destruction of Larvae

Coatrey (1968) had pointed that was easier and more effective to kill mosquitoes in their larval forms than as adults and several methods are used with success:

a. Oiling: The breeding places of *Anopheles* are sprayed with petroleum oils. The oil films formed on the surface of water asphyxiate the larvae and pupae.

b. Panama larvicide: This is a mixture of caustic soda, resin, and phenol in water. It has been used most effectively in the Panama region. The Panama larvicide mixes well with water and kills both the larvae and the algae on which they feed. One part of Panama larvicide is sufficient for 10,000 parts of water.

c. **Paris green:** This is a powder of arsenic mixed with fine dust; one part of powder with 100 parts of dust. This can be thrown in the wind and it will cover the surface of a pond; it is insoluble in water and remains floating and is eaten by surface feeding larvae of *Anopheles;* it will kill the larvae but not pupae.

2.8.3.2 Natural Enemies

Some fishes are of great value in control of mosquito larvae in natural waters. The viviparous *Gambusia affinis* is valuable because of its hardiness on feeding on larvae. Many other water inhabitants attack mosquito larvae or eggs, including predaceous insects, bugs, mites, etc. Bladderwort and *Utriculatia* capture the larvae in their trap like bladders, and surface covering plants such as *Lemna* (duckweed) by preventing the larvae from getting access to air (Edington *et al*., 1976).

2.8.3.3 Elimination of Breeding Places

For those mosquitoes which breed in rain–filled containers and cisterns, emptying of water is effective. For large ponds and swamps digging a sloping ditch removes large volumes of water; small ponds can be filled up with mud (Edington *et al*., 1976).

2.8.4 Malaria Transmission

Malaria is caused by species of *Plasmodium,* which are transmitted via the bites of infected *anopheline* mosquitoes. In the human body, the *Plasmodium* multiply in the liver, and then infect red blood cells (Taiwo, 2010). A single bite of *Anopheles* can introduce the life threatening *Plasmodium,* which if not promptly treated can lead to death or cerebral disorder due to their complex make up that tend to quickly attack the human brain (Florence, 2007).

Malaria is the world's most important tropical disease. It is endemic in the poorest countries of the world causing 300 to 500 million deaths each year (W.H.O, 2001). More than 90% of malaria deaths occur in sub-Saharan Africa and almost all the deaths are children younger than 5 years (Macdonald, 1982). Malaria is a major cause of infant mortality and is the only insect – borne parasitic disease comparable in impact to the world's major killer transmissible diseases (Curtis, 1996).

Over the last two decades, morbidity and mortality from malaria have been increasing due to deteriorating health systems (Brogdon and McAllister, 1998). Quantitatively, the number of foci of intense malaria transmission is increasing because of changing environmental conditions, especially in areas of intense economic development (WHO, 1993).

Determination of risk of malaria transmission requires quick and accurate methods of assessing transmission intensity, which is a product of man-biting rates and rate of infectivity of vectors, especially when targeting a vector control (WHO, 1975).

To determine the infection status of suspected mosquito vector species, microscopic examinations and dissection are accurate but time-consuming methods. However, more rapid test kits and automated procedures are now available (Marcel and Don, 2008).

These include the standard circum sporozoite (CS) protein by ELISA for malaria parasite detection anopheline in mosquitoes, considered to be the 'gold standard' although it takes 4 – 6 hours to run, requires equipment, electric power supply, refrigerated storage of reagents and specialized personnel (Beier and Koros, 1991). *Anopheles* infection rate, together with concurrent human-landing density data provide valuable parameters for estimating the

intensity of transmission and entomological inoculation rates to serve as a relative measure of malaria risk among human populations (Killen *et al*., 2000).

Long ago, malaria was differently named as dengue fever, marsh fever, intermittent fever, jungle fever, etc. The name malaria (Gr. mala=bad; aria=air) was given by Maccloch (1827) on the presumption that it was caused by the bad air of marshy localities. Laveran (1880) first reported the malaria parasites in the blood of a malaria patient. But the way of entry of the parasite into human blood remained a mystery for a long time. However, Pfeiffer (1892) suggested the role of some blood sucking insect in the transmission of malaria. Patrick (1894) suggested the role of mosquitoes in the transmission. Working on this suggestion, Sir Ronald Ross succeeded in establishing that malaria parasites are sucked up by female *Anopheles* mosquito and later on injected in the human blood. Thus, mosquito – malaria relationship was established. Then, Grassi (1898) worked out the complete life cycle of the human malaria parasite in the *Anopheles* mosquito.

Short (1948) and Gornham (1954) discovered that the parasite first enters the liver cells to undergo pre-erythrocytic schizogony before invading the red blood capsules (Jordan and Verma, 2011).

2.8.5 Trophozoite-induced Malaria

Man acquires infection by the bite of infected female *Anopheles.* However, infection may also be transmitted by:

i. Transfusion of blood from a malaria patient. This is known as transfusion malaria. *Plasmodia* can remain viable in refrigerated blood for up to 10 days.

ii. Transmission of infection to foetus in uterus through some placental cells. This is known as congenital malaria.

iii. By the use of contaminated syringes particularly in drug addicts.

The above conditions are known as trophozoite – induced malaria (Arora and Arora, 2010). In this condition there is no primary and secondary erythrocytic schizogony, incubation period is short and there is no relapse (Sarvice, 1980).

After an incubation period of 12 days for *P. falciparum,* 13 – 17 days for *P. vivax* and *P. ovale,* and 28 – 30 days for *P. malaria*, the patient develops malaria. The typical picture of malaria consists of febrile paroxysm, anaemia and splenomegaly (Clark and Alleva, 2004).

Febrile paroxysm begins in the early afternoon and comprises of three successive stages – cold stage, lasting for 15 – 60 minutes, when the patient experiences intense cold and shivering. This is followed by hot stage, lasting for 2 – 6 hours, when the patient feels intense heat. The patient develops high fever, severe headache, nausea and vomiting. Thereafter, fever leads to crisis accompanied by profuse sweating (Macdonald, 1982).

After a few paroxysms, anaemia develops. *P. vivax* and *P. ovale* infect only reticulocytes, therefore, the parasitaemia is usually limited to around 2 – 5% of available Red Blood Cells. *P. malariae* invades primarily the older RBCs, so that the number of infected cells is limited. *P. falciparum* tends to invade all ages of RBCs, and the proportion of infected cells may exceed 50% (Brogdon and McAllister, 1998). Splenomegaly is due to massive proliferation of macrophages which phagocytose both parasitized and non-parasitized red blood cells.

After a few paroxysms, spleen gets enlarged and becomes palpable (Brogdon and McAllister, 1998).

2.8.6 Pernicious Malaria

Pernicious malaria is a complex of life-threatening complications that sometimes supervene in acute *P. falciparum* malaria; it is due to heavy parasitization and is of three types (cerebral malaria and algid malaria as described by Clark and Alleva, (2004).

2.8.7 Cerebral Malaria

Cerebral malaria is a severe complication of *P. falciparum* malaria and frequently leads to death, even when appropriate therapy has been given. It is characterized by hyperpyrexia, coma and paralysis. Capillaries of the brain are plugged with parasitized red blood cells. In holoendemic areas of malaria, it occurs in children between 6 months and 5 years (Arora and Arora, 2010).

2.8.8 Innate Immunity

This refers to inherent, non immune mechanisms of host defense against malaria which is due to age of red blood cells, nature of haemoglobin, enzyme content of red blood cells and presence or absence of certain factors as described by Clark and Alleva (2004) and Arora and Arora (2010). They have proceeded to categorised them as;

- **Age of red blood cells:** *Plasmodium falciparum* infects both young and old erythrocytes while *P. vivax* and *P. ovale* infect only young erythrocytes and *P. malariae* infects only young erythrocytes.
- **Nature of Haemoglobin:** Presence of abnormal haemoglobin like thalassemia haemoglobin and foetal haemoglobin confers resistance against all *Plasmodium*

species, while sickle cell anaemia trait and haemoglobin E protect against *P. falciparum* and *P. vivax* respectively.

- **Enzyme Content of Red Blood Cells:** A genetic deficiency known as glucose – 6 – phosphate dehydrogenase (G6PD) trait confers some protection against *P. falciparum* infection. This enzyme is essential for respiratory process of the parasite.
- **Presence or absence of certain factors:** Presence of the Duffy factor increases the susceptibility to malaria. It is believed that Duffy factor present on the surface of erythrocytes acts as receptor for attachment of malaria parasite.

2.8.9 Acquired Immunity

Acquired immunity in malaria involves both humoral and cellular immunity. (Arora and Arora, 2010) who further explained that the antibodies against sporozoites of sexual and asexual blood stages develop in malaria patients where a variety of cellular mechanisms may play role in conferring protection against malaria. These include production of natural killer activity and activated macrophages. The latter phagocytose and induce extra cellular killing of target cells (Arora and Arora, 2010).

Immunity produced following infection with malaria parasites is species specific, stage – specific and strain – specific and the immunity lasts only till original infection remains active. This is known as concomitant immunity (Peter *et al*., 1998).

Parasite antigens on the infected red cell surface are targets for naturally acquired immunity to malaria. *Plasmodium falciparum* erythrocyte membrane protein1 (PfEMP1) is a potentially important family of target antigens, because these proteins are inserted into the

red cell surface and are prominently exposed and because they are highly polymorphic and undergo clonal antigenic variation. Anti-PfEMP1 antibodies agglutinate infected erythrocytes in a variant-specific manner. The apparent selective pressure exerted by established anti-PfEMP1 antibodies on infecting parasite supports the idea that such responses provide variant-specific protection against the infection (Peter *et al*., 1998).

2.9 Genetic Factors that Protect Against Malaria

Plasmodium vivax is rarely found in West Africa or other places where the red blood cells of the population lack the Duppy blood group antigens Fya and Fyb. The glycophorin receptors which *P*. *vivax* needs to attach to and invade red cells are missing on Duffy negative red cells. The protection is absolute and afforded only to homozygotes.

Persons with the haemoglobin genotype HbAS (sickle cell trait) are protected against severe *falciparum* malaria. Parsitemia is lower because HbAS cells sickle in the circulation and are removed by the spleen before the parasite can develop into schizonts. Sickle cell anaemia (HbSS) is not protective and can cause fatalities in young children with *falciparum* malaria (Toply, 2008).

Persons with ovalocytosis have lower parasite densities of *P*. *falciparum* and *P*. *vivax* Elliptical red cells resist parasitic invasion. In areas of South East Asia and Papua New Guinea where *falciparum* is endemic there is a high prevalence of ovalocytosis.

Newborn infants have protection against malaria in their first months of life when there is a high concentration of HbF in their red cells. Malaria parasites do not grow well in red cells containing HbF (Arora and Arora, 2010).

Possession of certain human leucocyte antigens are also thought to protect against severe malaria, such as class I antigen HLA-BW53 and class II antigen HLA-DRB1 1302 (Arora and Arora, 2010).

2.10 Malaria, a Fatal Disease

Over the last two decades, morbidity and mortality from malaria have been increasing due to deteriorating health systems. It is the world's most important tropical disease. It is endemic in the poorest countries of the world, causing 300 to 500 million deaths each year (WHO, 2011). Curtis (1996) reported that more than 90% of malaria deaths occur in sub – Saharan Africa and almost all the deaths are children younger than 5 years. Malaria is a major cause of infant mortality and is the only insect borne parasitic disease comparable in impact to the world's major killer transmissible diseases (WHO, 2011).

Malaria is a devastating disease that causes more than one million deaths per year, mainly among children in third world countries. Despite many efforts to control the disease with antimalarial drugs and insecticides to eliminate mosquito vectors (Ana and Wing, 2008).

Worldwide, some two billion individuals are at risk; 100 million develop overt clinical disease and 1.5–2.7 million die every year. Nearly 85% of the cases and 90% of carriers (many asymptomatic) are found in tropical Africa. The incidence of malaria is increasing due to resistance of vectors to insecticides and drug resistant parasite (Clark and Alleva, 2004).

Historically, malaria has rivaled tuberculosis as the leading cause of human death by infectious diseases. The incidence of malaria was greatly diminished in the 1960s by insecticides that reduced carrier populations of *Anopheles* mosquitoes and by drugs that

killed *Plasmodium* in humans. But the emergence of resistant varieties of both *Anopheles* and *Plasmodium* has led to a resurgence of malaria. About 300 million people in the tropics are now infected (Neil *et al.,* 2008).

Malaria is a major global public health burden with 3.3 billion people (half the world´s population) living in areas at risk of malaria transmission in 106 countries and territories (Azoma and Job, 2013). As at 2010, it was estimated that malaria caused an estimated 216 million clinical episodes and 655,000 deaths, all from Africa. About 86 per cent of deaths globally were in children. More than 90% of the current annual malaria incidence is in Africa where there are 500 million clinical cases with up to 2 million deaths (Martinez – Torres *et al.,* 2002).

Kenya, like many other of its sister African countries, faces its own malaria burden as about 39,000 children reportedly die of malaria every year. Available statistics reveal that malaria prevalence in the country is 20 per cent, affecting mostly young children and pregnant women (Azoma and Job, 2013).

In Nigeria, malaria is known to affect a high population of people. Women, particularly pregnant women, and children aged 0–5 years are among the worst hit by the mosquito – borne ailment (Florence, 2007). One out of every three children in Jigawa State that dies before his or her fifth birthday is killed by malaria. Eleven per cent of the maternal mortality in the state is also due to malaria, and households spend at least 25 per cent of their income on malaria treatment (Taiwo, 2010).

2.11 Control of Malaria

Malaria is a global problem and certain countries like India face a widespread infection with this disease (Jordan and Verma, 2011). With the assistance of WHO, the Ministry of Health Government of India started a National Malaria Control Programme (NMCP) in the year 1953. Under this programme effective measures were taken and malaria was almost controlled because DDT and other insecticides used were very much effective in eradicating the mosquitoes. But in recent years, the cases of malaria are frequently witnessed and are rapidly increasing again. It appears that the mosquitoes have developed resistance and immunity to DDT and similar insecticides (Jordan and Verma, 2011).

Reducing the suffering and loss of life caused by malaria is possible, providing the financial, political, and technical commitment to achieve this is strengthened (Cheesbrough, 2005). The WHO/UNICEF/UNDP and World Bank *Roll Back Malaria Partnership*, the *Global Fund to fight AIDS, Tuberculosis, and Malaria*, the *Medicines for Malaria Venture*, the *Gates Malaria Partnership* and the *Multilateral Iniative* on *Malaria* have been established to reduce the burden of malaria by:

- implementing malaria control stratagies,
- improving health infrastructures,
- raising awareness of malaria and its effects on poverty and development,
- mobilizing communities to combat malaria,
- raising funds to effect and sustain malaria control programmes and the development of antimalarial drugs and vaccines.

2.12 Indoor Residual Spraying (IRS)

Indoor residual spraying is the practice of spraying insecticides on the interior walls of homes in malaria affected areas. Efforts to eradicate malaria by eliminating mosquitoes in conjunction with the monitoring and treatment of infected humans have been successful (Njau, 2009). After feeding, many mosquitoes rest on a nearby surface to digest the blood meal; so if the walls of dwellings have been coated with insecticides, the resting mosquitoes will be killed before they can bite another victim, transferring the *Plasmodium* (Alexandra, 2009).

Rulisa (2009) reported that the first pesticide used for Indoor Residual Spray (IRS) was DDT. Although, it was initially used exclusively to combat malaria, it is quickly spread to agriculture. In time, pest-control, rather than disease control, led to the evolution of resistant mosquitoes in many regions. The DDT resistance shown by *Anopheles* mosquitoes can be compared to antibiotic resistance shown by bacteria. During 1960s, awareness of the negative consequences of its indiscriminate use increased, ultimately leading to bans on agricultural application in many countries in 1970s.

World Health Organization currently advises the use of 12 different insecticides in IRS operations, including DDT as well as alternative insecticides such as the pyrethtroids, permethrin and deltamethrin (Njau, 2009).

One problem with all forms of IRS is insecticide resistance via evolution of mosquitoes. The descendents of endophilic species are trending towards becoming exophilic species, meaning that they are not as affected – if affected at all – by the IRS, rendering it somewhat useless as a defense mechanism (Njau, 2009).

2.13 Mosquito Nets and Bed clothes

Mosquito nets help keep mosquitoes away from people and greatly reduce the infection and transmission of malaria. Insecticide – treated nets (ITNs) are estimated to be twice as effective as untreated nets and offer greater than 70% protection compared with no net (Rulisa, 2009).

The United Nations International Children's Emergency Fund (UNICEF) has stated that malaria kills 660,000 people every year, most of them African children, adding that universal coverage of insecticide treated nets is the key in tackling the menace (Ibrahim, 2013). In 2004, there were just 5.6 million bed nets in sub – Saharan Africa, but by 2010, bulk buying made up the figure to 145 million (Ibrahim, 2013).

2.14 Transmission Blocking Vaccines

There is an urgent need for new strategies to control malaria, but there is lack of detailed knowledge of the basic biological process of *Plasmodium* that would allow faster development of anti-malaria drugs and vaccines (Ana and Wing, 2008). Despite very intensive research since the mid 1970s, no effective malaria vaccine is yet available (Arora and Arora, 2010).

The search for malaria vaccine has been hampered by the fact that *Plasmodium* lives mainly inside cells, hidden from the host's immune system. And like trypanosomes, *Plasmodium* continually changes its surface proteins. The urgent need for treatments impaired an ambitious effort to sequence *Plasmodium's* genome. Research has now tracked the expression of most of the parasite's genes at numerous points in its life cycle. This research could help identify vaccine targets (Neil *et al.*, 2008).

A new vaccine initiative has been launched to try to prevent the malaria parasite from developing inside mosquitoes. Once a mosquito bites a vaccinated person, it picks up a substance that interferes with the *Plasmodium* growth (Rhoel, 2010). There are different approaches to malaria vaccines. The malaria vaccines that are currently being struggled outside of this transmission – blocking vaccine are targeted toward different stages of the parasite life cycle, while the previous efforts targeted prevention of liver stage infection and blood stages of the parasite. So, if a mosquito bites an infected person who has been vaccinated, the parasite would not be able to develop inside host's blood.

Joe (2010) revealed that some vaccines are designed to target a mosquito molecule. So the vaccines intended to generate antibodies in the immunized individual, when taken up into the mosquito, along with the *Plasmodium* in the blood, will mask the molecule in the mosquito that the parasite needs to invade.

2.15 Traditional Medicine

The use of plants for medicinal purposes dates back to antiquity and has been very important in health care delivery of every nation at one stage or another. Herbs dispensed through the traditional way have been a good source of medication to sick people in almost all the societies of the world, despite the emergence of the orthodox medicine (Musa, 2013). The value of plants in traditional medicine cannot be over emphasized and is still the first point of healthcare for many people in Sub-Saharan Africa (WHO, 2007).

Most of the drugs that are use today are becoming less effective due to the problem of drug resistance. The spate of drug resistance by *Plasmodium* has necessitated the scientific evaluation of many traditional medicinal plants for an alternative antimalarial drug that is

effective, safe and affordable. Plants still provide a source for effective lead compounds against malaria (McMorran, 2009).

2.16 *Plasmodium*

In 1880, a French army surgeon, Charles Laveran found and described *Plasmodium.* The life cycle of *Plasmodium* was described by Italian scientists Amico Bignami, Battista Grassi, and Giovanni Bastianelli in 1898. Ronald Ross, an army surgeon in Indian Medical Service demonstrated that mosquitoes of *Anopheles* species act as vectors of *Plasmodium.* In 1976, Trager and Jensen cultured *Plasmodium in vitro* for the first time (Arora and Arora, 2010). Romanowsky developed a staining method of malaria parasite in 1891.

There are approximately 156 named species of *Plasmodium* which infect various species of vertebrates. Four are known to infect humans: *P. falciparum, P. malariae*, *P. ovale* and *P. ovale* and *P. vivax* (WHO, 2009). Of recent, *P. knowlesi*, a simian species has been implicated to cause infection in humans.

Plasmodium is an important protozoan parasite of man and the best known genus of the class Telosporea. One of the widespread diseases of man, malaria is caused by *Plasmodium.* Species of *Plasmodium* have been reported from reptiles, birds, and various mammals. *Plasmodium* is widely distributed in tropical and temperate countries of the world but no longer a problem in the colder countries of the world (Jordan and Verma, 2011). It is found in regions lying roughly between latitudes 60^{o} N and 40^{o}S (Coatney, 1968; Fong *et al.*, 1971).

The arthropod hosts are females of certain species of *Anopheles* mosquito. The predominant vectors are *An. gambiae, An. funestus, An. darlingi* and *An. punctulatus (*Gordon, 1962). Mc Gregor (1971) and Wilson (1973) described three main types of *P. falciparum* antigens, L (labile), R (resistant) and S (stable) by investing their storage properties. Butcher (1972) showed that chronic malarial infection lead to synthesis of antibodies with wide cross-reactivity, and felt that this may be encouraging from the point of view of malaria vaccine production.

2.17 Pathological Role of *Plasmodium*

Plasmodium depends upon the host for its nutritional requirements. It follows therefore, that the development of *Plasmodium* must, in part, depend on successful competition with the host for certain substances required equally by both. Direct observation of the circulation in severe malaria has shown that at a certain stage of infection a substance which has been identified as fibrin appears as a fine precipitate around the erythrocytes. The appearance of this fibrin is followed by clumping of the erythrocytes. The red cells coated with the precipitated "fibrin" become sticky to macrophages and phagocytosized as both parasitized and unparasitized cells (Edington and Gilles 1976). It is also possible that pyrexia develops as a sensitivity reaction to parasite protein which is realised together with the residual body. It has been shown that as the *Plasmodium* grows in the erythrocyte there is a drastic reduction in the haemoglobin content and pigment.

The symptoms of malaria appear when the merozoites, along with toxins, are liberated into the blood; they are then deposited in the spleen, liver and under skin. The accumulated toxins

cause fever in which the patient suffers from chills, shivering and high temperature with convulsion followed by profuse sweating (Jordan and Verma, 2011).

2.18 *Plasmodium* species and Distribution

Plasmodium falciparum and *Plasmodium vivax* are known to be widespread while *Plasmodium malariae* and *Plasmodium ovale* are underspread species. The genus *Plasmodium* is subdivided into the sub-genera, *Laverania* and *Plasmodium*.

2.18.1 *Plasmodium falciparum*

Plasmodium falciparum is found mainly in the hotter and more humid regions of the world. It is the main species found in tropical and subtropical Africa and parts of Central America and South America, Bangladesh, Pakistan, Afghanistan, Nepal, Sri Lanka, South East Asia, Indonesia, Philippines, Haiti, Solomon Islands, Papua New Guinea and many Islands in Malasia. It also occurs in parts of India, the Middle East and Eastern Mediterranean (Cheesbrough, 2005).

Plasmodium falciparum is very common in tropics; incubation is 1/6 to 1/5 of the erythrocyte, often there are two trophozoites in one corpuscle; schizont is 2/3 to ¾ of erythrocyte which is not enlarged; haemozin is black; erythrocytes not enlarged, they may even shrink and become greenish, they have no Schuffner's dots; in blood the schizont forms 8 to 36 merozoites which are not seen in peripheral circulation; gametocytes are crescentic occupying one side of the erythrocyte. It causes pernicious malaria or malignant tertian malaria fever almost continuously or from 24 to 48 hours. A very serious result is black water fever, a condition when wholesale destruction of patient's erythrocytes occurs and the liberated haemoglobin is excreted in urine (Jordan and Verma, 2011). Is the most widely

spread species in Nigeria and Jigawa State (Cheesbrough, 2005) and (Umar and Ginsau, 2012).

2.18.2 *Plasmodium vivax*

Plasmodium vivax is capable of developing in mosquitoes at lower temperatures than *P. falciparum* and therefore has a wider distribution in temperate and sub-tropical areas. It is the main *Plasmodium* species in South America (occurring as far as northern Argentina), Mexico, the Middle East, northern Africa, India, Pakistan, Sri Lanka, Papua New Guinea and the Solomon Islands. It is also found in parts of South East Asia, Indonesia, Philippines, Madagascar, Tropical and sub-tropical Africa, Korea and China (Cheesbrough, 2005).

Plasmodium vivax lives as an intracellular parasite in the red blood cell corpuscles (R.B.Cs) of man in the form of its mature adult condition, called trophozoite. The trophozoite is amoeboid, uninucleated having vacuolated and granular cytoplasm (Jordan and Verma, 2011).

The life cycle of *P. vivax* is digenic involving two hosts, man is known as the primary host and mosquito as the secondary host. The asexual phase of its life cycle is completed in man by schizogony (differentiated into exoerythrocytic schizogony involving pre and post – erythrocytic schizogonic cycles and erythrocytic schizogony) and sexual phase of its life cycle is completed in female *Anopheles* by gametogony, syngamy and sporogony (Cheesbrough, 2005).

2.18.3 *Plasmodium malariae*

Plasmodium malariae has a much lower prevalence than *P. falciparum* and *P. vivax.* It is found in tropical and sub-tropical regions. In tropical Africa it accounts for upto 25% of *Plasmodium* infections. It is also found in Guyana, India, Sri Lanka and Malaysia where it accounts for less than 10% of *Plasmodium* infections (Cheesbrough, 2005).

Plasmodium malariae has an incubation period is 27 to 37 days; ring–shaped trophozoite, is 1/3 to ½ the size of the erythrocyte; schizont fills the erythrocyte which is not enlarged; haemazoin is dark brown; erythrocyte has no Schuffners dots; in blood the schizont forms 6 to 12 merozoites. It causes quartan malaria fever every 72 hours.

2.18.4 *Plasmodium ovale*

Plasmodium ovale has a low prevalence. It is found in West Africa where it accounts for upto 10% of malaria infections and has also been reported from other parts of Africa and from the Philippines, Indonesia, China and parts of the Fareast, South Asia and South America (Cheesbrough, 2005).

Plasmodium ovale is sporadic in tropical and subtropical zones; incubation period 14 days; ring – shaped trophozoite is 1/3 to ½ of the erythrocyte; schizont fills ¾ of the erythrocyte which become enlarged and irregular in shape; haemozoin is dark brown; the enlarged erythrocytes have Schiffner's dots; in blood the schizont forms 6–12 merozoites; gametocytes are round almost filling the enlarged and irregular–shaped erythrocytes. It causes ovale or mild tertian malaria fever every 48 hours (Jordan and Verma, 2011).

2.19 Life-Cycle of *Plasmodium*

The complete life cycle of human malaria parasite embraces:

i. A period of development within the mosquito and

ii. A period of infection in man

After ingestion of human infected blood a period of development lasting 10–14 days occurs in the mosquito resulting in the production of sporozoites. A bite infects the human host with these forms which remain in the circulating blood for 30 minutes or less, then enter tissue cells notably in the liver, where the pre-erythrocytic cycle takes place.

During the succeeding 7–9 days the sporozoites develop in the parenchyma cells of the liver. The stage of development is known as the pre-erythrocyte cycles. The cryptozoic schizoants thus formed rupture and release numerous merozoites most of which enter the circulation to invade the erythrocytes, thus starting the erythrocytic cycle. Attempts have been made to investigate the metabolism of the parasite existence. The liberation of the merozoites from the liver cells and their entry into the blood stream initiates the erythrocytic cycle. The *Plasmodium* first appears in red cells as a small speck of chromatin surrounded by scanty cytoplasm, and soon becomes a ring-shaped trophozoite. As the parasite develops, pigment particles appear in the cytoplasm prominent. Chromatin division then proceeds and when complete there is formed the mature schizoant containing daughter merozoites the majority of which re-enter erythrocytes to re-enter erythrocytes to re-initiate erythrocytic cycle takes 36 – 46 hours (subtartian); in *P. vivax* and *P. ovale* infections 48 hours (tertian); and *P. malariae* 72 hours (quarta) (Cheesbrough, 2005).

In response to an unknown stimulus, a number of the merozoites released after erythrocytic schizogony develop into male and female forms, known as gametocytes. Gametocytes are believed to be inert in man. They provide the reservoir of infection enabling mosquitoes to

perpetuate the malaria cycle, and remain within the red cell for the duration of their survival i.e. up to 120 days (Cheesbrough, 2005).

A certain portion of the merozoites liberated from the cryptozoic schizoants of the pre-erythrocytic phase do not enter the blood stream but re-enter the parenchymal cells of the liver to produce the secondary or metacryptozoic schizonts which are responsible for the persistence of the exo-erythrocytic cycle (Jordan and Verma, 2011).

2.20 Detection of *Plasmodium* from *Anopheles*

Molineaux and Grammicia (1980) and Alaribe (2007) employed the dissection of the *Anopheles* salivary gland to detect *Plasmodium* (sporozoite stage) in West Africa and Cross Rivers State respectively.

Onyabe *et al.* (2003), Ndams (2004) and Tukur (2010) used ELISA techniques to detect *Plasmodium* from *Anopheles* in parts of Nigeria. ELISA has been a valuable tool in epidemiological studies and in assessing the risk and identification of vectors. The Vec Test^{TM} antigen panel assay is the rapid wicking assay that identifies the presence or absence of *Plasmodium* in *Anopheles.* The assay is a rapid, one strip, one step procedure using wicking test strip. Rapid results, ambient storage and lack of specialized equipment needed in testing samples are big advantages of the wicking over ELISA, and prior training is not necessary.

Maxwell *et al.* (2003) had compared the Vec Test^{TM} dipstick assay for detection of *Plasmodium* sporozoites in *Anopheles* vectors of malaria with standard circum sporozoite (CS) microplate ELISA for detection of *Plasmodium falciparum* circum sporozoite protein

(Pfcsp) in *Anopheles* mosquitoes. The sensitivities of the two assays were 88.8% for Vec Test and 100% for visual ELISA.

Detection of malaria-causing *Plasmodium* species within their vectors is an essential component of vector control programmes. Several PCR protocols have been developed for this purpose. Many of these methods, while sensitive, require multiple PCR reactions to detect and discriminate all four *Plasmodium* species (Chris *et al*., 2008). A new assay based on TaqMan SNP genotyping was developed to detect all four *Plasmodium* species and was found to be highly specific when using *Plasmodium* genomic DNA as template.

The infection status of a mosquito is usually assessed by presence or absence of *Plasmodium* sporozoites in the salivary glands. Traditionally, this was done by dissection and visual assessment of glands using a microscope. This requires skilled personnel, time consuming and does not determine which *Plasmodium* species is present. It has, therefore, been largely suspended by more rapid immunological and molecular approaches. One of the widely adopted of these higher-throughput approaches is the circumsporozoite protein enzyme-linked immunosorbent assay (CSP ELISA) (Burkot *et al*., 1984; Wirtz *et al*., 1987). Although CSP ELISA has proven to be relatively robust and cheap, there are a number of potential drawbacks in using this approach. Firstly, there have been several reports that it overestimates true salivary gland infection rates and this may be linked to the spread of circumsporozoite protein throughout the mosquito after being shed from sporozoites while migrating through the mosquito (Beir and Koros, 1991; Fonteniile *et al*., 2001; Ponnudurai *et al*., 1991; Posthuma *et al*., 1989). Secondly, mosquitoes often need to be collected and stored for later analysis and this is usually done by either drying on silica gel or keeping in ethanol

or isospropanol. While the former is not inhibitory to ELISA the latter approach renders the specimens unsuitable for ELISA testing. Thirdly, although monoclonal antibodies have been produced for all four *Plasmodium* species that cause human malaria each assay must be run separately and therefore in practice many studies only test for the presence of one or two species. Finally the CSP ELISA may alsobe relatively insensitive to very low-level infections (Arez, *et al*., 2000).

The ELISA has many potential uses in malaria field studies, provided it is used accurately with an awareness of how ELISA results can be interpreted. One potential misconception is that ELISA tests on whole mosquitoes can totally replace dissections for determining sporozoite rates in vectors. Because the ELISA detects a large proportion of infected mosquitoes before sporozoites reach the salivary glands. This assay overestimates the actual sporozoite rate. If study objectives demand the precise determination of the sporozoite rate, the ELISA can best be employed by cutting specimens at the thorax and testin the head portions. Valuable information regarding parity, age-grading, and oocyst infections are also lost if programs rely solely on the ELISA. Another misconception is that considerable time is saved with ELISA. This is not the case for individual mosquitoes but would be true for pools of mosquitoes (appropriate in areas with infection rates <1%). The ELISA has wide applicability (Wirtz *et al*., 1985).

CHAPTER THREE

MATERIALS AND METHODS

3.1 The Study Area

The study was conducted in 24 locations in Jigawa State, Nigeria (Figure 1); the geo-coordinates and the vegetation type of the locations are listed in Table 3.1. The area is located between latitude 12˚00´N and longitude 9˚45´ W. It has a total land area of 23,154km². To the west, it shares boundary with Kano and Katsina States, Bauchi to the east and Yobe State to the northeast. It shares its northern boundary with the Republic of Niger (David, 2008). Its topography is generally characterized by undulating land, with sand dunes of various sizes spanning several kilometers. The ancient Pre-cambrian rocks of the basement complex are separated from the younger sediment of the Chad formation by a hydrological divide, which runs through Kiyawa, Dutse and Yankwashi (Zakari, 2006).

Two seasons may be recognized in Jigawa State, the dry season (November – April) and the wet season (May– October). The rain usually starts in May and ends in September. The average annual rainfall is about 22 – 40 inches (550 – 1000mm). The relative humidity is eight per cent (Zakari, 2006).

The main rivers (Figure 2) are Hadejia, Kafin Hausa, Miga, Guri and Kirikasamma with a number of tributarries. Hadejia – Kafin Hausa River transverses the state from west to east through Hadejia – Nguru wetlands and empties into Lake Chad Basin (David, 2008). There are also water bodies such as dam located at Hadiyin in Kaugama Local Government Area that runs through Auyo and rice fields.

Most parts of the State lie within the Sudan Savannah with elements of Guinea Savannah in the southern part (David, 2008). The total vegetation cover is much below national average of 14.8%. The vegetation cover is being depleted, making northern part highly vulnerable to desert encroachment (David, 2008).

3.2 Collection and Rearing of *Anopheles* Larvae/Pupae

In each location searching for breeding site was conducted within 100m of each of the houses. Sampling was done twice a month during the dry season (November – April) and rainy season (May – October) between 2010 and 2011. Larvae/pupae were collected between 09:00 am to 11:00 am hours and 04:00 pm to 06:00 pm hours.

On sighting a large object like human shadow, ordinarily, *Anopheles* larvae swim to the bottom of water body. To avoid this, the collector faced the Sun, thereby allowing shadow to be formed behind him and not on the water surface. Using a ladle, *Anopheles* larvae/pupae were scooped from ponds (Plates II), rice fields (Plate III), and pool (Plate IV) in the study locations together with water they were living in and placed in small, plastic buckets covered with bed net (1mm x1mm) to allow exchange of gases from the surroundings.

Twenty four mosquito rearing cages (2m x 1m x 1m) (Plate I) were constructed, each for a study location. The bucket was placed in a cage and the mesh that covered its top was removed. The larvae/pupae were fed with ground fish diet powder. The water was replaced every other day (Mark, 1997). A light spray with pyrethrin against the net killed the adults and made them fall on the white cloth beneath the cage. They were removed from the cage and preserved in separate Eppendorf tubes containing calcium sulphate for further studies.

Figure 1: Map of Jigawa State showing Study Areas

Source: Modified from the Administrative Map of Jigawa State

Table 3.1: Locations in the study area

Location	Geocoordinates	
	Latitude	**Longitude**
Agubu	12° 45′	11° 10′
Auyo	12° 36′	1 ° 96′
Azamu	12° 46′	1 ° 11′
Birniwa	10° 48′	7 ° 43′
Dubantu	12° 36′	9° 94′
Gandun Sarki	12° 50′	10 ° 09′
Garki	12° 43′	9 ° 18′
Guri	10° 23′	13° 46′
Hadejia	12° 45′	10° 05′
Jahun	12° 36′	10° 00′
Kadume	12° 45′	10° 08′
Kafin Hausa	12° 68′	10° 26′
Kaugama	12° 46′	10° 05′
Kirikasamma	12° 23′	9° 91′
Kiyawa	12° 11′	8° 18′
Ku´it	12° 24′	9° 08′
Makarahuta	12° 45′	10° 06′
Makaddari	12˚ 54´	10˚ 06´
Makwalla	12° 36′	9° 95′
Mallam maduri	12° 56′	10 ° 09′
Mawadachi	12° 51′	10° 01′
Miga	12° 23′	9° 68′
Ringim	68° 10′	9° 7′
Taura	10° 53′	9° 05′

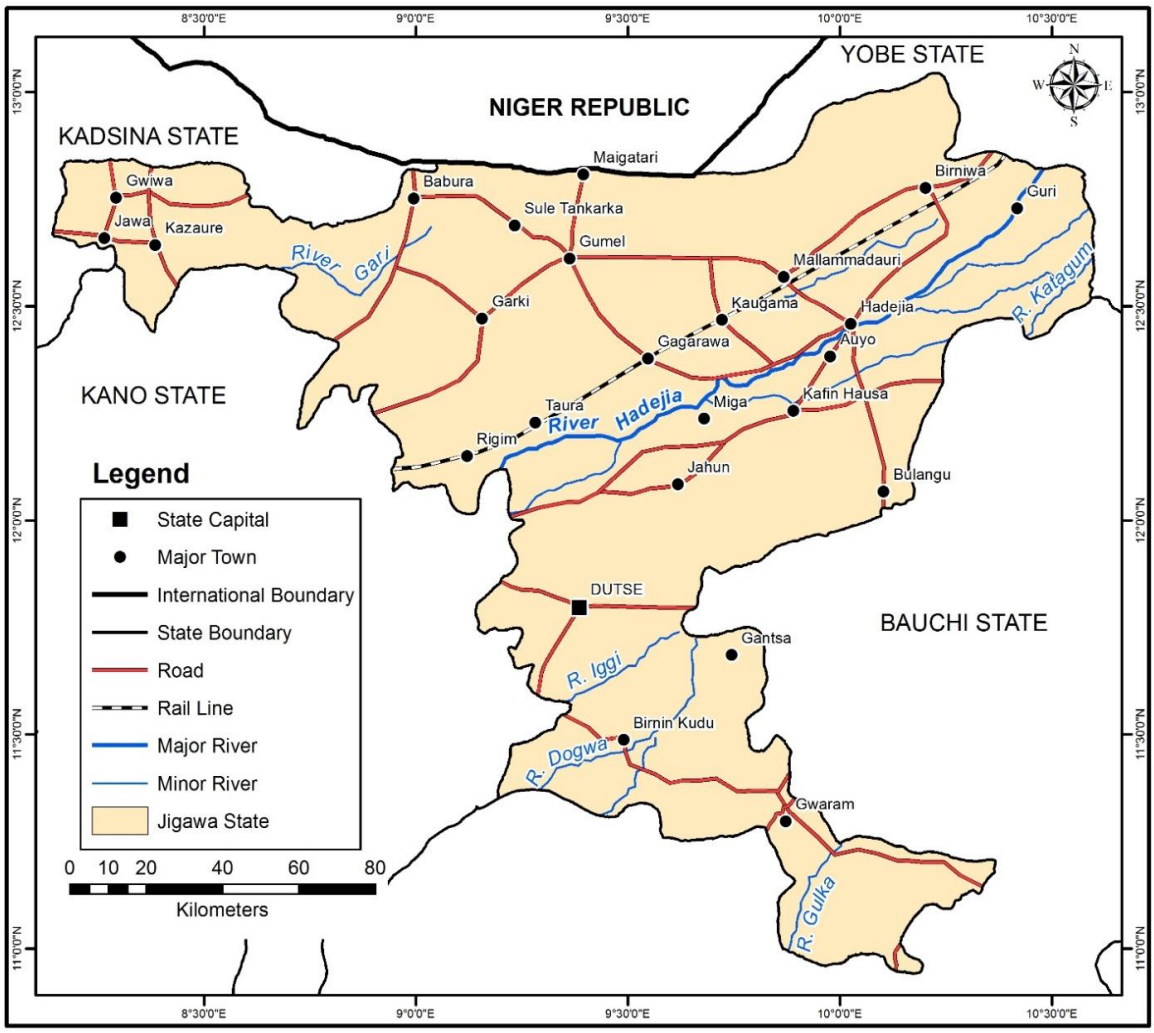

Figure 2: Map of Jigawa Sate showing the Rivers

Source: Modified from the Administrative Map of Jigawa State

Plate I: A Mosquito rearing cage

Plate II: A pond in the study location

Plate III: Part of a Rice field in the study location

Plate IV: A pond in the study location

Plate V: A pool in the study location

3.3 Indoor Collection of Adult Mosquitoes

Due to the difficulty in getting the consent of the inhabitants of the households, seventy two rooms, from the locations were used for the collections of adult *Anopheles*. The collections were made between 6.00 and 10.00am in rooms that were vacated and emptied with doors and windows shut. A sheet (4m x 4m) of white cloth was spread on the floor of the rooms for easy recognition of dead *Anopheles,* and this was followed by spray of pyrethrin (W.H.O, 1992). After 10 minutes, the mosquito that fell onto the sheet were hand picked using forceps and stored in separate Eppendorf tubes containing calcium sulphate according to date of collection and location (Gilles and De Meillon,1968).

Consecutive indoor collections of indoor resting *Anopheles* were carried out in each location twice a month during the dry and rainy seasons between 2010 and 2011 and the rooms were visited once a day.

3.4 Identification of *Anopheles* Mosquitoes

3.4.1 Morphological Identification

Using light microscope with x20 and x40 objectives the mosquitoes were identified morphologically using the keys of Gilles and Coetzee (1987). Wing margins have with light spots (Plates VIa and VI b); palps have pale bands (Plates VIIIa and VIII b) and legs have light spots (Plates IXa and IX b). In *An. funestus* the hind tarsi are narrow and apical with tarsi 1– 4 with light bands at least on the apices, the light spots are only on the upper margins of the wings and the pale bands on the palps are at different distances while *An.gambiae s.l.* has only one light spot on its tarsus; the light spots are on both upper and lower margins of the wings.

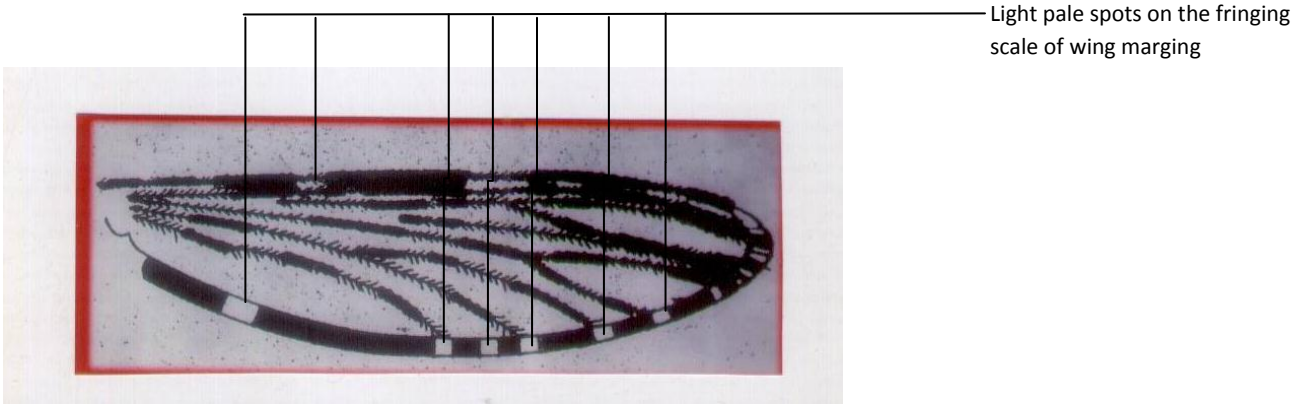

Plate VIa: A wing of *An.gambiae s.l* showing light spots with vien six completely dark with no pale spot on the fringing scale on the wing marging

Source: Gilles and Coetzee (1987)

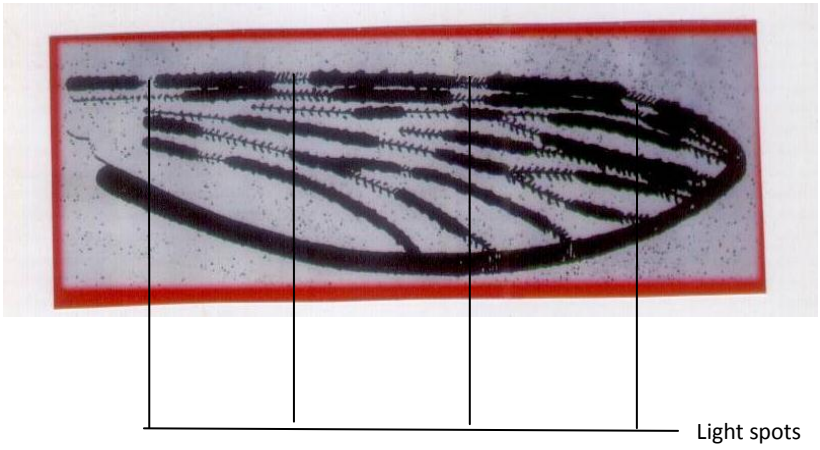

Plate VIb: A wing of *An. funestus* with light spots with vien six completely dark with no pale spot on the fringing scale on the wing margin
Source: Gilles and Coetzee (1987)

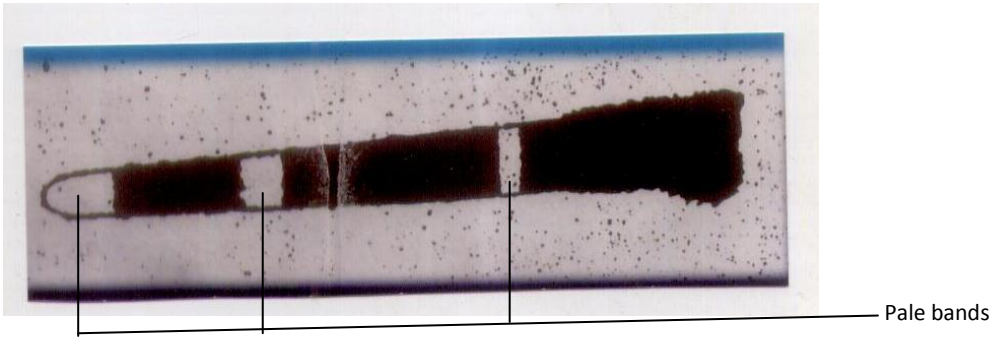

Plate VIIa: A palp of *An. funestus* with pale bands

Source: Gilles and Coetzee (1987)

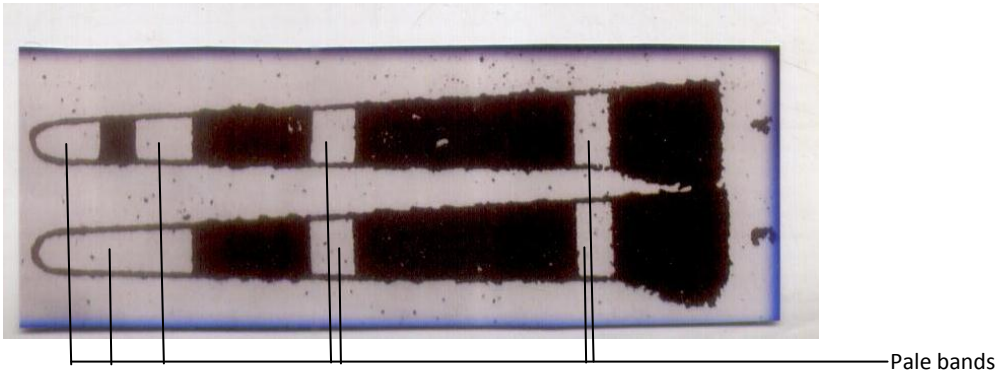

Plate VIIb: Palps of *An. gambiae s.l* with pale bands

Source: Gilles and Coetzee (1987)

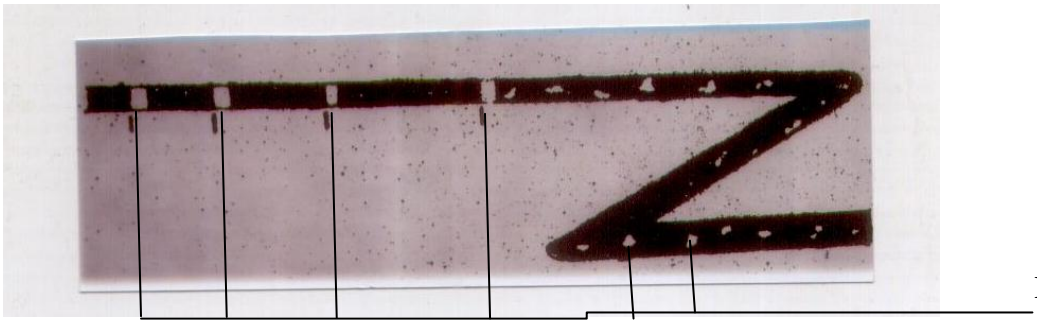

Plate VIIIa: A hind leg of *An. gambiae* with light spots

Source: Gilles and Coetzee (1987)

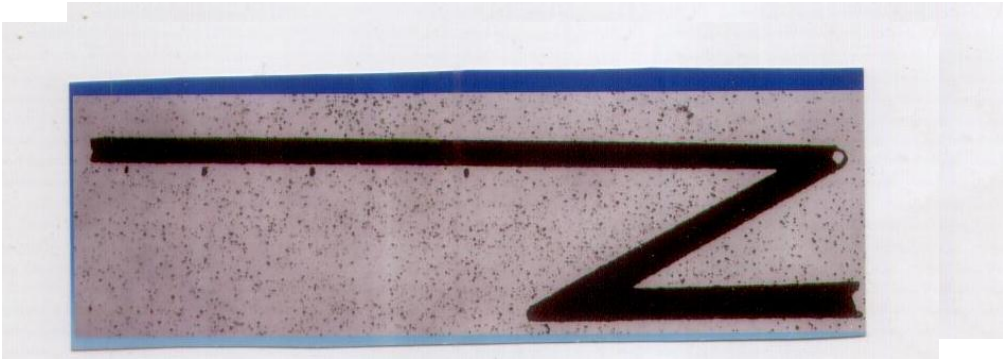

Plate VIIIb: A hind leg of *An. funestus* with no light spots

Source: Gilles and Coetzee (1987)

3.4.2 Molecular Identification

The essence of PCR amplification is to identify species complex of morphologically identified *Anopheles* mosquitoes.

Deoxyribonucleic acid (DNA) Extraction

The method of Collins *et al.* (1988) was employed for the *Anopheles* DNA extraction. Either the wing, leg or abdomen of mosquito was put inside eppendorf tube and 50µl of DNA extraction buffer (phenyl chloroform) was added into the eppendorf tube then vortexed thoroughly for 10 – 15 seconds. The mosquito part was ground with pestle then incubated at 95^{o}C for 15 seconds.

Polymerase Chain Reaction (PCR) Master Mix

Polymerase is an enzyme processes that ensures the making of copies of DNA molecules using the original template. It requires nucleotide bases which supply a form of basic building blocks of DNA and oligonucleotide primer (a short stretched of the copied DNA) which initiates the amplication. The DNA template obtained above was mixed with commercially obtained species – specific complementing primers (made from ribosomal DNA) namely universal primer (UN), Gambiae primer (GA), Merus primer (ME), Quadriannulatus primer (QA) and Arabiensis primer (AR) (Table 3.2). The mixture was mixed well and 12.5µl of this PCR master mix was put into eppendorf tube. The volume of quantity of master mix depends on the number of *Anopheles* to be examined. The UN anneals to the same position (16) of the rDNA of all the four species, GA anneals specifically to *An. gambiae s.s,* ME anneals to both *An. meras* and *An. melas* while AR anneals to *An. arabiensis* (Scott *et al.*, 1993).

Table 3.2: Species – Specific Primers

Primer	**Primer Sequence**						
Universal (UN)	GTG	TGC	CCC	TTC	CTC	GAT	GT
Gambiae (GA)	CTG	GTT	TGG	TCG	GCA	CGT	TT
Merus (ME)	TGA	CCA	ACC	CAC	TCC	CTT	GA
Arabiensis (AR)	AAG	TGT	CCT	TCT	CCA	TCC	TA

Source: Scott and Collins (1993)

PCR Amplification

The PCR amplification was done using PCR machine primus 96 plus in three phases, which make up one complete PCR cycle that lasts for less than 2 minutes. The nucleotide bases and the oligonucleotide are renewed after 30 cycles. The three phases are: Denaturation in which the original DNA is heated to a temperature from 90°C to 95°C for 30 seconds causing the individual strands to separate, then the annealing phases in which the temperate is lowered to 55°C over a 20 second period, allowing the oligonucleotide primers to bind to the separated DNA strands and the polymerization phase in which the temperature is raised to 75°C, a temperature at which the polymerase made copy the DNA rapidly (Scott *et al.,* 1993). The portion of the gene amplified was 165 of rDNA.

PCR Agarose Gel Electrophoresis

Agarose Gel

Two litres of 1x Trisboric ethidium (X1 TBE) containing 0.5µg/ml ethidium bromide was prepared. Ethidium bromide is a radioactive compound which fixes the nucleotide making them fluoresce in presence of ultraviolet light and is mutagenic.

One hundred millilitres of 1% agarose gel was melted in 1XTBE buffer in the microwave to form 1% gel (1.0g of agarose in 100ml of 1XTBE/EtBr). The gel tray was made in set position in gel box and the comb was at proper height above the tray. The agarose gel was poured into the gel tray and was allowed to cool slowly for 20 minutes. When the gel hardened forming a gel slab, the comb was pulled out from it and the gel slab was placed in

electrophoretic tank containing 1x TBE/EtBr running buffer so that the gel slab submerged under approximately 0.5 inches of running buffer.

Loading of DNA Samples

Using a fresh 1.7ml microcentrifuge tube, 10µl of PCR product was added to 8µl of distilled water and 2µl of loading dye (Bromophenol blue) and these made 20µl. Using a P-20 Bipetmen set at 20µl, the DNA samples were loaded into the wells. In addition, two lanes each of 20µl of pre-made DNA size standards known as 1kb plus Ladder were loaded. The loading dye is denser than electrophoresis buffer and will make the DNA sample to sink (Scott *et al.*, 1993).

Movement of DNA Material

After loading the gel, the lid was attached to the gel box ensuring that they were connected to the proper places and 100 volts was applied. The difference in speed of migration of the DNA is the principle used in electrophoresis. Since DNA is negatively charged, it moved to the red-coloured anode. The migration lasted for one hour.

Photomicrography

When the dyes and DNA samples had migrated to appropriate distances, depending on the weight of the fragment, the power was switched off and the gel was removed and photographed with a polariod camera.

Determination of the DNA Size

By comparing the distance that the fragments migrated to the DNA standards, the approximate distances were estimated and labelled.

3.5 Examination of Indoor Caught *Anopheles* for *Plasmodium* infection

The indoor caught *Anopheles* mosquitoes were examined for *Plasmodium* by Enzyme Linked Immunosorbent Assay (ELISA) (Wirtz *et al.*, 1987) as described below.

3.5.1 Preparation of Mosquito Sample

Head and thorax of *Anopheles* were separated from the abdomen using a razor blade. They were placed in a 1.5ml tube with 50µl Grinding buffer (BB-NP40) and squeezed with pestle. The pestle was rinsed with Blocking Buffer (BB) to a final volume of 200µl. The pestle was removed from the tube and washed three times with washing solution to avoid contamination. The ground specimen was tested immediately or stored at -70^{o}C for later use.

3.5.2 The ELISA Test Procedure

50µl of mosquito titurate was put per ELISA well. Each ELISA plate has 96 wells; 1 is positive, 7 are negative controls and 88 wells for specimens. The 7 negative controls were the triturate of laboratory reared, known uninfected female *Anopheles.*

1µl of monoclonal antibody (MA-b) stock was added to 125µl phosphate buffer saline (PBS) and 0.2mg/50µl solution of *P. falciparum*. 50µl of this solution was put per well, covered and incubated for 30 minutes at 4^{o}C. The contents of the wells were aspirated and filled with 20µl blocking buffer (BB) and incubated for 1 hour at room temperature. Then the well contents were aspirated and 50µl *Anopheles* triturate added per well. The positive and negative controls were respectively put and incubated for 2 hours at room temperature. The wells were washed with PBS-TWEEN 20 after which 50µl of MAb peroxide was put in each well and incubated for 1 hour at room temperature. Colour change (dark green) occurred within

minutes. The plate was read at 405 – 414nm using ELISA plate reader (model) 30 or 60 minutes after adding the substrate.

Absorbency cut off value to rule out positive specimens was calculated as 2 times the mean values for the 7 negative controls. All the specimens with absorbency values 2 times the mean values of the 7 negative controls were considered positive.

3.6 Data Analysis

The data obtained were subjected to Two way Analysis of variance (ANOVA) to determine differences between location and *t*-test was used to compare difference between the seasons INSTAT 3 statistical software for Windows version (2003) to compare statistically if there was any significant difference. $P<0.05$, was considered significant.

Prevalence, defined as number of individual of a host species infected with a particular parasite species divided by number of host examined and mean intensity, mean number of individuals of a particular parasite speices per infected host in a sample (Margolis *et al.,* 1982 and Bush, *et al.,* 1997) were also employed.

CHAPTER FOUR

RESULTS

4.1 Abundance of *Anopheles funestus*

Anopheles funestus was abundant in all the 24 study sites but in very disproportionate numbers from both indoor and larval/pupal collections (Tables 4.1 and 4.2). The abundance was high in only seven of the location: Birniwa, Garki, Guri, Kafin Hausa, Kirikasamma, Miga and Taura (indoor collections collections only) and nine of the 24 sites by rearing of larvae/pupae: Birniwa, Garki, Guri, Kaugama, Kafin Hausa, Kirikasamma, Kiyawa, Miga and Taura. However, there was no *Anopheles funestus* collected indoors from two localities: Jahun and Makaddari. The locality with most abundant indoor collections (Table 4.1) was Kafin Hausa while Kaugama for reared larvae/pupae (Table 4.2).

Overall, there was significant difference ($P<0.05$) the abundance among the indoor caught *An. funestus* across the sites (Table 4.1). Also there was high significant difference ($P< 0.05$) the *Anopheles* molecular among the reared larvae/pupae across the sites (Table 4.2).

Table 4.1: Abundance of *Anopheles funestus* collected indoor from the study sites of Jigawa State, Nigeria

Sites	No. Collected	Prevalence	Mean intensity
Agubu	2	1.11	0.6
Auyo	4	2.23	0.6
Azamu	3	1.67	0.6
Birniwa	17	9.49	0.6
Dubantu	3	1.67	0.6
Gandun Sarki	1	0.55	0.6
Garki	20	11.17	0.6
Guri	13	7.26	0.6
Hadejia	7	3.91	0.6
Jahun	0	0.00	0.6
Kadume	3	1.67	0.6
Kafin Hausa	24	13.40	0.6
Kaugama	5	2.80	0.6
Kirikasamma	12	6.70	0.6
Kiyawa	7	3.91	0.6
Kuit	2	1.11	0.6
Makarahuta	3	1.67	0.6
Makaddari	0	0.00	0.6
Makwalla	7	3.91	0.6
Malam Maduri	6	3.36	0.6
Mawadachi	3	1.67	0.6
Miga	17	9.49	0.6
Ringim	9	5.02	0.6
Taura	11	6.14	0.6
Total	**179**	**100**	**14.4**
Mean	7.5	4.16	0.6
95% Confidence	4.7		
N	24		
T	5.5		
P	1.333 E-05		

Table 4.2: Abubdance of *Anophele funestus* reared from larvae/pupae from the study sites of Jigawa State, Nigeria

Sites	No. Collected	Prevalence	Mean intensity
Agubu	5	1.55	0.31
Auyo	8	2.48	0.31
Azamu	9	2.79	0.31
Birniwa	30	9.32	0.31
Dubantu	1	0.31	0.31
Gandun Sarki	4	1.24	0.31
Garki	22	6.83	0.31
Guri	28	8.69	0.31
Hadejia	12	3.73	0.31
Jahun	3	0.93	0.31
Kadume	7	2.17	0.31
Kafin Hausa	20	6.21	0.31
Kaugama	40	12.42	0.31
Kirikasamma	28	8.69	0.31
Kiyawa	25	7.76	0.31
Kuit	3	0.93	0.31
Makarahuta	4	1.24	0.31
Makaddari	3	0.93	0.31
Makwalla	3	0.93	0.31
Malam Maduri	11	3.41	0.31
Mawadachi	6	1.86	0.31
Miga	19	5.90	0.31
Ringim	3	0.93	0.31
Taura	28	8.69	0.31
Total	**322**	**100**	**7.44**
Mean	13.4	4.16	0.31
95% Confidence	8.6		
N	24		
T	5.8		
P	7.315 E-06		

4.2 Abundance of *Anopheles gambiae s. l*

PCR results depicted that both *An. gambiae ss* 16s rDNA 300 bp Plate X and 300bp for *Anopheles arabiensis* based on indoor collections (Table 4.3), *An. gambiae s. l* were abundant in all the location with exception of Malam madori. The abundance was highly statistically significant ($P< 0.05$) among the sites. Five sites were with a fewer number: Ku´it, Dubantu, Jahun, Makaddari and Gandun Sarki.

Anopheles gambiae s.l reared from larvae/pupae (Table 4.4) in the 23 location while none was collected or reared in Malam madori. The abundance was significantly ($P< 0.05$) among 4 sites: Dubantu, Jahun, Makaddari and Azamu. The result further revealed that Kirikasamma, Ringim and Guri with most abundant reared larvae/pupae (n=92), (n=88) and (n=83) respectively, than the other locations/breading sites where the *Anopheles* were collected.

4.3 Abundance of *Anopheles arabiensis*

Polymerase chain reaction test for the *Anopheles gambiae* complex revealed that the abundance of *An. arabiensis*. Plate X is one of the PCR test results showing the diagnostic banding patterns of *An. arabiensis* on the PCR gel slab. It indicated that the PCR product depicted lanes 1, 2, 5, 7, 8, 9, 10 and 11 as *An. arabiensis* where 16s rDNA is 300bp. The results for all the tests were presented in Tables 4.5 and 4.6. No *An. arabiensis* collected indoors from seven localities: Auyo, Ku´it, Makwalla, Dubantu, Malam madori, Mawadachi and Azamu (Table 4.5) but were reared from larvae/pupae collected from these sites except from Malam madori (Table 4.6). Although the abundance was not uniform across the sites based on indoor collections, it was specifically high in four sites: Garki (n=10), Guri (n=12), Kafin Hausa (n=10) and Kirikasamma (n=12) (Table 4.5).

Fewer number was obtained from Agubu and Gandun Sarki (n=1) each but most abundant at Miga (n=16) (Table 4.6). In all, there was significant difference ($P< 0.05$) between the abundance of *An. arabiensis* among the 24 sites for both indoors and larval/pupal collections.

Table 4.3: Abundance of *Anopheles gambiae s.l* collected indoor from study sites of Jigawa State, Nigeria

Sites	No. Collected	Prevalence	Mean intensity
Agubu	12	2.91	0.24
Auyo	17	4.12	0.24
Azamu	10	2.42	0.24
Birniwa	14	3.40	0.24
Dubantu	4	0.97	0.24
Gandun Sarki	8	1.94	0.24
Garki	19	4.61	0.24
Guri	27	6.55	0.24
Hadejia	20	4.85	0.24
Jahun	2	0.48	0.24
Kadume	10	2.42	0.24
Kafin Hausa	32	7.76	0.24
Kaugama	29	7.03	0.24
Kirikasamma	30	7.28	0.24
Kiyawa	38	9.22	0.24
Kuit	4	0.97	0.24
Makarahuta	21	5.09	0.24
Makaddari	7	1.70	0.24
Makwalla	13	3.15	0.24
Malam Maduri	0	0.00	0.24
Mawadachi	9	2.18	0.24
Miga	33	8.00	0.24
Ringim	12	2.91	0.24
Taura	41	9.95	0.24
Total	**412**	**100**	**5.76**
Mean	17.2	4.16	0.24
95% Confidence	12.2		
N	24		
T	7.1		
P	2.3 E-07		

Table 4.4: Abundance of *Anopheles gambiae s.l* reared from larvae/pupae from the study sites of Jigawa States, Nigeria

Sites	No. Collected	Prevalence	Mean intensity
Agubu	17	1.79	0.10
Auyo	20	2.10	0.10
Azamu	13	1.36	0.10
Birniwa	60	6.31	0.10
Dubantu	10	1.05	0.10
Gandun Sarki	16	1.68	0.10
Garki	70	7.36	0.10
Guri	83	8.73	0.10
Hadejia	41	4.31	0.10
Jahun	11	1.15	0.10
Kadume	19	2.00	0.10
Kafin Hausa	60	6.31	0.10
Kaugama	80	8.42	0.10
Kirikasamma	92	9.68	0.10
Kiyawa	64	6.73	0.10
Kuit	18	1.89	0.10
Makarahuta	30	3.15	0.10
Makaddari	13	1.36	0.10
Makwalla	17	1.79	0.10
Malam Maduri	0	0.00	0.10
Mawadachi	19	2.00	0.10
Miga	70	7.36	0.10
Ringim	88	9.26	0.10
Taura	39	4.10	0.10
Total	**950**	**100**	**2.4**
Mean	40.75	4.16	0.1
95% Confidence	28.6		
N	24		
T	6.9		
P	4.8 E-07		

Table 4.5: Abundance of molecular identified *Anopheles arabiansis* collected indoor from study sites in Jigawa State, Nigeria

Sites	No. Collected	Prevalence	Mean intensity
Agubu	3	3.40	1.13
Auyo	0	0.00	1.13
Azamu	0	0.00	1.13
Birniwa	4	4.54	1.13
Dubantu	0	0.00	1.13
Gandun Sarki	1	1.13	1.13
Garki	10	11.36	1.13
Guri	12	13.63	1.13
Hadejia	3	3.40	1.13
Jahun	2	2.27	1.13
Kadume	1	1.13	1.13
Kafin Hausa	10	11.36	1.13
Kaugama	3	3.40	1.13
Kirikasamma	12	13.63	1.13
Kiyawa	5	5.68	1.13
Kuit	0	0.00	1.13
Makarahuta	2	2.27	1.13
Makaddari	6	6.82	1.13
Makwalla	0	0.00	1.13
Malam Maduri	0	0.00	1.13
Mawadachi	0	0.00	1.13
Miga	8	9.09	1.13
Ringim	2	2.27	1.13
Taura	4	5.54	1.13
Total	**88**	**100**	**27.12**
Mean	3.7	4.16	1.13
95% Confidence	1.98		
N	24		
T	4.5		
P	0.00015		

Table 4.6: Abundance of molecular identified *Anopheles arabiensis* reared from larva/pupae from study sites Jigawa State, Nigeria

Sites	No. Collected	Prevalence	Mean intensity
Agubu	1	0.75	0.75
Auyo	4	3.00	0.75
Azamu	3	2.25	0.75
Birniwa	11	8.27	0.75
Dubantu	5	3.76	0.75
Gandun Sarki	1	0.75	0.75
Garki	3	2.25	0.75
Guri	6	4.51	0.75
Hadejia	9	6.76	0.75
Jahun	2	1.50	0.75
Kadume	2	1.50	0.75
Kafin Hausa	4	3.00	0.75
Kaugama	7	5.26	0.75
Kirikasamma	9	6.76	0.75
Kiyawa	10	7.51	0.75
Kuit	3	2.25	0.75
Makarahuta	3	2.25	0.75
Makaddari	8	6.01	0.75
Makwalla	6	4.51	0.75
Malam Maduri	0	0.00	0.75
Mawadachi	3	2.25	0.75
Miga	16	12.00	0.75
Ringim	10	7.51	0.75
Taura	7	5.26	0.75
Total	**133**	**100**	**18**
Mean	5.50	4.16	0.75
95% Confidence	3.90		
N	24		
T	6.98		
P	4.01 E-07		

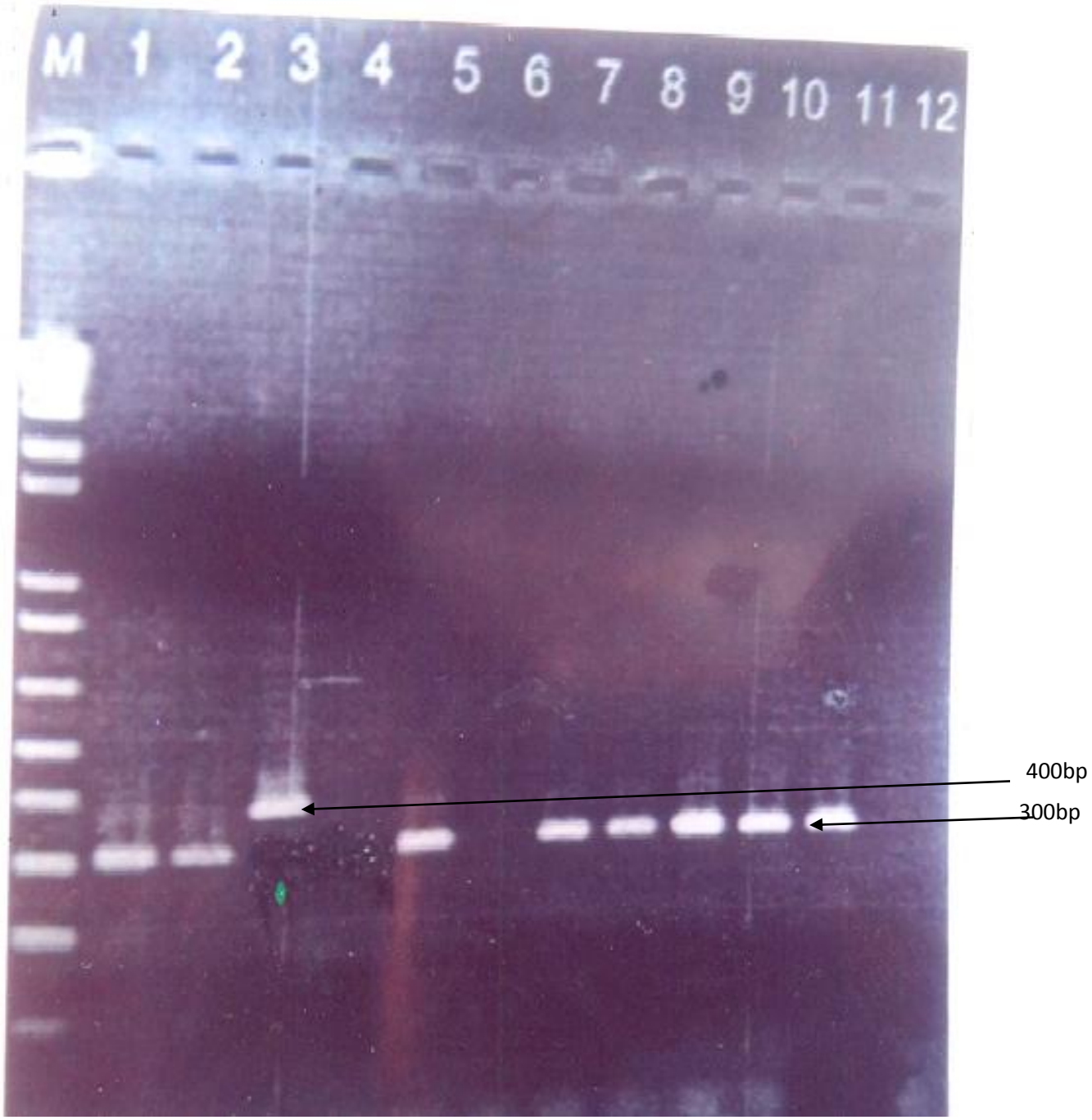

Plate X: PCR product depicting sibling species of *Anopheles gambiae s. l*: lane M is reference ladder; lane 3 is *An. arabiensis*; lanes 1, 2, 5, 6, 7, 8, 9, 10 and 11are *An. gambiae s.s*

4.4 Abundance of *Anopheles gambiae s. s*

The diagnostic banding patterns of *An. gambiae s. s* were shown on Plates X and XI. On plate X, only lane 3 whose fragment length was 400bp *An. gambiae s. s* while Plate XI indicated that the test lanes (2 – 20) were *An. gambiae s.s* and for detail of the results were presented in Table 4.7.

There was no *An. gambiae s. s* indoors from Malam madori. But for the remaining 23 sites, there was significant variation. Four sites: Garki (n=54), Guri (n=61), Kafin Hausa (n=50) and Ringim (n=57) were the sites with most abundant *An. gambiae s. s*. Moreover, there was moderate abundance at Birniwa (n=22), Kaugama (n=29), Kirikasamma (n=33), Kiyawa (n=37), Miga (n=23) and Taura (n=23). A fewer abundance was observed from Makwalla (n=4), Dubantu (n=3) and Makaddari (n=2) (Table 4.7). Overall, there was high significant difference ($P< 0.05$) between the abundance of indoor collected *An. gambiae s. s.*

On the other hand, the abundance of *An. gambiae s. s* reared from larvae/pupae was reported in Table 4.8 and revealed that there was significant difference ($P>0.05$) among the study sites. No *An. gambiae s. s* was obtained from two sites: Kiyawa and Malam madori. One site, Hadejia has the highest abundance (n=70) and was followed by Kafin Hausa (n=68). A fewer number was reported from Dubantu and Jahun.

4.5 Abundance of M-form (*Anopheles coluzzi*)

Lanes 5, 6, 7, 8 and 9 with 400bp fragment length on Plate XII were the PCR products depicting M-form. The detail of the overall result was presented in Table 4.9. The abundance of M-form was allover the 24 study sites. However, a distinct variation was observed among the sites. There was higher abundance at 4 sites: Garki, Kafin Hausa, Miga and Taura while

Jahun, Agubu and Gandun Sarki were the sites with least. There was no M-form obtained from Malam madori. Furthermore, the abundance was sympatric at 3 sites: Makwalla, Makaddari and Kadume with n=4 each. Also sympatry was observed at 2 sites: Dubantu and Azamu with n=3 each and of both M- and S-forms. The overall abundance showed that there was significant difference ($P< 0.05$) between the sites.

Table 4.7: Abundance of molecular identified *Anopheles gambiae s.s* collected indoor from study sites in Nigeria in Jigawa State, Nigeria

Sites	No. Collected	Prevalence	Mean intensity
Agubu	10	1.99	0.20
Auyo	12	2.39	0.20
Azamu	9	1.79	0.20
Birniwa	22	4.38	0.20
Dubantu	3	0.59	0.20
Gandun Sarki	10	1.99	0.20
Garki	54	10.75	0.20
Guri	61	12.15	0.20
Hadejia	18	3.58	0.20
Jahun	7	1.39	0.20
Kadume	7	1.39	0.20
Kafin Hausa	50	9.96	0.20
Kaugama	29	5.77	0.20
Kirikasamma	33	6.57	0.20
Kiyawa	37	7.37	0.20
Kuit	8	1.59	0.20
Makarahuta	11	2.19	0.20
Makaddari	2	0.39	0.20
Makwalla	4	0.79	0.20
Malam Maduri	0	0.00	0.20
Mawadachi	13	2.58	0.20
Miga	23	4.58	0.20
Ringim	57	11.35	0.20
Taura	22	4.38	0.20
Total	**502**	**100**	**4.80**
Mean	20.9	4.16	0.2
95% Confidence	13.1		
N	24		
T	5.54		
P	1.25 E-06		

Table 4.8: Abundance of molecular identified *Anopheles gambiae s.s* reared from larvae/pupa from study sites in Jigawa State, Nigeria

Sites	No. Collected	Prevalence	Mean intensity
Agubu	15	3.44	0.16
Auyo	21	3.41	0.16
Azamu	11	1.78	0.16
Birniwa	37	6.01	0.16
Dubantu	6	0.97	0.16
Gandun Sarki	12	1.95	0.16
Garki	22	3.57	0.16
Guri	31	5.04	0.16
Hadejia	70	11.38	0.16
Jahun	2	0.33	0.16
Kadume	19	3.10	0.16
Kafin Hausa	68	11.05	0.16
Kaugama	28	4.55	0.16
Kirikasamma	50	8.13	0.16
Kiyawa	0	0.00	0.16
Kuit	11	1.78	0.16
Makarahuta	35	5.69	0.16
Makaddari	12	1.95	0.16
Makwalla	20	3.25	0.16
Malam Maduri	0	0.00	0.16
Mawadachi	12	1.95	0.16
Miga	56	9.10	0.16
Ringim	31	5.04	0.16
Taura	46	7.48	0.16
Total	**615**	**100**	**3.84**
Mean	25.63	4.16	0.16
95% Confidence	17.07		
N	24		
T	6.20		
P	2.54 E-06		

Table 4.9: Abundance of *Anopheles gambiea s.s* (M-from) molecular forms collected from study sites of igawa State, Nigeria

Sites	No. Collected	Prevalence	Mean intensity
Agubu	2	0.9	0.45
Auyo	5	2.25	0.45
Azamu	3	1.35	0.45
Birniwa	13	5.85	0.45
Dubantu	3	1.35	0.45
Gandun Sarki	2	0.90	0.45
Garki	21	9.46	0.45
Guri	16	7.21	0.45
Hadejia	11	4.95	0.45
Jahun	2	0.90	0.45
Kadume	4	1.80	0.45
Kafin Hausa	18	8.10	0.45
Kaugama	14	6.30	0.45
Kirikasamma	15	6.76	0.45
Kiyawa	10	4.50	0.45
Kuit	6	2.70	0.45
Makarahuta	7	3.15	0.45
Makaddari	44	1.80	0.45
Makwalla	4	1.80	0.45
Malam Maduri	0	0.00	0.45
Mawadachi	5	2.25	0.45
Miga	28	12.61	0.45
Ringim	10	4.50	0.45
Taura	19	8.56	0.45
Total	**222**	**100**	**10.8**
Mean	9.25	4.16	0.45
95% Confidence	6.15		
N	24		
T	6.18		
P	2.64 E-05		

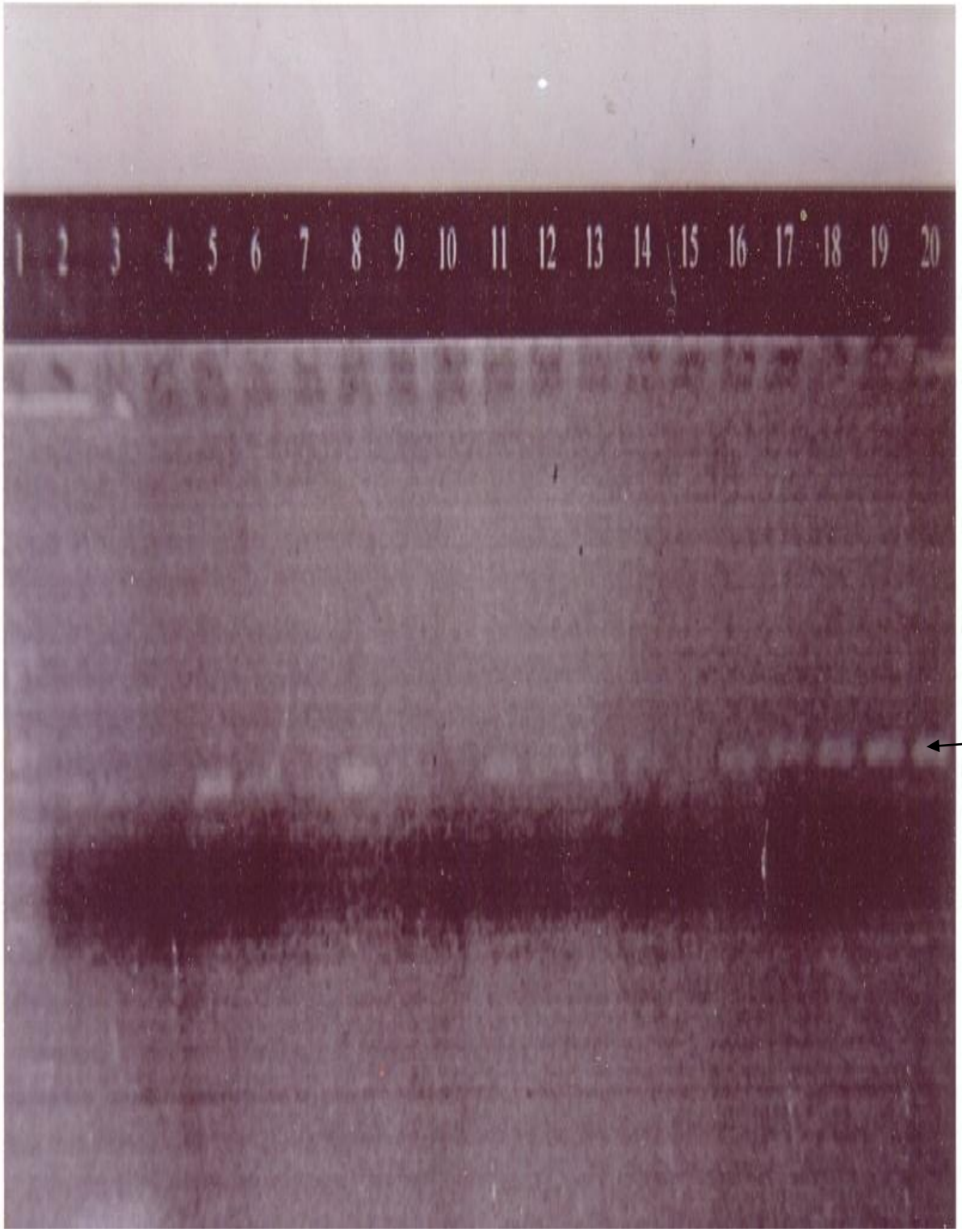

Plate XI: PCR product indicating a single species: Lane 1 is Reference ladder while lanes 2-20 depict *An. gambiae s.s* (400bp).

4.6 Abundance of S-form (*Anopheles s.s*)

Plate XII, shows that lanes 1, 2, 3 and 4 with DNA fragment length of 400bp were the PCR products depicting M-form and the detail was presented in Tables 4.10 and 4.11. In all, the abundance was high and across the sites with exception of Malam madori where no S-form was collected. Kaugama and Kirikasamma were with highest abundance, (n= 85) and (n= 86) respectively. These were followed by Kiyawa (n=77) and Ringim (n=78). The abundance was low (n= 6) at Dubantu. A similar proportion was collected from two sites: Makwalla and Mawadachi. Moreover, there was greater distinct proportion between Kirikasamma and Dubantu (n=86 versus n=6). There was high significant difference ($P< 0.05$) beween the sites.

4.7 Hybrid (M/S)

Plate XII shows that lanes 5,6,7 and 8 with fragment length of 250bp were the PCR products depicting M/S hybrid and was only reported from Auyo.

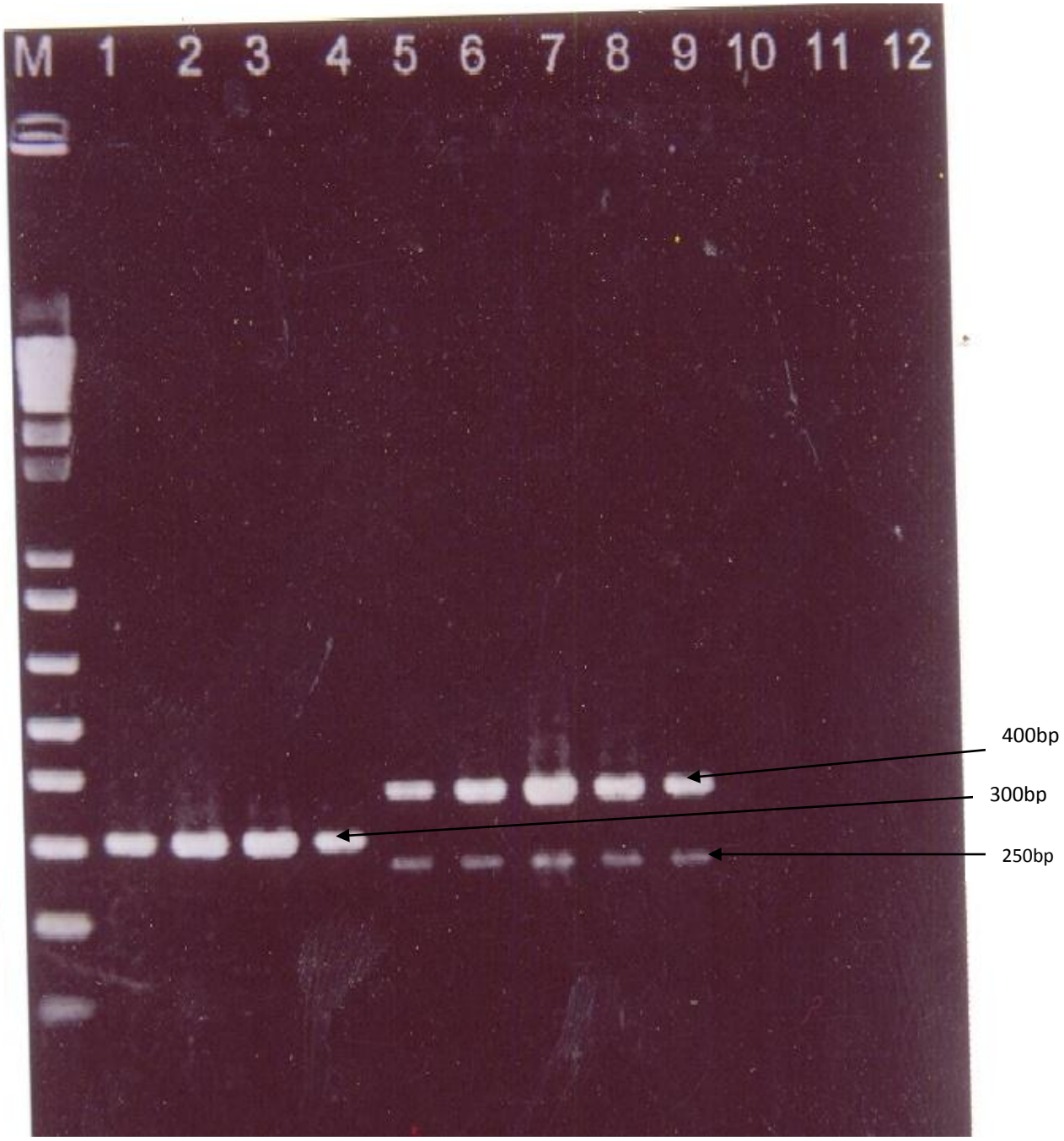

Plate XII: PCR product depicting *An. gambiae s.s* siblings 300bp: lanes 1, 2, 3 and 4 depict *Anopheles gambiae ss* M-forms; lanes 5, 6, 7, 8 and 9 are (300bp) *An. gambiae ss* S-forms and lanes 5-9 depict (400 and 250bp) M/S hybrid while lane M is ladder.

Table 4.10: Abundance of *Anopheles gambiae s.s* (S-Form) molecular forms collected from study sites of Jigawa State, Nigeria

Sites	No. Collected	Prevalence	Mean intensity
Agubu	23	2.49	0.11
Auyo	28	3.02	0.11
Azamu	17	1.83	0.11
Birniwa	46	4.96	0.11
Dubantu	6	0.64	0.11
Gandun Sarki	20	2.15	0.11
Garki	55	5.93	0.11
Guri	76	8.20	0.11
Hadejia	38	4.09	0.11
Jahun	7	0.75	0.11
Kadume	22	2.37	0.11
Kafin Hausa	60	6.47	0.11
Kaugama	85	9.16	0.11
Kirikasamma	86	9.27	0.11
Kiyawa	77	8.30	0.11
Kuit	13	1.40	0.11
Makarahuta	39	4.20	0.11
Makaddari	10	1.08	0.11
Makwalla	20	2.15	0.11
Malam Maduri	0	0.00	0.11
Mawadachi	20	2.15	0.11
Miga	51	5.50	0.11
Ringim	78	8.41	0.11
Taura	50	5.39	0.11
Total	**927**	**100**	**2.6410.8**
Mean	38.63	4.16	0.11
95% Confidence	27.13		
N	24		
T	6.95		
P	4.39 E-07		

Table 4.11: Abubance of overall *Anopheles gambiea s.s* molecular forms collected from study sites of Jigawa State, Nigeria

Sites	No. Collected	Prevalence	Mean intensity
Agubu	25	2.18	0.08
Auyo	33	2.87	0.08
Azamu	20	1.74	0.08
Birniwa	59	5.13	0.08
Dubantu	9	0.78	0.08
Gandun Sarki	22	1.91	0.08
Garki	76	6.61	0.08
Guri	92	8.00	0.08
Hadejia	49	4.26	0.08
Jahun	9	0.78	0.08
Kadume	26	2.26	0.08
Kafin Hausa	78	6.79	0.08
Kaugama	99	8.61	0.08
Kirikasamma	101	8.79	0.08
Kiyawa	87	7.57	0.08
Kuit	19	1.65	0.08
Makarahuta	46	4.00	0.08
Makaddari	14	1.22	0.08
Makwalla	24	2.08	0.08
Malam Maduri	0	0.00	0.08
Mawadachi	25	2.18	0.08
Miga	79	6.87	0.08
Ringim	88	7.68	0.08
Taura	69	6.00	0.08
Total	**1149**	**100**	**1.92**
Mean	47.87	4.16	0.08
95% Confidence	38.21		
N	24		
T	12.30		
P	2.53 E-07		

4.8 Seasonal Abundance of *Anopheles* caught during the study

Figure 3 illustrates the trend in the abundance of *Anopheles* mosquitoes collected during the two years, (2010 and 2011). It was observed that S-form was the most abundant of all three species collected. It was also the species that was abundant throughout the study period. However its proportion changed significantly within the 24 months. Its high preponderance was highest in August, 2010 and September, 2011 but lowest between Febuary and April in 2010 and 2011.

The prevalence of *An. funestus* followed a similar pattern as S-form but they differed in the sense that *An. funestus* was not abundant in later the later dry season period (January, Febuary, March and April) each year. It was more abundant than *An. arabiensis* even though they were almost equal in April, May and December, 2010; January and April, 2011; and December, 2011.

Each of *An. arabiensis* and M-form was prevalent over the other in at least two months each year. The two species were not abundant in January, February, March and April each year. The abundance of M-form was greater than that of *An. arabiensis* between May and November 2010 and vice- versa in December, 2010 January; 2011; between April and June, 2011 and between August and November, 2011.

All the species were abundant in 2011 than in 2010. In 2010, the prevalence declined in August while in September, 2011. Another observation was that there was rapid increase in May, 2011 than in May, 2010.

It was clearly observed that both species were more prevalence in rainy season than in dry season. Only S – form and a few *An. funestus* were present in dry seasons. Rapid increase in

abundance among all the species was observed during rainy season April, May and June, 2010 and May, June and July, 2011.

However, increase in prevalence declined as dry season was approached each year (October, 2010 and October, 2011).

Both the species were found in lower prevalence in January, February, March and April each year while abundance reached its peak between July and September.

4.9 Prevalence of *Plasmodium* among indoor caught *Anopheles* species

Of the 591 indoor – caught female *Anopheles* analyzed for *Plasmodium* by Enzyme – linked immunosorbent assay, 43 (7.28%) were positive (Table 4.12). *Anopheles* was not infected by *Plasmodium*. In other words, it was not found transmitting *Plasmodium.* The infection among other species revealed 10 (23.26%) *An. arabiensis*, 5 (6.81%) M – form and 28 (9.46%) S – form.

Plasmodium falciparum and *P. malairae* were found positive in *Anopheles* mosquitoes assayed *Plasmodium falciparum* (32/43) was more prevalent than *P. malairae* (11/42). *Plasmodium falciparum* was prevalent in 9(20.93%) *An. arabiensis*, 4(5.48%) *An. gambiae ss* M – form and 19(6.44%) *An. gambiae ss*- S – form while *P. malariae* who prevalent in 1(2.33%) *An. arabiensis*, 1(0.14%) *An. gambiae* ss- M – form and 9(3.04%) S – form. No *P. falciparum* and *P.malariae ss* were found in *An. funestus* during the study.

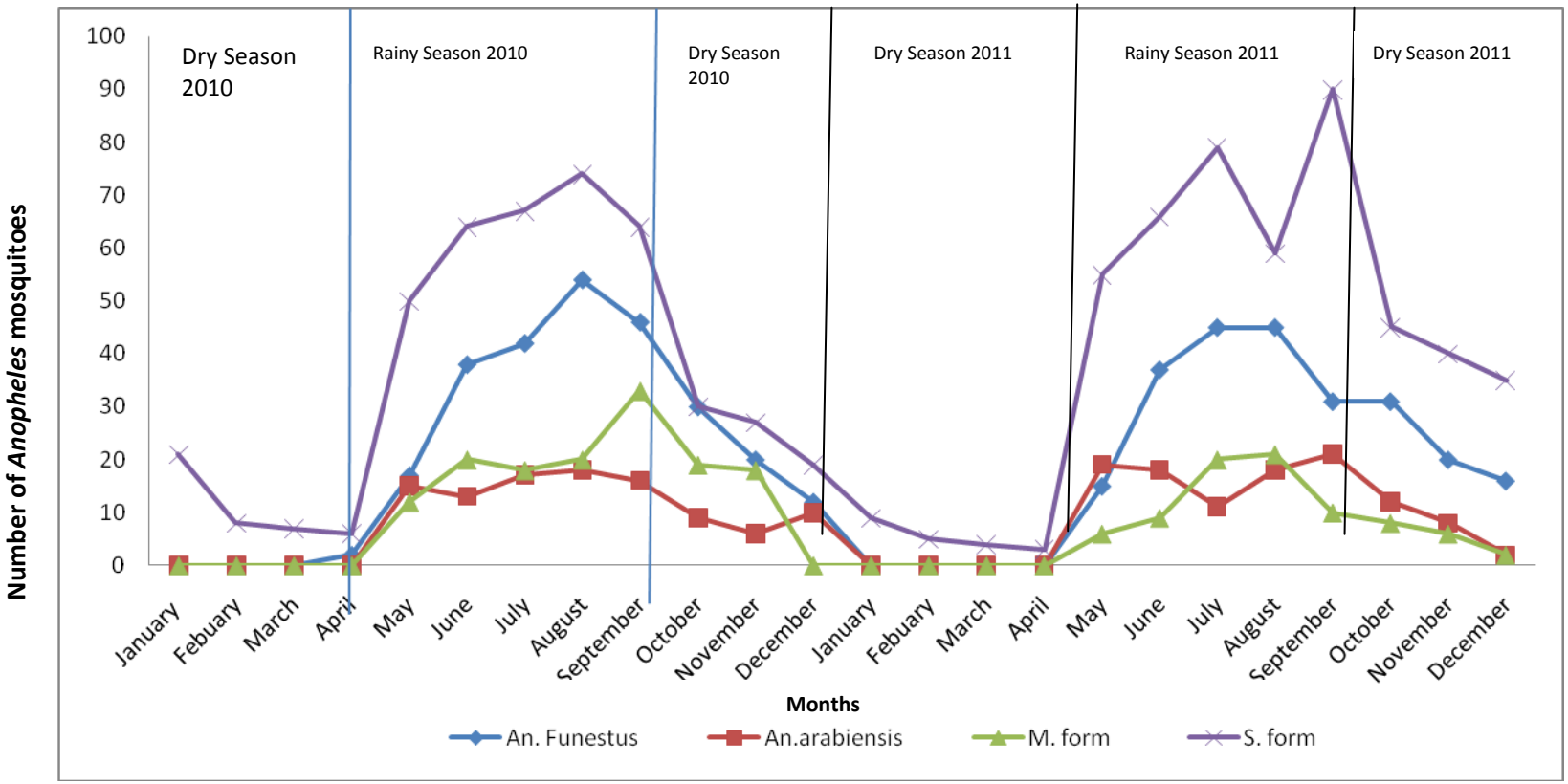

Figure 3: Seasonal abundance of *Anopheles indoor* caught during the study

Table 4.12: Percentage Infection prevalence of *Plasmodium* among the indoor caught *Anopheles*

	***Anopheles* species**				
			An. gambiae s.s		
	An. funestus	***An. arabiensis***	**M**	**S**	**Total**
No. examined	179	43	73	296	591
Plasmodium falciparum (%)	0(0.00)	9(20.93)	4(5.48)	19(6.42)	32(5.42)
Plasmodium malariae (%)	0(0.00)	1(2.33)	1(0.14)	9(3.04)	11(1.86)
Total infection (%)	**0(0.00)**	**10(23.26)**	**5(6.85)**	**28(9.46)**	**43(7.28)**

4.10 Prevalence of *Plasmodium* among indoor caught *Anopheles* in the study sites

Table 4.13 revealed that the distribution of infection among indoor caught female *Anopheles* mosquitoes in the study sites. The infection was all over the 24 study sites. Kirikasamma has the highest number of infected *Anopheles* 6 (13.95%) and 3 of the 4 species found in this site were infected. Only one species, M–form was found infected in 4 sites: Kuit, Garki, Guri and Kadume. Only *An. arabiensis* was found infected in Agubu, Miga and Ringim. Where both *An. arabiensis* and S–form were infeceted was Taura 5 (11.60%).

4.11 Seasonal prevalence of *Plasmodium* among the indoor caught *Anopheles* during study

Seasonal prevalence of *Plasmodium* by indoor caught female *Anopheles* was presented in Figure 4. Transmission was more in rainy season than in dry season in 2010 while it was more in dry season than in rainy season in 2011. For the two year seasons, there was more transmission in 2011 than in 2010. *An. funestus* were not found transmitting in all of the season. Transmission varied significantly among the *Anopheles* species. *An. arabiensis* transmitted more (between April and October 2011 while only in August and September 2010. In contrast to *An. arabiensis*, M – form transmitted more in 2010 than in 2011. There was exponential increment of transmission between April and October each year. Transmission was much for *An. arabiensis* in May, July and August 2011. S–form transmitted more each year.

Table 4.13: Percentage Infection among indoor caught *Anopheles* in the study sites

Sites	*An. funestus*	*An. arabiensis*	M-form	S-form	Total (%)
Auyo				2	2 (4.65)
Ku'it			1		1 (2.33)
Makwalla				1	1 (2.33)
Dubantu				1	1 (2.33)
Jahun				1	1 (2.33)
Birniwa				1	2 (4.65)
Garki				2	2 (4.65)
Guri			1		1 (2.33)
Hadejia				2	2 (4.65)
Kaugama				2	2 (4.65)
Kafin Hausa				3	3 (6.98)
Kirikasamma		2	1	3	6 (13.95)
Kiyawa				3	3 (6.98)
Malam Madori				1	1 (2.33)
Makaddari				1	1 (2.33)
Mawadachi				1	1 (2.33)
Makarahuta				1	1 (2.33)
Kadume			2		2 (4.65)
Agubu		1			1 (2.33)
Azamu				1	1 (2.33)
Gandun Sarki				1	1 (2.33)
Miga		3			3 (6.98)
Ringim		1			1 (2.33)
Taura		3		2	5 (11.60)
Total		**10**	**5**	**28**	**43**

Legend: *An. = Anopheles*

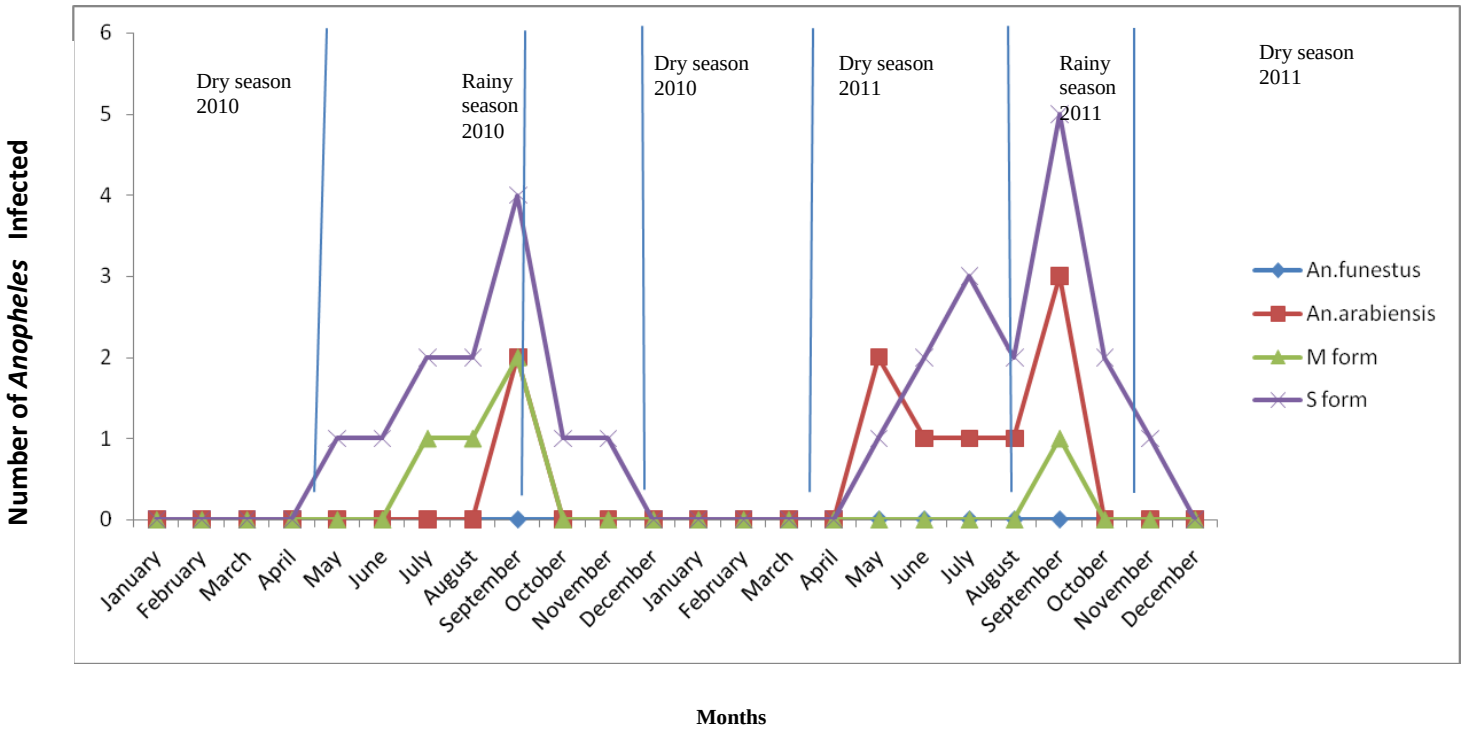

Fig. 4: Seasonal transmission of *Plasmodium* among the indoor caught *Anopheles* during the study

CHAPTER FIVE

DISCUSSION

The results of both morphological examination and molecular analysis of the *Anopheles* caught indoor and reared larvae from sites have revealed that the presence of six *Anopheles* species. The predominant species from morphological examination of adults reared from larvae were *An. gambiae s.l* over *An. funestus*. For example, Kaugama had the highest number of *Anopheles* reared from larvae while Dubantu has the least, no indoor – resting females of *Anopheles gambiae s.l* were caught from Malam Madori and no indoor – resting female *An. arabiensis* was encountered in Auyo, Kuit, Makwalla, Dubantu, Malam Madori, and Azamu. The observation can be explained in the light that generally *An gambiae s.l* are associated with human and therefore tend to inhabit the houses and breed in closed association breeding sites created by pools/ponds and water collections. *An funestus* is known to be a dried season breeding in rivers and stream patched of water might have been the reason of its low abundance in the indoor collection in those localities. The absence of *An. Arabiensis* in the seven localities but caught in others may be due to the present of the preferred host in those areas where they were not encountered. Service (1980) has associated *An. arabiensis* with zoophilism so would prefer to bite outside where there are animals outdoors. This behaviour might have excluded them during the collection period. These results seem to corroborate with those of Coluzzi *et al*. (1979) who reported that the distribution of *An. gambiae* and *An. arabiensis* across the ecological zones of Nigeria was puzzling.

The high observed abundance reached peak during the rainy seasons (in August, 2010 and September, 2011). Preponderance of *An. gambiae s.l* in larval collections than the proportions of *An. funestus* in all sites was observed during the study. It was the most abundant of the species encountered and this agrees with earlier reports that *An. gambiae s.l* is the most widely distributed species in sub – Saharan Africa, due to its genetic heterogeneity which enables it to adapt to many ecological zones (Service, 1985). The patchy collections of the three anopheline mosquitoes in all the localities during the study seems to fit with the anology that *An. gambiae s.l* is heterogeneous due to the complexity in its genetic composition in its existence in the Sahara region in sub-Saharan Africa. In 2001 Onyabe and Conn reported on the distribution of *Anopheles* in Nigeria based on larval collection only. Unlike Onyabe and Conn (2001) who reported only on the indoor population, this study reports on larval and indoor collection, thus combined larval and indoor collections reported more *Anopheles* species and advance more reason that not all mosquitoes breeding in a localities can be caught all indoors are shown by the absence of *An. arabiensis* in both larval population but not indoors collections in localities.

Anopheles funestus was abundant in all the 24 sites but in disproportionate numbers. It was found in higher abundance in Birniwa, Garki, Guri, Kafin Hausa, Kirikasamma, Miga and Taura. No *An. funestus* was caught indoors in Jahun and Makaddari. This might be due to its biting habit by both Anthrophilic and zoophilic (Service 1980). This agrees with findings by Ndams (2004) when *An. funestus* was in relatively low proportions in indoor collection. Moreover, this study reported the abundance of *An. funestus* from both larval and indoor collections across the study sites and this contradicts the findings by Ndams (2004) that *An. funestus* was not found in the larval collections because more *An. funestus* was more

predominant in the Sudan Savanna than Guinea Savanna and its absence may be due to the fact that it breeds in more permanent large bodies of waters like rivers and lakes (Molineux and Gramiccia, 1980), furthermore, the abundance of *An. funestus* may be associated with animals like cattle, which were seen within the vicinity of the study sites; especially in Auyo, Kirikasamma, Ringim and Taura. This report therefore supports Molineaux and Gramiccia (1980) who have reported that *An. funestus* is both zoophilic, anthropophilic and endophilic in there work several decades ago in this area.

Anopheles gambiae s.l collected from Malam Madori five sites with low prevalence: (Kuit, Dubantu, Jahun Makaddari and Gandun Sarki) and sites with higher prevalence (Kirikasamma, Ringim and Guri) could be associated with their feeding and resting habits where *An. gambiae s.l* is both anthropophilic and zoophilic (Service, 1980). The study sites are rural and agrarian with herds of cattle, sheep and goats kept by the inhabitants within their households.

In this study, four molecular forms were reported from the 16s rDNA amplification viz; *An. arabiensis*, and *An. gambiae s.s, An. coluzzi* (M – form) and *An. gambiae ss*- (S-form). Similarly, Onyabe and Conn (2001) used PCR technique and reported on the distribution and prevalence of *Anopheles gambiae s.s* and *Anopheles arabiensis* in Nigeria and their results compare favourably well with that obtained in this study. Nendantok (1991) reported only on the distribution of *Anopheles gambiae s.l* (complex) but his study has reported spilt of the complex into its siblings (*Anopheles gambiae s.s* and *Anopheles arabiensis*), thus highlighting the advantage of PCR technique over the morphological identification.

More *Anopheles gambiae s.s* reported than *Anopheles arabiensis* in all the localities. The result is not expected since the study area is Sudan Savanna and *An. arabiensis* is more adaptable to drier condition than *An. gambiae*. However, it could be that *An. gambiae ss* has invaded the Sudan Savanna because of its heterogeneity that cut across all vegetation zones of west Africa as posited by Caluzzi (1978). This agrees with findings of Coluzzi (1978), Molineaux and Grammiccia (1980), Onyabe *et al*. (2003), Ndams (2004) and Tukur (2010). Also in this study more *An. gambiae* S–form were reported than *An. gambiae* M – form and this disagrees with findings of Cuamba *et al*. (2003).

Anopheles arabiensis was more proponderant in Garki, Guri and Kafin Hausa, with slightly variation among the sites. It was not abundant in seven sites namely Kuit, Makwalla, Dubantu, Malam madori, Mawadachi, Auyo and Azamu by indoor collection. Based on collections of indoor-resting females, Coluzzi *et al*. (1979) concluded that the distribution of *An. arabiensis* across ecological zones of Nigeria was puzzling. Similarly, Onyabe and Conn (2001) reported that *An. arabiensis* exists in several localities but in a very disproportionate numbers. The variation in *An. arabiensis* may be due to enthoanthropogenic factors including construction of houses, clearing of bush for agricultural purposes and to some extent transportation as has been suggested by Carter *et al*. (2000). Coluzzi *et al*. (1979) and Onyabe and Conn (2001) have reported on the occurrence of *An. arabiensis* in some localities in the Southern Guinea savannah and suggested that *An. arabiensis* might have extended its range from the northern guinea savanna due to largely human activities creating breeding sites conducive for *An. arabiensis*.

One site, Hadejia has the highest number of *An. gambiae s.s* reported from this study while none was reported from Malam madori. There was more *An. gambiae s.s* than *An. arabiensis*

in the study sites. Molineaux and Grammiccia (1980) and Tukur (2010) reported similar abundance from Garki. The predominance of *An. gambiae s.s* obtained in Hadejia may be due to its ability to adapt to near all habitats notably in drier savanna (Coluzzi *et al*., 1987). It could also be that *An. gambiae s.s* is both anthropophilic and zoophilic. Combination with high anthropophily confirms the abundance of *An. gambiae s.s* as the primary vector in this site. This result therefore indicate widening of the geographic range of *An. gambiae ss* within which has established as the main malaria vector in the sites.

Anopheles gambiae S – form was the most prevalent among all the specimens encountered in this study. It was also abundant throughout the study period. Kaugama and Kirikasamma have the highest abundance. Tukur (2010) reported on the distribution of M – and S – forms in different vegetation zones of Nigeria. Also Onyabe *et al*. (2003) reported on the distribution of S – forms across Nigeria but limited in a scope determining the relationship between the chromosomal and molecular forms. There was a wide gap between the abundance of S – form and M – form in this study in favour of S – form. This agrees with findings of Ndams (2004) who reported that the distribution of M and S molecular forms was generally lopsided (ratio of 1:3 obtained in favour of S – form). He also reasoned that the pattern may be due to selection and or competition of one from over the other, which is characteristic of a non – panmictic population. Competition or selection was responsible for the distribution patterns of M and S – forms (Della Tore et.al, 2001). Whereas this result contradicts the results obtained by Onyabe *et al*., (2003) who reported a ratio of 1:8 in favour of M – form from southern guinea savanna. Suleman *et al*. (2014) futher reported approximately 77% of the *Anopheles* obtained from Auyo were M – form and no *An.* S – form was detected. They reasoned that M – form was predominant due to resistance to

lambda-cyhalothrin (86.8%) and other insecticides. M-and S – forms were sympatric at four sites: Kirikasamma, Kaugama and Ringim. This agrees with findings by Martinez – Tortes *et al*. (2002) that M-and S – forms were sympatric at 4 sites in Angola.

Overall, the composition of *Anopheles gambiae ss* obtained in this study indicates that M - and S – forms in Nigeria is lopsided with apparent relationship to ecological transition as earlier reported by (Onyabe *et al*. 2003) and Cuamba *et al*. (2003) also reported that *An. gambiae s.s* identified to molecular form were M – form.

The more of *Anopheles* obtained was in the wet than in the dry season in this study. This may be due to breeding sites are provided during the wet season as indicated by the ponds/ditches near the houses where the mosquitoes were caught. This concurs with the observations of Molineaux and Grammicia (1980) that there are large seasonal, yearly and local variations in the level of abundance in the Sudan Savanna. In most sites, the number of *Anopheles* dropped below critical level during the dry seasons. The variations from year to year are relatively important. These numbers increased markedly during the wet season, the time lag between rainfall and mosquito density depends on the species. For example, *Anopheles funestus* was abundant throughout the years in this study whereas the findings of Molineaux and Grammicia (1980) revealed *An. gambiae s. l* throughout the years. Moreover, when the two species occur in sympatry, large changes in species composition often occur, with *An. gambiae s.l* predominating during the dry season and both the species becoming more abundant during the rainy season. Large fluctuations in species abundance are also known to occur seasonally (Service, 1970; White and Rosen, 1973; DiDeco *et al*., 1981). In contrast, Coluzzi *et al*. (1979) reported individually no *An. arabiansis* in eight localities, two of which were sampled in both the dry and rainy seasons, in the southern guinea savanna.

The shifts in species abundance between the two years in this study have also been observed in other studies (Faye *et al*., 1997, and Toure *et al*., 1998). Large fluctuations in species composition are also known to occur seasonally (Service, 1970). Differences in sampling technique may also explain the disagreement between this study and that of Molineaux and Gramiccia (1980). We employed both indoor and larval samplings whereas Molineaux and Gramiccia (1980) collected indoor resting female *Anopheles.* Adult female collections may be biased against *An. arabiensis* because this species is thought to be more zoophilic and less endophilic than *An. gambiae s.l* (White and Rosen, 1973). Moreover, it is not clear how these different sampling methods affect estimates of species abundance and composition.

The amplification of the two fragments sizes, one with 400bp and the other 250bp clearly show the M/S hybrid of *An. gambiae ss* and to our knowledge is reported for the first time in Jigawa state. The hybrid was only reported from Auyo while it was not reported in the rest of the 23 sites. All current work on M/S hybrids assumes the two rarely hybridize (Talihun, 2010) but further explained that there is a significant exchange of genes between the two forms therefore, could exist between them. Even though the hybrids have reduced fitness and the gene exchange process is spatially and temporarily dynamic. The M – and S – forms have been the focus of intense study by malaria researchers and evolutionary biologists interested in ecological speciation (Lanzaro and Lee, 2013).

The M/S hybrids encountered in this study differed from those of Molineaux and Gramiccia (1980) and Tukur (2010) who did not report on M/S hybrid from Garki and Kiyawa, Suleman *et al*. (2014) has recently reported of single hybrid M/S (<1.0%) from Auyo thus confirms our findings on occurence M/S hybrid from the same site.

With regards to transmission, the Enzyme – linked immunnosorbent assays have shown that members of *An. gambiae* complex were positive to *P. malariae* and *P. falciparum* circum sporozoite (CSP) antigens, whereas *An. funestus* was negative to all the CSP antigens. No *Anopheles* species was positive to *P. vivax* vk210 and *P. vivax* vk247 CSP antigens. This shows that the main vectors of human malaria in the study area were members of *An. gambiae s.l* species only; this disagrees with the findings of Molineaux and Gramiccia (1980) who reported that the main vectors of human malaria in Garki were *An. gambiae s.l* and *An. funestus.* This could be attributed to the fact that *An. funestus is* more zoophilic and less endophilic than *An. gambiae s. l* (Coluzzi, 1984). Most *Anopheles* are not exclusive anthropophilic or zoophilic. However, the primary malaria vectors in Africa, *An. gambiae s.l* and *An. funestus* and are strongly anthropophilic and consequently, are two of the most efficient malaria vectors in the world (Steven, 2010). The result also contradicts the one by Anna (2003) that *An. funestus* is a major vector of malaria in Africa.

The prevalence of *Anopheles* positive with *Plasmodium* in this study is similar to the one reported by Molineaux and Gramiccia (1980) even though they employed classical mosquito dissection by microscopy while we employed ELISA. This means that the ELISA adapted for the same purpose and proved equality reliable (Wirtz *et al*., 1987). In this study, 7.28% of the *Anopheles* examined for the presence of *Plasmodium* found positive. This is in agreement with report by Pringle (1996) that in Africa, *An. gambiae* and *An. funestus* usually contain fewer than 5% sporozoites. Closely related, Mahaptra *et al*. (2006) reported 3.4% of the species identified to be *P. falciparum*. The sporozoite infection rate of 7.28% in this study was low. This could be attributed to limitations of indoor collection and the disadvantaged low number of female *Anopheles* caught which again may be as a result of sampling error,

which was influenced by many factors. Among which will be lack of co–operation of the members of households who may decide not to wholly empty their rooms or may decide to keep the windows open very early in the mornings before *Anopheles* collection. Accurate collection and assessment of vector in human habitation is crucial to the understanding of transmission (Coetzee, *et al*, 2000). For example, the number of vectors inside houses and the time they remain in them affect the effectiveness of assessing sporozoite in them.

This study indicated that the *An. gambiae ss* molecular forms were positive to *P. falciparum* and *P. malariae* and are invovled in the transmission of malaria. This confirms the report of Ndams (2004) who explained that *An. gambiae s.s* and *An. arabiensis* were positive to *P. falciparum* and *P. mlariae* CSP antigens. Similarly, absence of the *P. vivax* vk210 and *P. vivax* vk247 in the entire indoor – caught *Anopheles* in this study was reported because *P. vivax* is prevalent in temperate countries.

This study suggests that members of the *An. gambiae* complex are the important vectors of malaria in the study sites. Two species complex, *An. gambiae s.s* and *An. arabiensis* are both the most broadly distributed and the most efficient vectors of malaria (Coetzee *et al*, 2000). Members of *An. gambiae s.l* which usually have high sporozoite rates ranging from 1% to 5% but may approach 10% to 30% in extreme cases, this is because they exhibit high degree of anthropophilism (Ndams, 2004) thus agree with findings of this study. The observation made in this study is similar to the one reported in Garki (Molineaux and Gramiccia, 1980). The prevalence of *P. falciparum* and *P. malariae* only in the *An. gambiae s.s* and *An. arabiensis* suggest that the two are the main transmitters in the study sites.

The infection among other species revealed 10 *An. arabiensis*, 5 M – form and 28 S-form. This showed that S – form was most infected species across the study sites. This could be attributed to the fact that the proportion of S – form was higher than that of *An. arabiensis* in all of the study sites and could be due to the fact that S – form (members of *An. gambiae s.s*) is known to exhibit more anthropophily and endophily (Coetzee, 2000).

Two species, *P. falciparum* and *P. malariae* were detected in favour of the former. This would correlate well with the observed species incidence in human population (1980 – 1983) of 92% *P. falciparum and P. malariae* (Wirtz *et al*., 1985). Umar and Ginsau (2012) similarly reported more cases of malaria due to *P. falciparum and P. malariae* in Jahun which confirmed that the vectors in the study area are transmitting *P. falciparum and P. malirae*. Furthermore Cheesebrough (2005) reported that *P. malariae* has much lower prevalence than *P. vivax* and that it accounts for 25% of malaria infections. Whereas *P. vivax* and *P. ovale* were not reported in this study, *P. vivax* is the main species in South America (occurring as far south as southern Argentina), Mexico, the Middle East, Northern Africa, India, Pakistan, Sri Lanka, Papua New Gunea and Solomon Islands. It was also found in parts of south East Asia, Indonesia, Philippines, Madagascar, tropical and subtropical Africa, Korea, and China but it is not found in West Africa (Cheesbrough, 2005). On the other hand, *P. ovale* has a low prevalence. It is found in West Africa where it accounts for up to 10% of malaria infections (Cheesbrough, 2005). Overall, *P. falciparum* was detected in indoor – caught female *Anopheles* from all the sites and it accounts for 32/43 of the infection while *P. malariae* accounts for 11/43. This agrees with reports mentioned above. However, it was not detected in *An. funestus* (n = 0) but it was detected most in S – form (n = 19).

Kirikasamma had the highest number of *Anopheles* positive with *Plasmodium* while Malam madori recorded zero. Difference in positive rates patterns is attributed to different vector species. Distinctive species composition in each municipality explains the differences in *Plasmodium* transmission (Manuela *et al*., 2014). In our study we found that no *An. gambiae s.l* was reported from Malam madori and could be the reason for variation. Moreover, 3 of the 4 *Anopheles* species found in Kirikasamma were positive. Only M – form was not infected in the 4 sites: Kuit, Garki, Guri and Kadume. Only *An. arabiensis* was found infected in Agubu, Miga and Ringim.

In this study, there were more infected *Anopheles* in rainy season than in dry season of 2010 while more in dry season than in rainy season of 2011. The prevalence during the rainy season of 2010 could be attributed to availability of breeding sites harbored by rain. Collections of water as a result of rain, irrigation of farmland as well as swamps used for rice cultivation enhance mosquito breeding sites (Mahapatra *et al*., 2014). In agreement with seasonal variation in abundance observed in this study, Coetzee *et al.,* 2000) reported that the range and relative abundance of *An. arabiensis* and *An. gambiae* appear to be strongly influenced by climatological factors, especially annual precipitation. Species composition can predominate during the rainy season or dry season and may sometimes be more abundant during the rainy season or vice – versa. *Anopheles arabiensis* was infected more between April and October, 2011 while only infected in August and September, 2010. In contrast, M – form was infected more in 2010 than in 2011. There was exponential increment of transmission between April and October each year. Suleman *et al*. (2014) has investigated the dynamics of species composition in northern Nigeria and has reported larger number of M – form than *An. arabiensis* during the rainy season of 2009 in Auyo, Jigawa State.

CHAPTER SIX

SUMMARY, CONCLUSIONS AND RECOMMENDATIONS

6.1 Summary

This study on *Anopheles* transmitting malaria in parts of Jigawa State revealed *An.gambiae s.l* and *An. funestus* by morphological identification while *An. arabiensis*, *An. gambiae s.s*, M-form and S-forms were identified by molecular analysis. A hybrid between the *An. gambiae s.s* M-form and S-form was encountered.

The abundance was more during the rainy season than in the dry season and varied among the study sites. Kirikasamma had the highest number of *Anopheles*.

Enzyme-Linked Immunosorbent Assay (ELISA) performed on indoor caught *Anopheles* revealed 43 (7.28%) infected with *Plasmodium*. *Anopheles gambiae s.l* and its members were found to be transmitting in the area but not *An*. *funestus*. *Plasmodium falciparum* and *P.malariae* were responsible for malaria infection in the study area.

6.2 Conclusions

The study concludes that:

i. *Anopheles funestus*, *Anopheles arabiensis*, *Anopheles s.*s M-forms and *An. gambiae ss* S-form are the major *Anopheles* mosquitoes collected indoors in study area. A hybrid of M-form and S-form *An. gambiae ss* was encountered in a single locality (Auyo).

ii. The *Anopheles* were more abundant in the rainy season than the dry season during the study.

iii. *Plasmodium falciparum* and *P. malariae* were the *Plasmodia* harboured by the *Anopheles arabiensis* S-and M-forms, and

iv. *Anopheles arabiensis, An. gambiae ss* M-form and *An. gambiae ss* S-form were positive to *P. falciparum* and *P. malariae* ELISA assay. Therefore, are suggested to be the main malaria vectors. Whereas *An. funestus* was a major *Anopheline* but was not implicated as a malaria vector during the study.

6.3 Recommendations

Malaria transmission in some villages of Jigawa State seems to be related to the *Anopheles* species present. The use of insecticide treated nets is recommended for local control. However, personal protection and treatment of breeding sites should be the main control strategy, alongside any complementary indoor control activities such as residual spraying among others. Further studies should be carried on the hybrid to understand their population, biology and vectorial capacity and insecticide ressistance.

REFERENCES

Agarwal, V.K. (2011). *Zoology for Degree Students.* S.Chand and Company Ltd, Ram Nagar, New Delhi, India. Pp131.

Alaribe, A.A.A. (1999). Dynamics of Malaria Transmission in Ekempon Village in Odukpani Local Government Area of Cross River State, Nigeria. Ph.D Thesis, University of Calabar, Nigeria, pp. 32 – 68.

Alexander, K.R. (2009). Caution is required when using health facility–based data to evaluate the health impact of malaria control efforts in Africa. *Malaria Journal*, **8**(209): 43 – 45.

Ana, R. and Wing, K.A. (2008). *Plasmodium* infection and endotoxic shock induce the expansion of regulatory dendritic cells. *Journal of Immunology*, **180** (2): 7116 – 7126.

Anara G. (2011). *Anopheles gambiae* complex. Walter Reed Army Institute of Research. www.wrbu.org/species.

Angus, S. (2008). *Oxford Dictionary of English.* Oxford University Press. Pp64

Arez, A. P., Lopes, D., Pinto, J., Franco, A. S., Snounou, G and do Rosario, V. E. (2000). *Plasmodium* species: Optimal protocols for PCR detection of low parasite numbers from *Anopheles* samples. *Express of parasitology*, **94**: 269 – 272.

Arora, D.E. and Arora, B.B. (2010). *Medical Parasitology*, Third edition, CBS publishers and distributors PVT. Ltd., India. Pp 71 – 78.

Awodebe, H.O., Bassi, P.U., Olumese, P.E. and Oyedeji, S.I. (2005).Comparative assessment of *Plasmodium falciparum* sensitivity to chloroquine and amodiaquine *in vitro. African Journal of Biotechnology*, **4** (11): 1317 – 1319.

Azoma C. and Job O. (2013). Malaria: A Major Global Public Health Burden. *Daily Sun,* **5** (8): 51.

Bangs, M. J., Rusmiarto, S., Gionar, Y. R., Chan, A. S. T., Dave, K and Ryan, J. R. (2002). Evaluation of a dipstic malaria sporozoite panel assay for detection of naturally infected mosquitoes. *Journal of Medical Entomology*, **39**(2): 324 – 330.

Beier, J.C. and Koros, J.K. (1991). Visual assessment of sporozoite and blood meal ELISA samples in malaria field studies. *Journal of Medical Entomology*, **28:** 805 – 808.

Beier, J. C and Koros, J. K. (1991). Anatomical dissemination of circumsporozoite protein in wild Afrotropical *Anopheles* affects malaria sporozoites determination by ELISA. *Medical Veterinary Entomology*, **5**: 81 – 85.

Besanky, N.J., Powell, J.R., Caccone, A., Hamm, D.M., Scott, J.A and Collins, F.H. (1994). Molecular Phylogeny of the *Anopheles gambiae* complex suggests genetic introgression between principal malaria vectors. *Proceedings National Academic of Science* ISA, **91** (15): 6885 – 8.

Brogdon, W.G and McAllister, J.C. (1998). Insecticide resistance and Vector Control. *Emerging Infectious Diseases*, **4**: 605 – 613.

Burkot, T. R., Williams, J. L and Schhneider, I. (1984). Idetification of *Plasmodium falciparum*-infected mosquitoes by a double antibody Enzyme-Linked

Immunosorbent Assay. *American Journal of Tropical Medicine and Hygiene*, **33**: 783 – 788.

Bush, O.A., Kevin, D.L., Jeffrey, M.L. and Allen, W.S. (1997). Parasitology meets Ecology on its Own Terms. *The Journal of Parasitology,* 8(4): 576 – 578.

Butcher, B. (1972).Transmission of *Plasmodium* by female *Anopheles. In:* Edington, G.M. and Gilles, H.M. (1976). *Pathology In The Tropics*, 2nd edition, Edward Arnold (Publishers) Ltd. Pp 11 –31.

Caputo, B. (2011). The ''far-west'' of *Anopheles gambiae* molecular forms. *PLuS ONE*, **6**(2): e16415.

Carter, R., Mendis, K.N. and Roberts, D. (2000). Spatial targeting of interventions against malaria. *Bulleting of the World Health Organization,* **78**: 401 – 1411.

Chandler, A.C. and Read, C.P. (1961). *Introduction to Parasitology,* Tenth Edition, Macmillan, New York. Pp 722 – 757.

Cheesbrough, M. (2005). *District Laboratory Practice in Tropical Countries,* Part I 2nd edtn. Cambridge University Press, pp. 239 – 258.

Chris, B., Nikou, D., Donnlly, M. J., Williamson, M. S., Ranson, H, Ball, A., Vontas, J and Field, L. M. (2007). Detection of knock down resistance (kdr) mutations in *Anopheles gambiae*: a comparison of two new high – through put assays with existing methods. *Malaria Journal*, **6**: 111.

Clark, I.A. and Alleva, L.M. (2004). Pathogenesis of malaria and clinically similar conditions.*Clinical Microbiology*, **17**: 509 – 539.

Coatney, H. (1968). Epidemiology of Malaria. *In:* Edington, G.M. and Gilles, H.M. (1976). *Pathology in the Tropics* 2nd Edition, Edward Arnold (publishers) Ltd. pp 11 – 13.

Coetzee, M., Craig, M. and Le Sueur, D. (2000).Distribution of Africa Malaria Mosquitoes belonging to the *Anopheles gambiae complex. Parasitology Today*, **16:** 67 – 93.

Coetzee, M. (2013).*Anopheles coluzzi* and *Anopheles emharicus*, new members of the *Anopheles gambiae* complex. *Zootaxa*, **3619** (2):246 - 274

Collins, F.J., Procell, P.M., Campbell, G.H. and Collins W.E. (1988).Monoclonal antibody-based ELISA for detection of *Plasmodium malariae. American Journal of Tropical Medicine and Hygiene,* **38:** 283 – 288.

Coluzzi, M. (1978). Morphological divergencies in the *Anopheles gambiae* complex. *Rivistadi Mariologia*, **43**: 197 – 232.

Cuamba, N., Choi, K.S., and Townson, H. (2001). Malaria vectors in Angola: distribution of species and molecular forms of the *Anopheles* complex their pyrethroid insecticide knockdown resistance (kdr) status and *Plasmodium falciparum* sporozoite rates. *Pubmed-indered forMEDLINE,* **(13):** 63-361.

Curtis, C.F. (1991). Can Molecular Biology contribute usefully to vector control? *Sweiz Medical Wochenschr,* **129:** 111 – 116.

Curtis, C.F. (1996). *Control of malaria vectors in Africa and Asia.* London, United Kingdom, Pp. 1–8.

Della Torre, A., Tu, Z and Petrarca, Z. (2005). On the distribution and genetic differentiation of *Anopheles gambiae s.s*. molecular forms. *Insects Biochemistry Molecular Biology*, **36** (7): 755 – 769.

Della Torre, A. (2001). Molecular evidence of incipient speciation within *Anopheles gambiae s.s*. in West Africa. *Insect Molecular Biology*, **10**(1): 9 - 18

David, C. (2008).Canback Global Income Distribution Database.*www.cgidd*.com

Davidson, G. (1964). The five mating types of the *Anopheles gambiae* complex. *Malariology*, **13**: 167 – 183.

Diabate, A., Dabire, R. K., Millogo, N and Lehmann, T. (2007). Evaluating the effect of postmating isolation between molecular forms of *Anopheles gambiae* (Diptera: Culicidae). *Journa lof Medical Entomology*, **44**(1): 60 – 64.

Edington, G.M. and Gilles, H.M. (1976). *Pathology in the Tropics* 2nd edition. Edward Arnold (publishers) Ltd., England. Pp 11 – 34.

Eric, C.,Van, M., Pham, O. M., John, F., Anderson, J and Ribeiro, M.C. (2009). ‘’The salivary gland transcriptome of the neotropical malaria vector *Anopheles darling* reveals accelerated evolution of genes relevant to hematology’’ *Genomics*, **10** (1): 57

Externe, A.K. (2011). *Anopheles gambiae:* First genome of a vector for a parasitic disease. http://www.cns.fr/externe.

Fanello, C., Santolamazza, F. and Della – Torre (2002).Simultaneous Identification of species and molecular forms of the *Anopheles gambiae* complex by PCR – RFLP.*Veterinary Entomology*, **16** (4): 461 – 473.

Favia, G., Della Torre A., Bagayoko, M., Lanfrancotti, A., Sagnon, N.F., Toure, Y.Y. and Coluzzi, M. (2001). Molecular identification of sympatric chromosomal forms of *Anopheles gambiae* and further evidence of their reproductive, isolation. *Insect Molecular Biology*, **6:** 377–383.

Favia, G., Lanfrancoti, A., Spanos, I and Louis, C. (2001). Molecular characterization of ribosomal DNA polymorphisms discrimination among chromosomal forms of *Anopheles gambiae s.s. Insect Molecular Biology*, **10** (1): 19 - 23

Florence, V. (2007). Guarding Against Malaria–using insecticide treated Nets. http://povertynewsblog.blogspot.com

Fong, E. (1971). Distribution of *Plasmodium. In:* Edington, G.M. and Gilles, H.M. (1976). *Pathology in the Tropics*, 2nd edition, Edward Arnold (Publishers) Ltd., Pp 11 – 34.

Fontenile, D., Meunier, J. Y., Nnkondjio, C. A and Tchuinkam, T. (2001). Use of circumsporozoite protein Enzyme-linked Immunosorbent Assay compared with microscopic examination of salivary glands for calculation of malaria infectivity rates in mosquitoes (Diptera: Culicidae) from Cameroun. *Journal of Medical Entomology*, **38**: 451 – 454.

Frederic, S., Anna, C., Jean-Claud, Pierre, K., Maurean, C. and Didier, F. (2003).Species identification within the *Anopheles funestus* group of malaria vectors in Cameroun and evidence for a new species. www.ajtmb.org/cgi/content

Garnham, M. (1954). *Plasmodium* first enters the liver cells. *In.* Jordan, E.L. and Verma P.S. (2011).*Invertebrate Zoology.*S. Chand and Company Ltd., Ram Nagar, New Delhi, India Pp 176 – 925.

Gilles, M.T. and Coetzee, M. (1987). *A supplement to the Anopheles of Africa South of the Sahara.*The South Africa Institute of Medical Research, Johannesburg, South Africa. Pp 12 – 79.

Gilles, M.T. and De Meillon B (1968). The *Anophelinae of Africa South of Sahara Ethiopian Zoogeographical Region,* 2nd edn., no. 54. The South African Institute for Medical Research. Johannesburg. Pp 34 - 343

Gordon, L.M. (1962). The arthropod hosts are females of certain species of *Anopheles* mosquito. *In:* Edington, G.M. and Gilles, H.M. (1976). *Pathology in the tropics*, 2nd edition, Edward Arnold (Publishers) Ltd. Pp 11

Hunt, R. H., Coetzee, M. and Fettene, M. (1998). Tha *Anopheles gambiae* complex: A new species from Ethiopia. *Transactions of the Royal Society of Tropical Medicine and Hygiene,* **92** :231 – 235.

Hunt, R.H., Coetzee, M., Craig, M., Le Sueur, D. (2000). Distribution of African malaria mosquitoes belonging to the *Anopheles gambiae* complex. *Parasitology Today*, **16:** 74 – 77.

Ibrahim, M.G. (2013). Malaria kills 660,000 people yearly UNICEF. *Daily Trust,* pp 12.

Joe, D. (2010). We are not after trying to eradicate mosquitoes. But we are actually just trying to make them less harmful to us. www.malariavaccine.RSS.org.

Johns, H. (2010). Our vaccine is not targeting the parasites, we are targeting a mosquito molecule Africa RSS Feed: *Malaria,* **1** (818): 73 – 15.

Jordan, E.L. and Verma, P.S. (2011). *Invertebrate Zoology.* S. Chad & Company Ltd, Ram Nagar, New Delhi, India. Pp 176 – 925.

Jyotsana, D., Hemlata S., Meenu, S., Manoj, K., Das, O. P., Singh, K., Raghavendra, N., Nanda, N., Aditya, P., Dash, D. N and Saksena, A. D. (2010). Phylogenetic inference of Indian malaria vectors from multilocus DNA sequences. *Infection, Genetics and Evolution*, **10**(6): 755 – 763.

Killen, G. F., McKenzie, F. E., Foy, B.D., Schieffelin, C., Billingsley, P.F and Beir, J. C. (2000). A simplified model for predicting malaria entomologic inoculation rates based on entomologic and parasitologic parameters relevant to control. *American Journal of Tropical Medicine and Hygiene*, **62**: 535 – 544.

Killen, G.F. (2003). Following in Sooper's footsteps: northeast Brazil 63 years after eradication of Anopheles gambiae. Lancet Infectious Diseases, 3 (10): 663-544.

Lanzaro, G. C and Lee, Y. (2013). Speciation in *Anopheles gambiae* – The distribution of populations. *Anopheles* mosquitoes: New insights into Malaria Vector. Ed Mangui (InTech. Rijeka.Croatia). *Lancet Infectious Disease,* **5** (7): 45-67.

Lavern, N. (1880). Malaria parasite (*Plasmodium)* in the blood of a malaria patient. In: Jordan, E.L. and Verma, P.S. (2011). *Invertebrate Zoology.* S. Chand and Company Ltd., Ram Nagar, New Delhi, India. Pp 176 – 925.

Lindsay, S.W., Parson, L. and Thomas, C.J (1998). Mapping the ranges and relative abundance of the two principal African malria vectors, *Anopheles gambiae sensu stricto* and *An. arabiensis*, using climate data. *Proceeding of Royal Society,* **265**:847 – 854.

Manuela, H., Lorena, I. O., Cilia, P., Jan, E. C and Martha, L. Q. (2014). *Anopheles* species composition explains differences in Plasmodium transmission in La Guajira, northern Columbia. *Memorias Instituto Oswaldo Cruz,* **109** (7): 952-956

Macculoch, J. (1827). Named "malaria" in: Jordan, E.L. and Verma, P.S. (2011).*Invertebrae Zoology*.S. Chand and Company Ltd., Ran Nagari New Delhi, India. pp 176 – 925.

Macdonald, G. (1952). The analysis of the sporozoite rate. *Tropical Disease Bulletein*, **49**: 569 – 586.

Mahapatra, N., Marai, N. S., Ranjit, M.R., Parida, S. K., Hansdah, D. P., Hazra, R. K and Kar, S. K (2006). Detection of *Plasmodium falciparum* infection in *Anopheles* mosquitoes from Keonjhar ditrict, Orissa, India. *Journal of Vector Borne Diseases*, **43**: 191 – 194.

Margolis, L., Esch, G.W., Holmes, J.C., Kuris, A.M. and Schad, G.A. (1982). The use of Ecological Terms in Parasitology. *The Journal of Parasitology,* 68(1): 131

Mark, Q.B. (1997). *Care and Maintenance of Anopheline Mosquito Colonies: The Molecular Biology of Insect Disease Vectors.* Springer Science and Business Media, B.V., Springer, Netherlands. Pp 347 - 350

Martinez – Torres, D., Chandre, F., Williamson, M.S., Darriet, F., Berge, J.B., Devonshine, A.L., Guillet, P., Pasteur, N. and Pauron, D. (2002). Molecular characterization of Pyrethroid Knockdown resistance (Kdr) in the major malaria vector *Anopheles gambiae.* www.ncbi-nlm.nih.

Maxwell, A.A., Kwabena, M.B., Samuel, D., Uris, S.M. Isace A., Elizabeth, O., Daniel, E.S., William, O.R., Kwado, K. and David, J.F. (2003). Detection of malaria sporozoite by standard ELISA and Vec – Test dipstick assay in field – collected *Anopheline* mosquitoes from a malaria endemic site in Ghana. *Tropical Medicine and International Health*, **8** (11): 1012 – 1017.

Mercel, T and Don, D.S. (2008). Malaria eradication back on the table. *Bulletin of the World Health Organization*, **86** (2): 82 – 83.

McGregor, M.G. (1971). The predominant vectors of malaria. *In:* Edinton, G.M. and Gilles, H.M. (1976). *Pathology in the Tropics,* 2nd edition. Edward Arnold (publishers) Ltd, Pp 18 – 33.

McMorran, B. (2009). New hope for better malaria treatment http://www.abc.net.au

Molineaux, L. and Grammicia, G. (1980). *The Garki project: The Epidemiology and Control of Malaria in the Sudan savanna of West Africa.* W.H.O, Geneva, Switherland. Pp 20 – 331.

Moreno, M., Marinotti, O., Krrzywinsky, J., Tadei, W.P., James, A.A., Achee, N. L and Conn, J. E. (2010). ‘’Complete mtDNA genomes of *Anophelesdarling* and an approach to anopheline divergence time’’. *Malaria journal*, **9**: 127.

Musa, H. (2013). Kano Sensitizes Traditional Medicine Practitioners. *Kano Chronicle*, **3**(8):2

Ndams, I.S. (2004). Studies on *Anopheles* and *Plasmodium* transmission in parts of Nigeria. Ph.D Thesis, Ahmadu Bello University, Zaria, Nigeria. Pp 98–117.

Neil, A.C., Jane, B.R., Lisa, A.U., Michael, L.C., Steven, A.W., Peter, V.M. and Robert, B.J. (2008).*Biology*, 8th edition, Benjamin Cummings Limited, New York, Pp 583 – 584.

Nendangtok, Y. I (1991). A Survey of Mosquitoes in Biolgical Sciences Department, Ahmadu Bello University, Zaria. B.Sc Thesis, Department of Biological Sciences, Ahmadu Bello University, Zaria. (Unpublished)

Njau, R.J. (2009). Implementation of an insecticide – treated net subsidy scheme under public – private partnership for malaria control in Tanzania challenges in Implementation. *Malaria Journal* **8** (201): 5 – 7.

Ogba, O.M., Alaribe, A.A., Ogban, G.I., Osuchukwu, N.C. and Inyang – Etoh, P.C. (2007). Malaria Transmission potential in Adim Commuity of Biase Local Government Area of Cross River State of Nigeria. *Journal of Medical Laboratory Science,* **16** (1): 26-34

Onyabe, D.Y. and Conn, J.E. (2000). Genetic Differentiation of the malaria vector *Anopheles gambiae* across Nigeria suggests that selection limits gene flow. *Heridity,* **87***:* 647 – 658.

Onyabe, D.Y., Vajime, C.G., Nock, I.H., Ndams, I.S., Akpa, A., Alaribe, A.A. and Conn, J.E. (2003).The Distribution of M-and S-Molecular forms of *Anopheles gambiae s.s* in Nigeria. *Transactions of the Royal Society of Tropical Medicine and Hygiene,* 97: 605 – 608.

Parmakelis, A., Rusello, M.A and Caccone A. (2008). Historical analysis of a near disaster: *Anopheles gambiae* in Brazil. *American Journal of Tropical Medicine and Hygiene,* **78** (1): 176 – 8.

Patrick, K. (1894). Role of mosquitoes in malaria Transmission. In: Jordan, E.L. and Verma, P.S. (2011). *Invertebrate Zoology*. S. Chand and Company Ltd., Ram Nagar, New Delhi, India. Pp 176 – 925.

Pennetier, C., Warren, B., Dabire, K.R., Russell, I.J. and Gibson G. (2009). Singing on the wing as a mechanism for species recognition in the malarial mosquito *Anopheles gambiae.Current Biology,* **61** (5): 78-93

Pfeifter, R. (1892). Role of some blood-sucking insect in the transmission of malaria. In: Jordan, E.L. and Verma, P.S. (2011). *Invertebrate Zoology*. S. Chand and Company Ltd., Ram Nagar, New Delhi, India. Pp 176 – 925.

Peter, C.B., Brett, S.L., Moses, K. C., Christotopher, S. A. and Kevin, I.N. (1998). Parasite antigens on the infected red cell surface are targets for naturally acquired immunity to malaria. *Natural Medicine,* **4** (3): 358 – 360.

Ponnudurai, T., Lensen, A. H. W., Vangemert, G. J. A., Bolmer, M.G and Meuwissen, J (1991). *Transaction of Royal Society of Tropicl Medicine and* Hygiene, **85**: 175 – 180.

Posthuma, G., Meis, J., Verhave, J. P., Gigengack, S., Hollingdale, M. R., Pondurai, T and Geuze, H. J. (1989). Immunological determination of *Plasmodium falciparum*

circumsporozoite protein in *Anopheles stephensi* salivary gland cells. *European Journal of Cell Biology*, **49**: 66 – 72.

Pringle, G. (1966). Aquantitative study of naturally-acquired malaria infections in *Anophelesgambiae* and *Anopheles funestus* in a highly malarious area of East Africa. *Transaction of Royal Society of Tropical Medicine and Hygiene,* **60**: 626 – 632.

Rhoel, D. (2010). New malaria Vaccine Initiative Aims to Stunt Parasite within mosquitoes. *Africa RSS Feed: Malaria*, **1** (18): 1 – 12.

Rudinska, A. and John, C. (1965).The ultra structure of *Plasmodium. In:* Jordan, E.L. and Verma P.S. (2011). *Invertebrate Zoology.* S. Chand and Company Ltd., Ram Nagar, New Delhi, India. Pp 176 – 925.

Rulisa, S. (2009). Malaria has no effect on birth weight in Rwanda. *Malaria Journal,* **8** (194): 5 – 7.

Scott, J.A., Brogdon, W.G and Collins, F.H. (1993). Identification of Single Specimens of the *Anopheles* complex by the polymerase chain reaction. *American Journal of Tropical Medicine and Hygiene,* **49:** 520 – 529.

Scott, J.A., Brogdon, W.G. and Collins, F.H. (1993).Identificaiton of single specimens of the *Anopheles gambiae* complex by the polymerase chain reaction. *American Journal of Tropical Medicine and Hygiene,* **49**: 520 – 529.

Service, M.W. (1980). *A Guide to Medical Entomology*. Macmillan International College Edition. Oxford Press Ltd, Pp 12 – 34.

Service, M.W. (1993). *Field Sampling Methods.* 2nd edition., Elsevier Applied Science, London. Pp 16 –47.

Service, M.W. (1970). Identification of the *Anopheles gambiae* complex in Nigeria by larvaland adult chromosomes. *Annals of Tropical Medicine and Parasitology*, **64**:131 – 136.

Shigeto, Y., Yohei S., Daisuke, K., Yoshiaki, K., Anil, K. G., Mercelo, J. L and Robert, E. S. (2007). Hemolytic C-type lectin CEL-III from sea cucumber expressed in transgenic mosquitoes impairs malaria parasite development. *PLUS Pathogens*, **3** (12): 192.

Short, T. (1948). Pathology of *Plasmodium.* In: Jordan, E.L. and Verma, P.S. (2011). *Invertebrate Zoology*. S. Chad and Company Ltd., Ram Nagar, New Delhi, India Pp 176 – 925.

Singh, N., Singh, P.O. and Sharma, V.P. (1996). Dynamics of malaria transmission in forested and deforested regions of Mandla district, Central India (Madhya Pradesh). *Journal America Mosquito Control Association*, **12** (2): 225 – 234.

Steven, L. (2010). ‘’*Anopheles* mosquito transmission of brain tumor’’. *Medical Hypotheses*, **74** (1): 167 – 168.

Suleman, S. I., Yayo, A. M., Zainab, T., Helen, I and Charles, S. W. (2014). High frequency of *kdr* L101F is associated with pyrethroid resistance in *Anopheles coluzzii* in Sudan Savannah of northern Nigeria, *BMC Infectious Diseases*, **10** (14): 441

Taiwo, O. (2010). Nigeria: Malaria Fighting for Jigawa’s Tomorrow. http://al;africa.com/stories

Toply, E.W. (2008). *Microbiology and Microbial Infection Parasitology.* Arnold E. co-publishers in the USA, Oxford University Press Incorporation. Pp 363.

Toure Y.T., Dolo, G., Petrarca, V. Traore S.F., Boure, M., Dao, A., Carnahan, J and Taylor, C.E. (1998). Mark-release-recapture expirements with *Anopheles gambiae* s. l. in Banambani Village, Mali, to determine population size and structure. *Medical Veterinary Entomology*, **12**: 74 – 83.

Tripet, F. (2001). DNA analysis of transferred sperms reveals significant levels of gene flow between molecular forms of *Anopheles gambiae*. *Molecular Ecology*, **10** (1): 1725 – 1732.

Tukur, Z. (2010). Resistance status of *Anopheles* mosquitoes to pyrethroid (Deltamethrin) in selected areas of Northern Nigeria. *Ph.D Dissertation*, Ahmadu Bello University, Zaria, Nigeria. Pp 50 – 78.

Turner, T. L and Hahn, M. W and Nuzhdin, S. V. (2005). Genomic islands of speciation in *Anopheles gambiae*. *PLUS Biology*, **3**(9): e285.

Turner, T. L and Hahn, M. W. (2010). Genomic islands of speciation orgenomic islands and speciation? *Molecular Ecology*, **19**(5): 848 – 850.

Tylor, C. (2001). Gene flow among populations of the malaria vector. *Anopheles gambiae* in Mali, West Africa. *Genetics*, **157**(2): 743 – 750.

Umar, A.A. and Ginsau, M.A. (2012). Rating of *Plasmodium* species in Malaria cases in Jahun Local Government Area, Jigawa State, Nigeria. *Biological and Environmental Journal for the Tropics,* **9** (1): 48 – 51.

Van Bortel, W., Trung, H. D., Roelants, P., Harbach, R. E., Beckljau, T and Coosemans, M. (2000). Molecular identification of *Anopheles smimus s.l*. beyond distinguishing the members of the species complex. *Insect Molecular Biology*, **9** (3): 335 – 340.

Wang, Y., Gilbreath, T. M., Kulkuta, P., Yan, G and Xu, J. (2011). Dynamic gut microbe across lifehistory of the malaria mosquito *Anopheles gambiae* in Kenya.*PLUS ONE*, **6** (9): 24-67.

W.H.O (1975). Manual on Practical Entomology in Malaria part II: Methods and Techniques 13. WHO, Geneva. Pp 453-501

W.H.O (1992). Learners Guide Entomological Field Techniques for Malaria Control. Part I: 1 - 7

W.H.O (1993). Global Plan of Action for Malaria control. 1993 – 2000. A WHO Study group on the implementation of the Global plan action for malaria control. Pp 78-92

W.H.O. (2001). Malaria at – a glance – roll back malaria: P. 1 – 5 Curtis, C.F. (1996). *Control of Malaria vector in Africa and Asia.* London, United Kingdom. Pp. 1 – 8.

W.H.O/TDR News (2007). TDRS Molecular Entomological Committee: Mosquito genomes secrets. TDR news No. **78**: 28 – 30.

W.H.O. (2009). Malaria fact sheet No. 94 updated January, 2009.

White, G. B. (1974). *Anopheles gambiae* complex and disease transmission in Africa. *Transaction of Royal Society of Tropical Medicine and Hygiene,* **68:** 278 – 301.

White, G. B and Rosen, P. (1973). Comparative studies on sibling species of the *Anopheles gambiae* Giles complex (Diptera: Culicidae). II. Ecology of species A and B in Savannah around Kaduna, Nigeria, during transition from wet to dry season. *Bulletin of Entomological Research*, **62**: 613 – 625.

William, N. and Wylie, T. (1983). Poverty, Distress, and Disease: Labour and Construction of the Rideu Canal. *Labour*/*LeTravail*, **11**:7 – 29.

Wirtz, R.A., Burkot, T. R., Andre, R. A., Rosenberg, R., Collins, W. E and Roberts, D. R. (1985). Identification of *Plasmoidum vivax* sporozoites in mosquitoes using an Enzyme-linked Immunosorbent Assay. *American Journal of Tropical Medicine and Hygiene*, **34**: 1048 – 1054.

Wirtz, R. A., Zavala, F., Charoenvit, Y., Campbell, G. H., Burkot, T.R., Schneider, I., Esser, K. M., Beaudoin., R. A and Andre, R. G. (1987). Comparative Testing of *Plasmodium falciparum* sporozoite monoclonal antibodies for ELISA development. *Bulletin of World Health Organization*, **65:**39– 45.

Wirtz, R. A., Burkot, T. R., Graves, P. M and Andre, R. G. (1987). Field evaluation of Enzyme-Linked Immunosorbent Assay (ELISA) for *Plasmodium falciparum Plasmodium vivax* sporozoites in mosquitoes (Diptera: Culicidae) from Papua New Guinea. *Journal of Medical Entomology*, **24**: (In press).

Witson, H. (1973). *The Distribution of Malaria Vectors*. In: Edington, G. M. and Gilles, H.M. (1976). *Pathology in the Tropics*, 2nd Edition. Edward Arnold (Publishers) Ltd. Pp 17 – 41.

Zakari, U. (2006). Settlements and settlers of Northern Nigeria.www.africahistory/nigeria.

APPENDICES

Appendix I: ELISA values obtained from examining the indoor caught *Anopheles*

	1	2	3	4	5	6	7	8	9	10	11	12
A	0.218	**0.207**	0.110	**0.242**	**0.261**	0.103	0.061	0.083	0.068	0.009	0.046	0.034
B	0.086	**0.229**	0.040	**0.203**	0.048	0.061	0.070	0.067	0.091	0.019	0.013	**0.226**
C	0.082	0.063	0.063	0.057	**0.208**	0.020	0.112	0.053	0.080	**0.200**	0.018	0.026
D	0.106	0.041	0.071	**0.227**	0.108	**0.286**	0.026	0.011	0.043	0.012	0.018	0.106
E	0.103	**0.232**	0.083	**0.199**	**0.278**	0.008	0.109	0.031	0.041	**0.214**	0.069	0.013
F	0.108	0.100	0.066	0.008	0.090	0.112	0.010	0.006	0.021	**0.245**	0.068	0.089
G	0.107	0.068	**0.215**	0.111	0.103	0.110	0.006	0.089	0.104	**0.200**	0.110	0.073
H	0.100	**0.248**	0.010	**0.216**	0.023	0.036	0.011	0.027	0.011	0.013	0.018	0.036

Cut off value = 0.198 Number of Positive Wells = 19

A1 is positive control B1 – H1 are negative controls prepared from laboratory reared *Anopheles*

Appendix II: Showing ELISA values obtained from examining the Indoor caught *Anopheles*

	1	2	3	4	5	6	7	8	9	10	11	12
A	0.199	**0.207**	0.110	**0.242**	**0.261**	0.103	0.061	0.083	0.068	0.009	0.046	0.034
B	0.086	**0.229**	0.040	**0.203**	**0.208**	0.061	0.070	0.067	0.091	0.019	0.013	**0.206**
C	0.082	0.063	0.063	**0.227**	**0.278**	0.020	0.112	0.053	0.080	**0.245**	0.018	**0.226**
D	0.106	0.041	0.071	**0.199**	0.108	**0.214**	0.026	0.011	0.043	0.012	0.018	0.106
E	0.103	**0.232**	0.083	**0.238**	**0.286**	0.008	0.109	0.031	0.041	**0.200**	0.069	0.013
F	0.108	0.100	0.066	0.008	**0.200**	0.112	0.010	0.006	0.021	**0.214**	0.068	0.089
G	0.107	0.068	**0.215**	0.111	0.103	0.110	0.006	0.089	0.104	**0.203**	0.110	0.073
H	0.100	**0.248**	0.010	**0.216**	0.023	0.036	0.011	0.027	0.011	0.013	**0.218**	0.036

Cut off value = 0.198 Number of Positive Wells = 24

A1 is positive control B1 – H1 are negative controls prepared from Laboratory reared *Anopheles*

Appendix III: Showing ELISA values obtained from examining the indoor caught *Anopheles*

	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
A	0.233	0.061	0.023	0.101	0.024	0.113	0.101	0.002	0.129	0.083	0.061	0.017
B	0.107	0.101	0.008	0.012	0.106	0.124	0.106	0.027	0.002	0.109	0.106	0.009
C	0.104	0.061	0.106	0.002	0.088	0.013	0.006	0.077	0.128	0.016	0.006	0.106
D	0.119	0.073	0.006	0.128	0.071	0.123	0.128	0.043	0.078	0.127	0.012	0.071
E	0.120	0.129	0.012	0.101	0.063	0.114	0.009	0.081	0.081	0.109	0.111	0.114
F	0.093	0.114	0.071	0.009	0.001	0.129	0.017	0.076	0.114	0.118	0.068	0.107
G	0.108	0.107	0.114	0.017	0.103	0.091	0.129	0.116	0.070	0.126	0.009	0.012
H	0.116	0.008	0.107	0.103	0.088	0.102	0.007	0.100	0.009	0.121	0.129	0.023

Cut off value = 0.219 Number of positive wells = 0

A1 is positive control B1 – H1 are negative controls prepared from Laboratory reared *Anopheles.*

Appendix IV: Showing ELISA values obtained from examining the indoor caught *Anopheles*

	1	2	3	4	5	6	7	8	9	10	11	12
A	0.181	0.107	0.110	0.053	0.008	0.034	0.051	0.068	0.103	0.107	0.013	0.112
B	0.106	0.006	0.034	0.105	0.079	0.049	0.013	0.051	0.063	0.113	0.056	0.063
C	0.103	0.0116	0.078	0.051	0.058	0.068	0.031	0.013	0.051	0.116	0.101	0.109
D	0.108	0.109	0.107	0.113	0.036	0.058	0.113	0.056	0.116	0.051	0.114	0.107
E	0.091	0.008	0.051	0.068	0.022	0.116	0.061	0.116	0.013	0.113	0.079	0.063
F	0.103	0.101	0.108	0.007	0.112	0.034	0.013	0.051	0.112	0.116	0.108	0.113
G	0.001	0.012	0.115	0.109	0.104	0.106	0.108	0.063	0.079	0.051	0.069	0.115
H	0.013	0.063	0.112	0.076	0.008	0.061	0.104	0.108	0.112	0.102	0.116	0.104

Cut off value = 0.150 Number of positive wells = 0

A1 is positive control B1 – H1 are negative controls prepared from Laboratory *Anopheles*

Appendix V: Showing ELISA values obtained from examining the indoor caught *Anopheles*

	1	2	3	4	5	6	7	8	9	10	11	12
A	0.253	0.046	0.016	0.103	0.057	0.010	0.032	0.073	0.019	0.010	0.093	0.010
B	0.068	0.012	0.047	0.006	0.058	0.100	0.018	0.100	0.058	0.060	0.103	0.058
C	0.114	0.057	0.078	0.007	0.013	0.093	0.047	0.023	0.011	0.098	0.101	0.023
D	0.108	0.088	0.093	0.047	0.088	0.008	0.101	0.105	0.105	0.008	0.102	0.007
E	0.101	0.105	0.088	0.078	0.006	0.105	0.093	0.100	0.068	0.100	0.068	0.093
F	0.091	0.047	0.057	0.081	0.093	0.007	0.098	0.011	0.007	0.045	0.105	0.105
G	0.120	0.105	0.101	0.008	0.0058	0.102	0.012	0.018	0.053	0.102	0.046	0.011
H	0.142	0.003	0.105	0.010	0.011	0.006	0.063	0.101	0.102	0.011	0.101	0.060

Cut off value = 0.213 Number of positive well = 0

A1 is positive control B1 – H1 are negative controls prepared from laboratory reared *Anopheles*

Appendix VI: Showing ELISA values obtained from examining the indoor caught *Anopheles*

	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
A	0.293	0.163	0.168	0.171	0.106	0.137	0.120	0.148	0.007	0.179	0.181	0.102
B	0.031	0.183	0.107	0.148	0.201	0.188	0.148	0.112	0.119	0.066	0.009	0.001
C	0.067	0.177	0.172	0.156	0.200	0.114	0.201	0.123	0.104	0.114	0.098	0.020
D	0.048	0.078	0.188	0.200	0.004	0.041	0.108	0.146	0.178	0.049	0.126	0.020
E	0.093	0.094	0.031	0.121	0.098	0.131	0.114	0.200	0.104	0.200	0.076	0.041
F	0.178	0.156	0.098	0.046	0.124	0.201	0.123	0.210	0.170	0.213	0.201	0.118
G	0.108	0.147	0.105	0.016	0.139	0.111	0.168	0.106	0.201	0.200	0.178	0.200
H	0.183	0.173	0.162	0.001	0.123	0.088	0.010	0.028	0.167	0.098	0.125	0.200

Cut off value = 0.202 Number of positive well = 0

A1 is positive control B1 – H1 are negative controls prepared from Laboratory reared *Anopheles*

Appendix VII: Showing ELISA values obtained from examining the indoor caught *Anopheles*

	1	2	3	4	5	6	7	8	9	10	11	12
A	0.253	0.102	0.012	0.078	0.0104	0.094	0.073	0.101	0.091			
B	0.146	0.060	0.098	0.003	0.066	0.102	0.103	0.090	0.061			
C	0.093	0.012	0.101	0.023	0.061	0.067	0.012	0.104	0.104			
D	0.069	0.009	0.060	0.046	0.102	0.011	0.056	0.001	0.068			
E	0.148	0.041	0.046	0.103	0.054	0.038	0.100	0.102	0.101			
F	0.140	0.103	0.092	0.091	0.061	0.100	0.018	0.068	0.043			
G	0.067	0.067	0.068	0.021	0.071	0.069	0.010	0.007	0.098			
H	0.073	0.088	0.068	0.102	0.031	0.101	0.102	0.040				

Cut off value = 0.210 Number of positive well = 0

A1 is positive control B1 – H1 are negative controls prepared from Laboratory reared *Anopheles*

Appendix VIII: ELISA values obtained from examining the indoor caught *Anopheles*

	1	2	3	4	5	6	7	8	9	10	11	12
A	0.218	**0.207**	0.110	**0.242**	**0.261**	0.103	0.061	0.083	0.068	0.009	0.046	0.034
B	0.086	**0.229**	0.040	**0.203**	0.048	0.061	0.070	0.067	0.091	0.019	0.013	**0.226**
C	0.082	0.063	0.063	0.057	**0.208**	0.020	0.112	0.053	0.080	**0.200**	0.018	0.026
D	0.106	0.041	0.071	**0.227**	0.108	**0.286**	0.026	0.011	0.043	0.012	0.018	0.106
E	0.103	**0.232**	0.083	**0.199**	**0.278**	0.008	0.109	0.031	0.041	**0.214**	0.069	0.013
F	0.108	0.100	0.066	0.008	0.090	0.112	0.010	0.006	0.021	**0.245**	0.068	0.089
G	0.107	0.068	**0.215**	0.111	0.103	0.110	0.006	0.089	0.104	**0.200**	0.110	0.073
H	0.100	**0.248**	0.010	**0.216**	0.023	0.036	0.011	0.027	0.011	0.013	0.018	0.036

Cut off value = 0.198 Number of Positive Wells = 19

A1 is positive control B1 – H1 are negative controls prepared from laboratory reared *Anopheles*

Appendix IX: ELISA showing the re-tested positive samples of appendix II

	1	2	3	4	5	6	7	8	9	10	11	12
A		0.207		0.242	0.261							
B		0.229		0.203	0.208							0.206
C				0.227	0.278					0.245		0.226
D				0.199		0.214						
E		0.232		0.238	0.286					0.200		
F					0.200					0.214		
G			0.215							0.203		
H		0.248		0.216							0.218	

Note: The samples remained positive after been re-tested.

Appendix X: Abundance *Anopheles* species in some villages of Jigawa State, Nigeria for the rainy and dry season, 2010 and 2011.

Season	***An. fun.***	***An. Arab.***	***An. gamb***	**Total**	**Mean±SEM**
Rainy	370	166	837	1373	475.65±198.60
Dry	131	47	312	490	163.33±78.19
Total	501	213	1149	1863	621.00±276.78
Mean±SEM	250.50±119.50	106.50±59.5	574.50±262.50	931.5±441.50	

Appendix XI: Mean annual values of *Anopheles* species in some villages of Jigawa State, Nigeria for 2010 and 2011 sampling years combined.

Year	***An. fun.***	***An. gamb***	***An. gamb***	**Total**	**Mean±SEM**
2010	261	104	577	942	314.00±139.09
2011	240	109	572	921	307.00±137.79
Total	501	213	1149	1863	621.00±276.78
Mean±SEM	250.50_10.50	106.50±2.50	574.50±2.50	931.50±10.50	

Appendix XII: Mean monthly values of *Anopheles* species in some villages of Jigawa State, Nigeria for 2010 and 2011 sampling years combined.

Month	***An. fun.***	***An. gamb***	***An. gamb***	**Total**	**Mean±SEM**
May	32	34	123	189	63.00±30.01
Jun	75	31	159	265	44.67±15.19
July	87	28	184	299	99.67±45.47
Aug	99	36	174	309	103.00±39.89
Sept	77	37	197	311	103.67±48.07
Oct	61	21	102	184	61.33±23.38
Nov	40	14	91	145	48.33±22.62
Dec	28	12	56	96	32.00±12.86
Jan	0	0	30	30	10.00±10.00
Feb	0	0	13	13	4.33±4.33
Mar	0	0	11	11	3.67±3.66
Apr	2	0	9	11	3.67±2.73
Total	501	213	1149	1863	621.00±276.78
Mean±SEMDD3[;’	250.50_10.50	106.50±2.50	574.50±2.50	931.50±10.50	

Appendix XIII: One-way Analysis of Variance (ANOVA)

The P value is < 0.0001, considered extremely significant.

Variation among column means is significantly greater than expected.

Tukey-Kramer Multiple Comparisons Test

If the value of q is greater than 4.723 then the P value is less

than 0.05.

Comparison	Mean Difference	q	P value
An. arabiansis indoor vs *An. gam*, ss indoor	-23.333	8.999	*** p<0.001
An ara indoor vs An. ara larv.	-12.208	4.708	ns p>0.05
An ara indoor vs An. gam. ss larv	-8.833	3.407	ns p>0.05
An ara indoor vs M form	-2.792	0.8791	ns p>0.05
An ara indoor vs S form	-32.167	10.129	*** p<0.001
An ara indoor vs An fun indoor	-1.000	0.3149	ns p>0.05
An ara indoor vs An gam sl indoo	-10.708	3.372	ns p>0.05
An ara indoor vs An fun larv	-6.958	2.191	ns p>0.05
An ara indoor vs An gam sl larv	-33.125	10.431	*** p<0.001
An ara indoor vs An ara infectio	4.458	0.7469	ns p>0.05

Appendix XIII: One-way Analysis of Variance (ANOVA) (*Cont.)*

An ara indoor vs M form infect	5.208 0.7879 ns p>0.05
An ara indoor vs S form infect	4.870 1.358 ns p>0.05
An. gam, ss ind vs An. ara larv.	11.125 4.291 ns p>0.05
An. gam, ss ind vs An. gam. ss larv	14.500 5.592 ** p<0.01
An. gam, ss ind vs M form	20.542 6.469 *** p<0.001
An. gam, ss ind vs s form	-8.833 2.782 ns p>0.05
An. gam, ss ind vs an fun indoor	22.333 7.033 *** p<0.001
An. gam, ss ind vs an gam sl indoo	12.625 3.976 ns p>0.05
An. gam, ss ind vs an fun larv	16.375 5.157 * p<0.05
An. gam, ss ind vs an gam sl larv	-9.792 3.083 ns p>0.05
An. gam, ss ind vs an ara infectio	27.792 4.656 ns p>0.05
An. gam, ss ind vs m form infect	28.542 4.318 ns p>0.05
An. gam, ss ind vs s form infect	28.203 7.867 *** p<0.001
An. ara larv. Vs gam. Ss larv	3.375 1.302 ns p>0.05
An. ara larv. Vs m form	9.417 2.965 ns p>0.05
An. ara larv. Vs s form	-19.958 6.285 *** p<0.00
An. ara larv. Vs an fun indoor	11.208 3.530 ns p>0.05
An. ara larv. Vs an gam sl indoo	1.500 0.4724 ns p>0.05
An. ara larv. Vs an fun larv	5.250 1.653 ns p>0.05
An. ara larv. Vs an gam sl larv	-20.917 6.587 *** p<0.001
An. ara larv. Vs an ara infectio	16.667 2.792 ns p>0.05

Appendix XIII: One-way Analysis of Variance (ANOVA) (*Cont.)*

An. ara larv. Vs m form infect	17.417	2.635	ns	p>0.05
An. ara larv. Vs s form infect	17.078	4.764	*	p<0.05
An gam. ss larv vs m form	6.042	1.903	ns	p>0.05
An gam. ss larv vs s form	-23.333	7.348	***	p<0.001
An gam. ss larv vs an fun indoor	7.833	2.467	ns	p>0.05
An gam. ss larv vs an gam sl indoo	-1.875	0.5904	ns	p>0.05
An gam. ss larv vs an fun larv	1.875	0.5904	ns	p>0.05
An gam. ss larv vs an gam sl larv	-24.292	7.649	***	p<0.001
An gam. ss larv vs an ara infectio	13.292	2.227	ns	p>0.05
An gam. ss larv vs m form infect	14.042	2.124	ns	p>0.05
An gam. ss larv vs s form infect	13.703	3.822	ns	p>0.05
M form vs s form	-29.375	8.011	***	p<0.001
M form vs an fun indoor	1.792	0.4886	ns	p>0.05
M form vs an gam sl indoo	-7.917	2.159	ns	p>0.05
M form vs an fun larv	-4.167	1.136	ns	p>0.05
M form vs an gam sl larv	-30.333	8.272	***	p<0.001
M form vs an ara infectio	7.250	1.161	ns	p>0.05
M form vs m form infect	8.000	1.166	ns	p>0.05
M form vs s form infect	7.662	1.903	ns	p>0.05
S form vs an fun indoor	31.167	8.500	***	p<0.001
S form vs an gam sl indoo	21.458	5.852	**	p<0.01

Appendix XIII: One-way Analysis of Variance (ANOVA) (*Cont.)*

S form vs an fun larv	25.208	6.875	***	p<0.001
S form vs an gam sl larv	-0.9583	0.2614	ns	p>0.05
S form vs an Ara infectio	36.625	5.865	**	p<0.01
S form vs m form infect	37.375	5.448	**	p<0.01
S form vs s form infect	37.037	9.198	***	p<0.001
An fun indoor vs An gam sl indoo	-9.708	2.648	ns	p>0.05
An fun indoor vs An fun larv	-5.958	1.625	ns	p>0.05
An fun indoor vs Angam sl larv	-32.125	8.761	***	p<0.001
An fun indoor vs An ara infectio	5.458	0.8741	ns	p>0.05
An fun indoor vs M form infect	6.208	0.9050	ns	p>0.05
An fun indoor vs s form infect	5.870	1.458	ns	p>0.05
An gam sl indoo vs An fun larv	3.750	1.023	ns	p>0.05
An gam sl indoo vs An gam sl larv	-22.417	6.113	**	p<0.01
An gam sl indoo vs An ara infectio	15.167	2.429	ns	p>0.05
An gam sl indoo vs M form infect	15.917	2.320	ns	p>0.05
An gam sl indoo vs s form infect	15.578	3.869	ns	p>0.05
An fun larv vs Angam sl larv	-26.167	7.136	***	p<0.001
An fun larv vs An ara infectio	11.417	1.828	ns	p>0.05
An fun larv vs M form infect	12.167	1.774	ns	p>0.05
An fun larv vs S form infect	11.828	2.938	ns	p>0.05
An gam sl larv vs An ara infectio	37.583	6.019	**	p<0.01

Appendix XIII: One-way Analysis of Variance (ANOVA) (*Cont.)*

An gam sl larv vs M form infect	38.333	5.588	**	p<0.01
An gam sl larv vs S form infect	37.995	9.436	***	p<0.001
An ara infectio vs M form infect	0.7500	0.08802	ns	p>0.05
An ara infectio vs S form infect	0.4118	0.06372	ns	p>0.05
M form infect vs S form infect	-0.3382	0.04792	ns	p>0.05