A STUDY ON THE MALARIA VECTOR (*ANOPHELES SPP)* IN A SUDANO-SAHELIAN SAVANNAH AREA OF BORNO STATE NORTH EASTERN NIGERIA AND THE INSECT GROWTH REGULATOR PYRIPROXYFEN (S-31183)

LAZARUS MUSA SAMDI

D.V.M (A.B.U), M.Sc. (JOS)

PGNS/UJ/14148/02

A Thesis in the Department of ZOOLOGY,
Faculty of Natural Sciences,
Submitted to the School of Postgraduate Studies,
University of Jos, in fulfillment of the
Requirements for the Award of the
Degree of DOCTOR OF PHILOSOPHY of the
UNIVERSITY OF JOS

AUGUST, 2012

CERTIFICATION

This is to certify that the research work for this thesis and the subsequent preparation of this thesis by **LAZARUS MUSA SAMDI** (PGNS/UJ/14108/02), were carried out under my supervision.

....

Prof. J.A. Ajayi

SUPERVISOR

DECLARATION

I hereby declare that this work is the product of my own research efforts, undertaken under the supervision of **Prof. J.A. Ajayi** and has not been presented elsewhere for the award of a degree or certificate. All sources have been duly distinguished and appropriately acknowledged.

…………………………………….

LAZARUS MUSA SAMDI

PGNS/UJ/14148/02

ACKNOWLEDGEMENT

With profound gratitude to God almighty. I am indeed grateful for the help, friendship and erudite support of my supervisor, Professor J.A. Ajayi, Prof. Gregory Ikechukwu Anyanwu. The late Professor Norman Baka Molta gave everything in support of this project. My gratitude starts from the roots, University of Jos. I appreciate all I have learnt from University of Jos and the support of all staff. I am also deeply grateful to Prof. N.N. James–Rugu for her detail constructive criticism and invaluable contribution to this work. Dr. (Mrs). G. Mwansat Head of Department Zoology sacrificed much to see this work become a reality .I am eternally grateful .Dr I.M.Watila State Specialist Hospital Maiduguri exposed me to field work in Damboa, Borno State.

I am also deeply indebted to the Management of University of Maiduguri Teaching Hospital especially Dr Steve Oguche the Head of Department Paediatrics University of Maiduguri Teaching Hospital Maiduguri and the management of Damboa General Hospital, Borno state. To all my field assistants in mosquito collection and slide collection in both General Hospital Damboa and The University Teaching Hospital Maiduguri especially Bunu, Ba'na and Musa. The Director-General Nigerian Institute of Medical Research Yaba, Lagos, Chief (Dr) Oni Idigbe gave me all travel grant for parts of this work conducted in Lagos.

To Dr .T.S. Awolola my internal Supervisor at NIMR lagos trained, sponsored and gave me all materials for the molecular and pyriproxyfen work in this project. Dayo Oduola and Judith Obansa of the Molecular Entomology/Parasitology Research Laboratory, Nigerian Institute of Medical Research Lagos who provided

the technical support for the molecular work in this project. Mrs C.N. Amajoh of the Federal Ministry of Health Abuja stood by me in all these years helping me to grow in my knowledge of Malaria Entomology. Thank you and God bless.

For the preliminary stage in our trial of pyriproxyfen. I thank the late Prof. Chris Curtis of the London School of Hygiene for his guidance on some aspects of this work from its inception, Dr M. Yapabandara of the regional office Anti malaria campaign, Matale, Sri Lanka was of tremendous help in obtaining the pyriproxyfen, Julie Grant of Samplerite London and Sumitomo Horatio house London supplied us the S-31183 Pyriproxyfen for the study. Helpful suggestions and corrections on the trial of pyriproxyfen were undertaken by Dr J.R. Lucas of Sumitomo Chemical (UK) Plc London, UK to whom we are indeed most grateful. Journal publications and other helpful materials were obtained from the United States Armed Forces ,U.S .Department of Defence Pest Management Board Washington and the Centre for Disease Control (CDC) Atlanta U.S.A. from whose publication (MR4 Methods in *Anopheles* Research edited by Mark Q Benedict) important *Anopheles* mosquito pictures in this thesis were sourced. Special gratitude to the MR4 team for giving me a written permission to use of their pictures in this thesis. This work is the way it is typographically because of the effort of my bossom friend Dear Petrus Inyama, I say thank you!

DEDICATION

For Professor Norman Baka Molta (22 August 1955 - 19th of April 2007) who gave every financial, academic and moral support possible for this work.

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Abstract

Malaria is a major problem in the Sudano-sahel Northeastern Nigeria with the highest prevalence of malaria in pregnancy of 64.5 percent. Little is known about the major malaria vector and its role in malaria transmission. Longitudinal entomological and parasitological surveys were conducted to better understand the relationship of the key components in malaria transmission dynamics .*Anopheles* mosquitoes were sampled using pyrethroid spray collection and identified morphologically and by molecular methods of Polymerase chain reaction (PCR). Enzyme linked immunosorbent Assay (ELISA) was used for the bloodmeal analysis and mosquito infectivity by circumsporozoite detection. Malariometric indices were determined following the World Health Organization procedures. A total of 1030 female *Anopheles* mosquitoes were caught consisting of five species, namely, 1026 (99.6%) of *Anopheles gambiae* complex further identification using PCR showed the predominant sibling species were *An. arabiensis* Patton 95%(n=221) and *An. gambiae s.s*. 5%(n=12). Other Anopheles mosquitoes collected were morphologically identified as *An. pharoensis*, *An. squamosus* and *An. rhodesiense*. Results showed that the population of *Anopheles arabiensis* was significantly higher than that of *Anopheles gambiae* s.s ($P<0.05$). Mosquito infection was determined by ELISA method for the detection of *Plasmodium falciparum* sporozoites 7(2.4%) were positive for *P.falciparum* circumsporozoite antigen. All seven were *An.arabiensis*. Indoor collection was significantly higher than the outdoor collection ($P<0.01$). Mosquito blood feeding source determined by direct ELISA showed human bloodmeal was (98%, 94/96) for indoor collections and (2%, 2/96) for outdoors ($P<0.01$). The Human Blood Index (HBI) was 0.98. The results implicate

An.arabiensis as the main malaria vector in the area. Of a total of 692 children consecutively screened over a period of one year, significant difference (p<0.05) in infection rates was observed between the males and the females. The levels of parasitaemia asexual parasite were significantly related to age (p<0.05).The majority of infected children (68.0%) were aged between 12-60 months and their asexual parasite density was between 1000-5000 of whole blood. The month of September recorded the highest geometric mean parasite density (GMPD) of 13,655 while the lowest parasite densities were observed during the dry season months of March, April, and May while gametocytaemia was not significantly affected by the age of the patients nor the season (p>0.05). Overall average Inhibition of Emergence (IE) rates were 86% for the first week (0-7days) then peaked to 100% during the second week (8-14days) and declined to 73% (15-21days) on the third week and finally to 36% on the fourth week (22-28day). Percentage Inhibition of Emergence between 0.1 and 0.5mg (a.i)/l treatments were not different (P> 0.05). In planning effective site specific malaria vector control programmes under the Integrated Vector Management (IVM) program of the Federal Government of Nigeria, results of this study has highlighted the need to give special consideration to the predominance of a single malaria vector *An. arabiensis* in the Sudanosahel and the strong seasonality of malaria in contrast to other regions of Nigeria. Findings also demonstrated the potentials of pyriproxyfen as an effective mosquitoes larvicide for consideration under the Integrated Vector Management (IVM) program for use in Sudanosahel Northeastern Nigeria.

CHAPTER ONE
INTRODUCTION

1.1 BACKGROUND OF THE STUDY

Malaria is by far the most important insect transmitted disease (Gilles and Warrell, 1993). Latest World Health Organisation estimates are that there are 300-500 million cases of clinical malaria per year, with 1.4-2.6 million deaths, many among African children. Malaria is therefore a major cause of infant mortality and is the only insect borne parasite disease comparable in impact to the Worlds major killer transmissible diseases: diarrhea, acute respiratory infections, tuberculosis and AIDS (Curtis, 2006). In Nigeria, up to 60% of outpatient attendance in health facilities is due to malaria and 30% of all hospital admissions.It is estimated that malaria is responsible for nearly 110 million clinical cases and an estimated 300,000 deaths per year, The disease is responsible for 25% infant mortality, 30% childhood mortality and is associated with 11% maternal deaths.The economic burden of this disease in Nigeria is estimated to be N132 billion lost annually in terms of treatment costs,prevention,loss of man hours etc (FMOH, 2005b; 2009d).Most malaria deaths occur at home hence are not reported (Rugemalila, 2006). The disease can be attributed almost entirely to the mosquitoes *Anopheles gambiae, An. arabiensis* and *An.funestus,* three of the most efficient malaria vectors in the world. All live almost exclusively in close association with humans and feed on blood, primarily from humans (Collins and Besansky 1994). The power of *An. gambiae* as a malaria vector for instance, is well illustrated by its accidental introduction into Brazil, where in 1938 after a series of small but intense local outbreaks, it caused the worst epidemic of malaria ever recorded there, with over 14,000 deaths in less than 8 months

(Collins and Besansky, 1994). In Africa alone, the economic burden is about US$12billlion annually. Malaria is estimated to slow economic growth in African countries by about 1.3% per year. Malaria constitutes a major economic burden on endemic communities in Africa including Nigeria. The disease thus constitutes a great burden on the already depressed Nigerian economy. Malaria causes great misery to sufferers, and adversely affects the social and psychological wellbeing of individuals, families and the nation at large (FMOH, 2004). Forty percent (40%) of the worlds population are at risk of malaria, of this number 500million are in Sub Saharan Africa.240 -400 million cases are recorded yearly with children experiencing between 1-9 attacks per child per year and adults experiencing between 1-4 attacks. Deaths due to malaria are between 1-2 million; at least 90% of this figure is in Africa. Malaria is directly responsible for 10% of deaths in children under the five years and indirectly responsible for 25% of all childhood deaths (Molta, 2000b).

Despite the fact that strong attempts to eradicate malaria have been made, the disease burden is still on the rise and some estimate that the number of cases could double in the next twenty years without the development of new methods of control (Sachs and Malaney, 2002). Aside from the human tragedy this predicts, an economic disaster is likely for the stricken countries (Cahill, 2004).

The malaria parasite also interacts with other afflictions, such as HIV and under-nutrition, in ways that are still not well understood. These estimates render malaria the prominent tropical parasitic disease and one of the top three killers among communicable diseases (Rugemalila *et al.,* 2006).

Malaria caused by *Plasmodium* species, notably *Plasmodium falciparum* is a major cause of morbidity and mortality in children and adults in Borno State. It is

responsible for 70 percent attendance in hospitals, 91 percent of eleven notifiable diseases, 83 percent of diagnosable infection and at least 32 percent of deaths. In this state, transmission of the disease occurs all-year-round with peaks were observed during the middle to late rainy season(August –October/November) and transmission declining during the dry season (December-April/May) thereby demonstrating strong seasonality (Pull and Grammicia, 1976; Sibina 1984; Molta *et al.,*1991; Molta *et al.,*1995;Oguche *et. al.*, 2001). *Plasmodium* infection ranged from 35.2% in Maiduguri to 70.2% in Damboa both located in Borno State. The overall rate of infection was 50% (Molta *et al.,* 1993, 2004) The possible malaria vectors in the sahel include *An. gambiae* s.l., *An. funestus, An. pharoensis, An. squamosis, An. coustani and An. ziemanni* (Gadzama, 1983; Bariki, 1988; Sara,1990 and Kalu, 1992).

The national malaria control strategic plan aims to reduce by 50% malaria related morbidity and mortality in Nigeria by 2010 and sustain the level to 2013 to minimize the socioeconomic impact of the disease; and to achieve the relevant millennium Development Goal i.e to have halted by 2015 and begun to reverse the incidence of malaria and other major diseases (FMOH, 2009c).

1.2 STATEMENT OF THE PROBLEM

Malaria is focal disease which differs from in its characteristics from country to country and even within the same country(Phillips ,2001).According to Federal Ministry of Health the highest case fatality ratio due to malaria in Nigeria was recorded in the Sahel, North-eastern (FMOH,1991), and recently the highest prevalence of malaria in pregnancy of 64.5 percent (FMOH, 2004). Since the report of Chloroquine resistance in the North-eastern Nigeria by Daniel and Molta (1989),

there have been evidence of the increasing resistance to Chloroquine and Sulphadoxine which has now been put at of an average of 50% up from 12.7% as at 1993 (Molta *et al*., 1993; Molta *et al.*, 2004; Watila *et al.,* 2006). Gametocyte carriage rate too increased from 2.7% to 10% in the Sahel indicating the possibility of a continual increase in antimalarial resistance (Watila *et al*., 2006).

The World Health Organisation recommends the implementation of vector control to curtail the spread of malaria where the parasite is resistant to antimalarial drugs. Vector control remains the most effective measure to prevent malaria transmission. It is also one of the four technical elements of the global malaria control strategy (WHO, 2003; 2006). Unfortunately as the time of this study no community based effort was operational to control *Anopheles* vectors in the study area nor is there adequate information on the malaria vectors of this part of Nigeria.

1.3 JUSTIFICATION FOR THE STUDY

Vector control is a major component of the national campaign against malaria in Nigeria.The global strategic plan for Roll Back Malaria recommends that by 2010, 80% of the population at risk need to be protected using effective vector control measures.To achieve this objective, there is a need to scale up all effective components of Integrated Vector Management (IVM) which include Long lasting Insecticidal Net (LLIN) and Indoor Residual Spraying (IRS) and larval source management (FMOH, 2009a).

Inspite of the evidence that vector control is crucially important in reducing malaria morbidity and mortality and a key objective of primary health care, not much is known about malaria vectors of of the Sudano-Sahelian part of Northeastern Nigeria. Although existing documented works involved mainly anophelines species

distribution. It is apparent that little or no information exists either on the precise identity of sibling species of the malaria vectors (members of *Anopheles gambiae* complex), their sporozoite positivity rate, disease transmission probability nor their involvement in malaria transmission. Yet, this basic information is crucially needed to properly devise and implement malaria vector control interventions and to assess their effectiveness (Gadzama, 1983; BruceChwatt, 1986; Gad, 1988;Service, 1989; Sara, 1990,Savage *et al.,* 1991; Kalu, 1992 and Molta *et al.,* 1999).

The identification of malaria vector species and their concomitant distribution are not simply of academic interest but are vital to effective malaria control measures.With species identification comes the associated knowledge of the biology of that species which in turn, dictates appropriate control measures. (Coetzee, 2004). If meaningful control strategies are to be formulated against the malaria vectors in the Sahel, studies to confirm the predominant sibling species of *An. gambiae sensu* lato must be carried out (Gadzama, 1983).

Earlier attempts (Gadzama, 1983; Gad, 1988; Sara, 1990; Kalu, 1992 and Molta *et al.,* 1999) at identifying member species of *An. gambiae* complex of the Sahel used keys based on morphological features which are slow and cumbersome with a high chance of error. It is therefore important that further studies take advantage of new emerging molecular tools (Polymerase Chain Reaction, PCR; enzyme-linked immunosorbent assay, ELISA; etc) to precisely identify the various vector populations of the Sahel using these high precision biomolecular tools given their advantage of Precision and speed (Molta *et al.*,1999). A longitudinal parasitological study to complement entomological studies in understanding the malaria transmission cycle in the Sudano-sahel for timely and correct application

vector control measures is needed to determine seasonal peaks of malaria transmission (WHO, 1995).

In areas of low seasonal transmission such as the Sahel, longitudinal monitoring of factors related to increases in transmission will identify optimal times in which to intervene and aid localized policy making, targeting key interventions (Mwerinde *et al,* 2005). It is important to know the duration, start and end of the malaria transmission season in terms of planning control strategies. For example to maximize impact, Indoor Residual Spraying(IRS) should be carried out prior to the onset of the malaria season ,also the cost–effectiveness of some malaria control strategies maybe influenced by the intensity of seasonality (Roca-Feltrer *et al.*, 2009).

1.4 LIMTATIONS OF THE STUDY

1. This work was limited by the inability to access quality and detailed satellite based meteorological data focusing on basic weather indices such as rainfall, humidity and temperature to correlate with the fluctuations of malaria vector and parasite densities
2. The possibility of the presence of some 3rd instar larvae amongst the 4th instar larvae used for the laboratory evaluation of pyriproxyfen cannot be ruled out ,this stage is far less sensitive (refractory) to juvenile hormone analogues like pyriproxyfen than the 4th stage larvae.

1.5 AIM AND OBJECTIVES OF THE STUDY

The aim of this study is to identify local malaria vectors, determining their vectorial importance/status and the suitability of the insect growth regulator for their control in North-eastern Nigeria.

1.5.1 Objectives

1. To identify the predominant sibling species of *Anopheles gambiae* complex in the study area using the polymerase chain reaction assay (PCR).
2. To carry out vector-incrimination study to identify the probable vectors responsible for malaria by determining the circumsporozoite (CSP) rate in the vector using the ELISA.
3. To determine the seasonal peaks of malaria transmission in the study area through a longitudinal parasitological study.
4. To evaluate the inhibitory action of the insect growth regulator pyriproxyfen (S-31183) on the 4th stage larvae of *An. gambiae* under laboratory conditions.

1.6 HYPOTHESIS

1. There is no difference in the species composition of the *Anopheles gambiae* sibling species in the study area.
2. The resting and feeding behaviors of all incriminated vectors in the study area are not different
3. The host and bloodmeal preference of all sibling species of *An.gambiae* in the study are the same
4. No difference exists in the malaria infection rates in the study area across all seasons.
5. No difference in the duration of malaria transmission during both the rainy and dry seasons
6. No difference in Inhibitory Emergence effect of pyriproxyfen on 4th stage larvae of *An. gambiae ss* at all the doses tried.

CHAPTER TWO
LITERATURE REVIEW

2.1 THE DISEASE MALARIA

Malaria, a mosquito borne, protozoal disease, is older than recorded history. It is believed that man and malaria evolved together, that populations of human malaria may have had their origin in West Africa (Rugemalila *et al.,* 2006).

Hippocrates was the first to describe the manifestation of the disease, and relate them to the time of the year and to where the patients live before this, the supernatural was blamed. The association with stagnant waters (breeding grounds for *Anopheles*) led the Romans to begin drainage programs, the first interventions against malaria .The word malaria meaning bad air has its origins there (Rugemalila *et al.*, 2006)

Not until 1889 was the protozoal cause of malaria elucidated by Laveran working in Algeria, and only in 1897 was the *Anopheles* mosquito demonstrated to be the vector of the disease. Ronald Ross of the Indian Medical Service, working on Manson's suggestion, succeeded in establishing the *Anopheles* mosquito-malaria relationship. He found oocysts of malaria parasites, *Plasmodium* on the stomach of the female *Anopheles* mosquito previously fed on the blood of a malaria patient. This discovery was made on August 29, 1897, ever since called the "mosquito day" (Jordan and Verma 2005; Rugemalila *et al.*, 2006) .At this point the major features of the epidemiology of malaria seemed clear, and control measures started to be implemented (Rugemalila *et al.*, 2006).

Malaria is caused by single celled protozoan parasites of the genus *Plasmodium*. According to Bruce-Chwatt (1986) these microorganisms are commonly

referred to simply as malaria parasites; this term is usually restricted to the family Plasmodiidae within the order Coccidiida, sub order Haemosporidiidea which comprises various parasites found in the blood of reptiles, birds and mammals. The classification of Haemosporidiidea as a suborder of the coccidiida is complex and controversial, an alternative system has been proposed by Levine. However, Garnham's classification of Haemosporidiidea into Plasmodiidae, Haemoproteidae and Leucocytozoidae has however been maintained (Bruce-Chwatt, 1986). The zoological family of Plasmodiidae includes the parasites, which undergo two types of multiplication by asexual division (schizogony) in the vertebrate host and a single sexual multiplication (sporogony) in the mosquito host. The genus *Plasmodium* has been defined on the basis of one type of the asexual multiplication by division occurring in the parenchymal cells of the liver of the vertebrate host (exoeythrocytic schizogony); the other characteristic of this genus is that the mosquito hosts are various *Anopheles*. There are nearly 120 species of Plasmodia, including at least 22 species found in primate hosts and 19 in rodents, bats or other mammals. About 70 other plasmodial species have been described in birds and reptiles. Plasmodia of the primate hosts are divided into three sub-genera and within the sub genus of *Plasmodium* there are four groups classified according to the periodicity of their erythrocytic schizogony (Bruce-Chwatt, 1986).

The zoological classification of *Plasmodia* is complex, and even today there is some difference of opinion with regard to taxonomic position of the parasite causing falciparum malaria. In this malaria parasite the cresentric shape and lengthy development of sexual erythrocytic forms has been accepted by some authors as a valid argument for recognition of the parasite as belonging to a separate genus,

Laverania falcipara. While this view may be correct in a context of zoological systematics, the rejection of the familiar name *Plasmodium falciparum* might be confusing and since the use of this well known name is still taxonomically permissible it is retained (Bruce-Chwatt, 1986). There are four generally recognised species of malaria parasites of man: *Plasmodium malariae* (Laveran) *P. vivax* (Grassiand Feletti,), *P. falciparum* (Welch), *P. ovale* (Stephens) (Bruce-Chwatt, 1986).Their worldwide distribution and characteristics are as follows:

P. falciparum or malignant tertian malaria (Together with *P. vivax*) are the most common human malaria parasite and are found in most malarious areas mainly tropical Africa, Asia and latin America including Haiti, Dominican Republic French Guinea, Surinam, parts of Asia and Papua New Guinea. *P. falciparum* is the most dangerous form of the disease; the high levels of parasites in the blood (parasitaemia) alone can result in death or produce fatal cerebral, renal or pulmonary complications, particularly in non-immune individuals.

P. vivax *or* tertian malaria is predominant in tropical zones worldwide as well as in some temperate areas, particularly Latin America, Turkey, the Indian subcontinent and China. *P. vivax* is not found in West Africa.

P. malariae or quartan is worldwide in occurring but very focal in distribution and is less common than either *P. vivax* or *P. falciparum*.

P. ovale **is** found chiefly in tropical Africa (West Africa). These three infections (*P. vivax, P. ovale and P. malariae*) are not generally life threatening, they can however, sometimes cause severe acute illness. *P. vivax* and *P. ovale* forms are characterized by relapses (reappearance of symptoms or parasitaemia followed by latent or symptomless periods of up to 5 years).

Recrudescences (repeated manifestations of infections after a relatively short latent period, usually between 3-12 months) may occur in falciparum and malariae malaria.

2.1.1 Malaria Epidemiology and Distribution

Malaria is widespread in the tropics and also occurs in subtropical and temperate regions, Globally Malaria is responsible for 273 million clinical cases and 1.12 million deaths annually. More than 40% of the global population (>2.1 billion people) is estimated to be at risk (Toure and Oduola, 2004). Malaria occurs in 100 countries, but is mainly confined to poor, tropical areas of Africa, Asia and Latin America (Figure 1). More than 90% of malaria cases occur in tropical Africa (Toure and Oduola, 2004). Malaria is among the most important causes of death and illness in Africa, especially among children and pregnant women. Travellers, tourists and immigrants may be at risk (Rozendaal, 1997). *Plasmodium falciparum* occurs throughout tropical Africa and in parts of Asia, the Western Pacific, South and Central America, Haiti and the Dominican Republic. *Plasmodium vivax* is almost absent from Africa but is the predominant malaria parasite in Asia and South and Central America. *Plasmodium malariae* is found worldwide but has a very patchy distribution. *Plasmodium ovale* occurs mainly in tropical West Africa and rarely in the western pacific (Rozendaal, 1997).

In Nigeria, malaria is caused by three species of *Plasmodium: P. falciparum* (80%), *P. malariae* (15%) and *P. ovale* (5%). Mixed infections with *P. falciparum* are common. The principal method of malaria transmission is through the bite of infected female Anopheles mosquito (Nnochiri, 1975; FMOH, 1991).

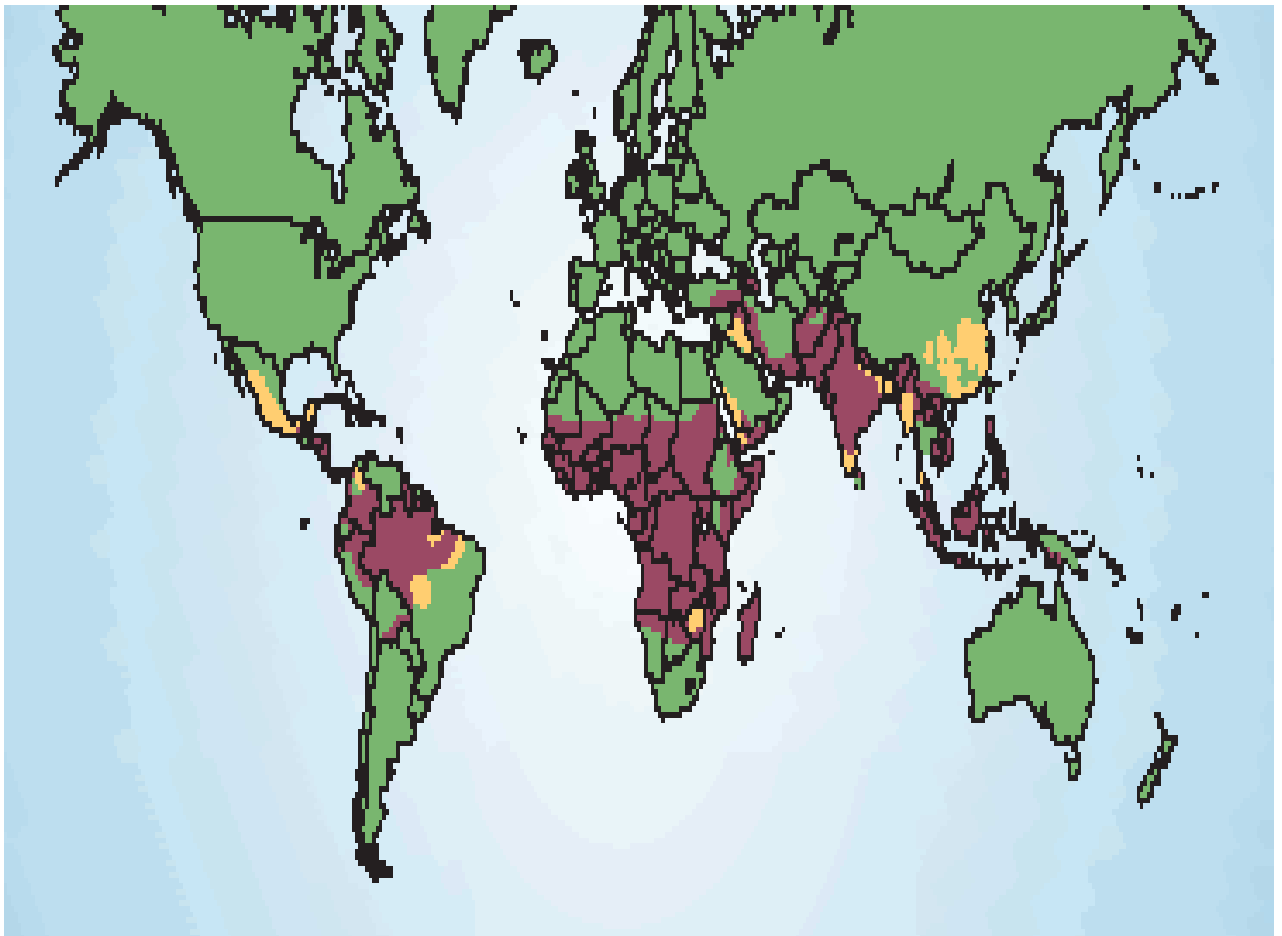


Figure 1: Global distribution of malaria (source: Toure and Oduola) 2004)

Areas where malaria transmission occur

Areas with limited risk

No malaria

2.1.2 Human Plasmodia

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, Phillipines, Haiti, Solomon Islands, Papua New Guinea and many Islands in Melanesia. It also occurs in parts of India, the Middle East, and eastern Mediterranean (Cheesebrough, 1997).

Of all the species of human plasmodia, *P. falciparum* is the most highly pathogenic, as is indicated by the name malignant often applied to the type of malaria associated with it. This, in non-immune subject, usually runs an acute course, and unless promptly treated with specific drugs, frequently terminates fatally. It is the chief infection in areas of endemic malaria in Africa, and is responsible for the great regional epidemics, which were a feature of malaria in North West India and Sri Lanka. It is generally confined to tropical or subtropical regions, because the development in the mosquito is greatly retarded when the temperature falls below 20°C. Even at this temperature, about three weeks are required for maturation of sporozoites (Bruce-Chwatt, 1986).

The asexual development of *Plasmodium falciparum* in the liver involves only a pre erythrocytic phase and the hypnozoites do not occur. The earliest forms hitherto seen in the liver are schizonts measuring about 30µm in diameter on the fourth day after infection. The number of merozoites in a mature schizont, which reaches some 60µm in size, is about 30,000 (Bruce-Chwatt, 1986).

The young ring forms of *Plasmodium falciparum*, as usually seen in the peripheral blood, are very small, measuring about one-sixth of the diameter of a red blood cell. In many of the ring forms there may be two chromatin granules, and marginal (accole) forms are fairly common. There are frequently several ring forms to be seen in a single host cell. Although marginal forms, rings with double chromatin dots and multiple infections of red cells may occur in other human plasmodia, they are much common in *Plasmodium falciparum* and their presence is an important aid to diagnosis (BruceChwatt, 1986).

Later in the attack the ring forms of *Plasmodium falciparum* may be considerably larger, measuring one quarter and sometimes nearly one half the diameter of the red cell, and may be mistaken for parasites of *P. malariae*. They may have one or two grains of pigment in their cytoplasm. In acute infections with numerous parasites, atypical forms are sometimes seen. The succeeding developing stages of the asexual erythrocytic cycle do not generally occur in the blood, except in severe cases. The presence of maturing or mature schizonts of *Plasmodium falciparum* in a blood film is therefore often an indication for prompt and vigorous treatment. The ring forms and older trophozoites usually disappear from the peripheral circulation after 24 hours and are held up (sequestered) in the capillaries of the internal organs, such as the brain, heart, placenta, spleen, intestine, or bone marrow, where their further development takes place (Bruce-Chwatt, 1986).Severe falciparum malaria is associated with cerebral malaria, severe anaemia, hypoglycaemia and complications in pregnancy.

2.1.3 The Malaria Parasite and its Life Cycle

Sporozoites, thought to be less than 100 on each occasion are released from the female mosquito" s salivary glands in her saliva, into the circulating blood of the host and within 30 to 45 minutes have entered hepatocytes. It is not clear how sporozoites squeeze through the sinusoid lining into the space of disse (or as might be the case, kupffer cells on the walls of the sinusoids to reach the hepatocytes or the precise nature of the sporozoites-hepatocytes ligand receptor interaction which enables the parasite to recognize the host cell. Peptides forming part of the major surface protein of the sporozoite, the circumsporozoite protein (CSP), have been suggested to interact with receptors on the hepatocytes. Growth and division in the liver for the human malaria parasites take from approximately 6 to 15 days depending on the species. At the end of the pre erthrocytic cycle, thousands of merozoites are released into the blood flowing through the sinusoids and within 15 to 20s, attach to and invade the erythrocytes. Recognition and attachment are via a receptor ligand interaction, and at least for *P. vivax* and *P. ovale,* some of the sporozoites appear to develop for about 24hours before becoming dormant as a hypnozoite stage; this form can remain as such for months or even years until reactivated to complete the liver cycle, releasing merozoites into the blood to precipitate a relapse of infection (Phillips, 2001).

The asexual erythrocytic cycle produces more merozoites that are released with the destruction of red blood cells after 48 or 72 hours for the human malaria depending on the species, and which then immediately invade additional erythrocytes. The asexual cycle usually continues until controlled by the immune response or chemotherapy or until the patient dies (in the case of *P. falciparum*).

Most malaria parasites developing in the host red blood cells grow in synchrony with one another, for at least some animal species apparently tuning into the hosts circadian rhythms. There is no compelling evidence as yet that,that is the case for human malaria parasites. Consequently, they complete schizogony together at the end of the asexual cycle, pyrogenic materials which induce the characteristic fever spike and clinical symptoms. The morbidity and mortality associated with malaria are derived solely from the erythrocytic stage (Phillips, 2001)

After invading red blood cells eventually some merozoites differentiate into sexual forms (gametocytes) and, following ingestion by another female mosquito will mature to male and female gametes in the blood meal. After fertilization the resulting zygote matures within 24hours and attach to the motile midgut wall to encyst on the basal lamina, the extra cellular matrix layer separating the haemocoel from the midgut. Within the developing oocysts full of sporozoites (Phillips, 2001). Rupture of the oocysts releases the sporozoites which migrate through the haemocoel to the salivary glands to complete the cycle approximately 7 to 18 days after ingestion, depending on host parasite combination and external environmental conditions. All stages in the life cycle are thought to be haploid, apart from the diploid zygote, which immediately after fertilization undergoes a two-step cycle meiotic division, the resulting cell containing a nucleus with four haploid genomes. The sexual process and meiotic division following fertilization allow genetic makeup of the sporozoites and together with mutations provides the raw material upon which selective pressures such as antimalarial drugs can work (loc.cit).

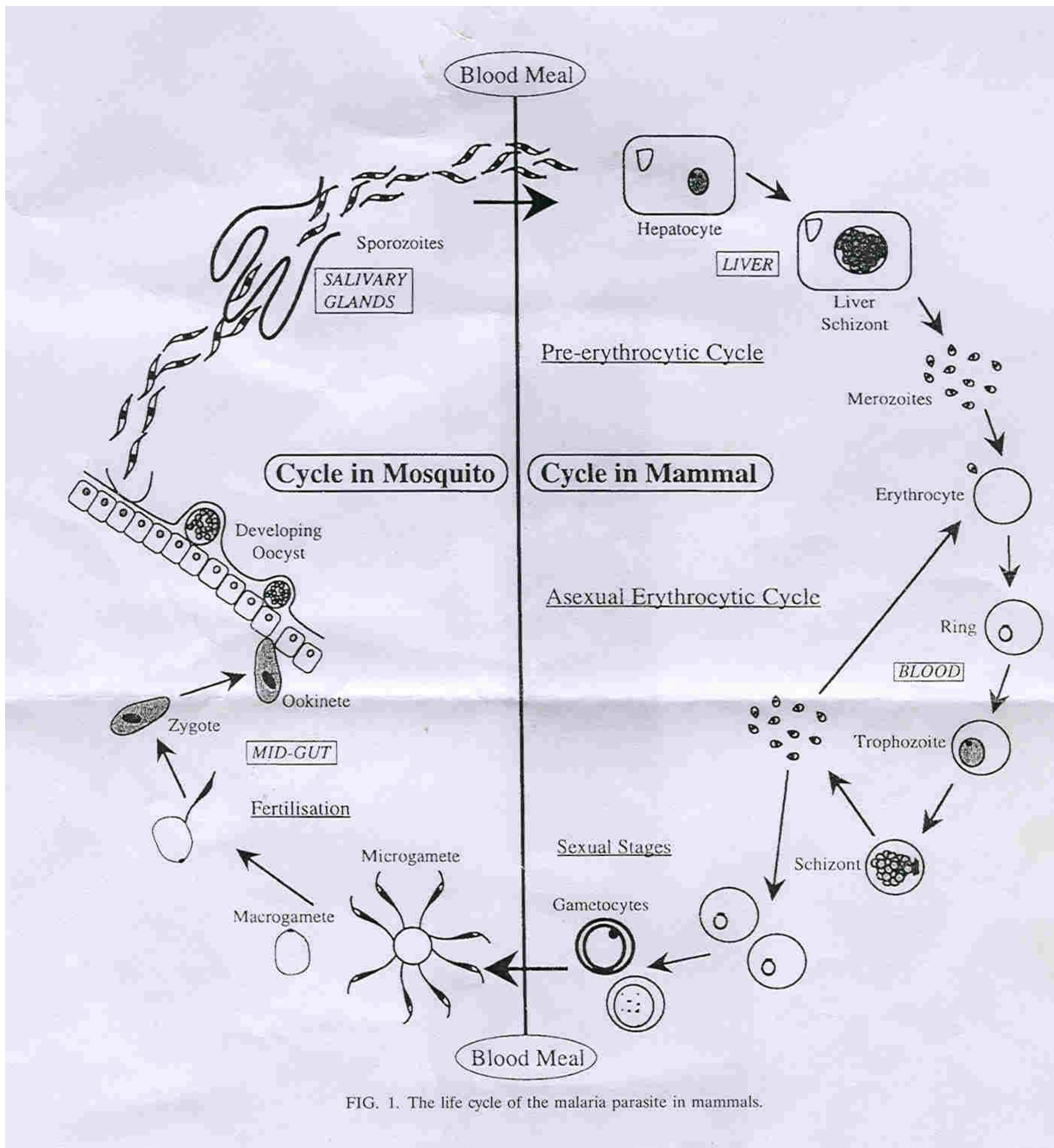


FIG. 1. The life cycle of the malaria parasite in mammals.

Figure 2: The life cycle of the Malaria Parasite.

(Source: Phillips 2001).

2.2 MALARIA TRANSMISSION

2.2.1 *Anopheles-Plasmodium*-Relationships and Impact on Malaria Transmission

The factors involved in malarial transmission and infact the other organisms are part of a natural ecological system. These factors include the status of the parasite, the vector and the human host which interact with one another and the human host which interact with one another and also with the biological and physical environment (Service, 1982). The transmission of malaria involves as much the transfer of the parasite from mosquito to man as from man to mosquito. The bite of an *Anopheles*, carrying sporozoites within its salivary glands, involves three distinct but linked processes which are at the sometime both distinct and related: the penetration and probing by the mouthparts into the skin of the buccal appendages (appendages), the injection of saliva containing sporozoites and blood feeding. Recent discoveries, concerning this infected bite as observed by Robert (2001) focused mainly on the following four points:

i. The number of sporozoites actually injected into the host is approximately ten, but can occasionally be several hundreds. This number is surprisingly small and is equivalent to about 1% of the number of sporozoites contained within the salivary glands.

ii. Sporozoites injected into the skin during probing seem to be the only ones capable of reaching the liver of the host. Sporozoites injected into venous blood during feeding have no effect on the parasitic cycle as they are immediately re-ingested by the mosquito in its blood feed.

iii. The success of a single bite in infecting a non-immune host is never absolute; it may be one chance out of two.

iv. The success of an anopheles biting behaviour of anopheline with the presence of sporozoites within its salivary glands appears modified, according to the vector species and perhaps also, depending on to the plasmodial species.

Factors modulating gametocytes infectivity for the vector are multiple and poorly understood (Robert, 2001). Six factors are described:

(i) Gametocyte density is probably the most obvious factor; the lowest sufficient gametocyte density is required for vector infectivity to have a reasonable weak probability to be infective for the vector success, but this condition is not enough, as in some cases, high densities are clearly not infective.

(ii) The sickle cell anaemia status of the gametocyte carrier can increases the infecting ability of these gametocytes.

(iii) The high proportion of male gametocytes also play a role in favouring successful the chances of infection.

(iv) Serum factors involving immunity by limiting or blocking transmission, can play an important role.

(v) The age of gametocytes is an important factor, very young gametocytes are non infective or poorly infective; likewise, several week old gametocytes also have reduced infectivity.

(vi) The direct or indirect impact of medicines anti malarial drugs can be of great importance. By way example, chloroquine has stimulating effect on the infectivity of gametocytes; the sulphadoxine-pyrimethamine has sporonticidal effect (Robert, 2001).

Transmission level is a determinant factor for the dynamics of immune acquisition morbidity and mortality: in area of high endemicity the youngest age groups are those which show the highest morbidity and mortality rates clearly more affected in areas of high transmission, whilst the consequences of malaria are distributed more equitably between the different age groups in the weaker transmission regions (Robert, 2001).

2.2.2 Malaria Transmission in Nigeria

'Malaria' is the leading cause of presentations at clinics and The main reason why malaria in tropical Africa is much worse than in other parts of the World is because two of the World's most efficient vectors of malaria alternate in abundance seasonally throughout the savannahs: *An. funestus* breeding prolifically in grassy swamps to produce peak population densities towards the end of the rainy season and into the dry season; members of the *An. gambiae* complex breeding opportunistically in freshwater temporary pools wherever they occur with rainfall, irrigation, burrow pits or other man made sites prone to flooding, such as footprints and road ruts. The two most important members of the *Anopheles gambiae* complex are *An. arabiensis*, with females blood-feeding on livestock or humans plentifully indoors or outdoors. Evidently these anophelines have coadapted to human ecosystems in the afrotropical savannah where their combined contributions to malaria transmission have apparently facilitated the evolution of falciparum malaria (Coluzzi, 1999; WHO, 2006). Due to their endophilic and anthropophagic behaviour, *An. funestus and An. gambiae* s.s seldom occur away from human habitations. Their exceptionally high vectorial capacity can be attributed to their endophilic resting

behaviour, allowing relatively longer survival rates than for exophilic adult mosquitoes, as well as their propensity to feed on humans repeatedly (WHO, 2006).

Several other species are found in Savannah areas including *An. nili* that may be a locally important vector, as well as *An. coustani* and *An. pharoensis*. Malaria transmission is typically intense, regular, long, perennial or seasonal, according to the rainfall pattern and presence of water bodies round human communities. Transmission is very much influenced by the local ecological situation of each village, nearby river, swamp, backwater and human ways of life – both on small scale (near pits from which soil is taken for making bricks, footprints in marshy ground, etc) as well as large scale(small or large dams, rice fields, etc). In these conditions, the annual entomological inoculation rate is very high, often between 50 and 350 infective bites/human, and prevalence of *Plasmodium* is variable during the year, from around 50% during the dry season to more than 80% at the end of the rainy season in children under five years (WHO, 2006)

In some ways, malaria is a simple disease – it is either in people or in mosquitoes. Transmission depends on three things infected people, infected mosquito, and the biology of the parasite in both its host (Knell, 1991).

Malaria transmission is intense and stable in Nigeria. It is perennial in the forest ecotype and subperennial in dry savannah ecotype where transmission is relatively low during the dry season (November/December to April/March). This malaria situation is sustained by the presence of very efficient vectors of malaria namely *Anopheles gambiae, Anopheles arabiensis and Anopheles funestus* as well as favourable environmental factors such as temperature (20°C-30°C) rainfall (mean monthly rainfall of greater than 10cm), relative humidity (greater than 60%) and

topography elevations less than 2000m above sea level (Amajoh, 1997). The malaria vectorial system in Nigeria and of course south of the Sahara is probably the most powerful available anywhere to human *Plasmodia*. The human vector contact, particularly with *An. gambiae* s.l., shows a remarkable stability and flexibility, producing extremely high inoculation rate in a range of geographic and seasonal ecological conditions (Amajoh, 1997).

Malaria transmission can be measured by entomological inoculation rate i.e. the number of infective anopheline bites per year. This rate is very high in Nigeria In the south of Nigeria; the mosquitoes seem to show only slight seasonal variation in their infectivity rates. This contrasts markedly with the situation in the north where the sporozoite rate of mosquitoes is much lower during the latter parts of the dry season. The sporozoites rates for *An. gambiae* are often higher than those for *An. funestus*. Sporozoites rates are higher in the wet season than in the season. *An. gambiae* are late feeders (2300-0300hrs).Times of biting can be epidemiologically important (Amajoh, 1997).

Malaria transmission is through the bite of infected female *Anopheles* mosquito while the main vectors of malaria in Nigeria are: *An. gambiae s.s* (sensu stricto), which is predominantly in humid areas. It is therefore, present in high density, anthropophilic and is a very important vector of malaria. *An. arabiensis* is more dominant in the savannah ecotype. It prefers arid environment. It is also more zoophilic and exophilic, while *An. melas* is the salt-water species. It is generally more exophagic and zoophilic and thus a poorer vectothan *An.gambiae* (Amajoh, 1997).

2.2.3 Malaria Transmission in Unstable (Epidemic Prone) Areas

The distribution of malaria is linked to climatic conditions and the geography of water supply. As a result there is a mosaic of epidemiological situations with: areas with perennial transmission, where malaria is hyperendemic (for example in equatorial areas, coastal and lagoon areas), Areas with seasonal fluctuations in transmission with a minimum and maximum (the rainy season and the establishment of breeding sites is dependent upon the extent of rainfall, and epidemic outbreaks (for example in the soudanian like savannah). Areas with intermittent transmission, in the dry areas with very short rainy seasons (for example in the sub Saharan region) (Molez *et al.,* 1996). Though currently, there is no clear definition of a malaria epidemic, posing serious operational implications for diseases control however for operational purposes, Githeko *et al.* (2001) defined an epidemic as incidence of inpatient malaria cases 15% above the annual mean value (Githeko *et al.*, 2001). The use of the term epidemic in this work is as defined by Bruce-Chwatt, (1986), Najera *et al.* (1998). In this context, an epidemic is referred as a seasonal or periodic or occasional sharp increase in the amount of clinical malaria in a given indigenous community with moderately endemic malaria (meso endemic) as a result of abnormal meteorological conditions, which temporarily change the equilibrium between hosts, vectors and parasites. The situation described above has been termed as 'true epidemics' (Najera *et al.,* 1998).

Over the past two decades, due to climate change, epidemics of *Plasmodium falciparum* malaria, often with high case fatality rates, have been common in areas of unstable transmission in Africa. These unstable areas include those where transmission is limited by temperature (e.g. the East African Highlands), by rainfall

(e.g Sahel) or by both (e.g the Southern African highland plateau) (WHO, 2001). Malaria epidemics most often occur in hypo or meso-endemic areas, where the disease is normally present with low endemicity and where an epidemic situation will be a departure of what could be could be considered normal local variability of incidence and what health services are prepared to cope with (Najera, 2000). Semi arid areas are characterized as areas of low moderate rainfall with high inter annual variability. Epidemics of malaria have recently occurred in a number of such situations in southern, Eastern, and West Africa: (e.g. Northern Botswana, 1996, Western Zimbabwe 1996, Northern eastern Kenya1998, and Northern central Mali 1999). Each of these situations concerns districts, which have had a history of malaria epidemics and alargely non-immune population (WHO, 2001). The incidence in Gezira is an instructive example. The Sennar Dam of Gezira in the Sudan was constructed for the purpose of reclaiming nearly half a million hectares of land for cotton cultivation. When latter allowed, this brought about major changes resulting in the availability of mere stagnant water. Coupled with a season of exceptionally heavy rains in 1950, the mosquito *An. gambiae* increased greatly infecting more than half of the labour force of the whole Gezira with the severest form of malaria *Plasmodium falciparum*. Hundreds of people died and a third of the crops went unharvested resulting in a significant economic loss of about 5 million dollars (Gadzama and Gadzama, 1987).

Populations affected by malaria live in highlands-or arid and semi arid areas. Here, unusual rainfall and/ or higher temperatures might play a strong role in triggering such epidemics, especially after an extended period of drought, thereby increasing general population vulnerability. Catastrophic epidemics have also been

recorded as a result of movements of non-immune populations to endemic regions because of civil war or local conflicts (Delacollette *et al.,* 2004). Simulations performed using five different climate change scenarios compared to the simulation done with the climatology for present conditions suggest an increase of the seasonal zones ranging from 16% to 55%. Conversely, all but one scenario generated result in a decrease of the perennial zones from 3% down to 41% (Martin and Lefebvre, 1995). The global increase of seasonal potential malaria transmission zones caused by encroachment of seasonal zones on perennial ones and by the expansion of seasonal malaria into areas formerly free of malaria is worrisome. Seasonal potential malaria transmission is most likely to foster epidemics, causing widespread debilitation, increased mortality, and high morbidity among unprepared or non immune populations (Martin and Lefebvre, 1995). Malaria epidemics occur mainly in hypo or mesoendemic areas. One of the characteristics of these of these epidemics their occurrence in cycles of 5-8 years; however, it is difficult to forecast a cyclical epidemic as the cycles are far from regular. The most obvious pointers to a possible epidemic are meteorological and environmental factors, but a reasonably good collection of vital statistical data may detect it at an early stage and facilitate the initiation of appropriate measures (Bruce-Chwatt, 1986).

Unstable malaria (epidemic prone) areas share the following:

Highly seasonal transmission.

Highly variable risk of malaria from year to year.

Explosive seasonal proliferation of vectors.

Low survival rate of vectors due to mostly hostile environment.

Climatic conditions favourable for short periods of transmission.

High density of anopheline required to sustain transmission (WHO, 2003).

According to WHO (2003) areas of unstable (epidemic) malaria can be classified into two distinct types of types of transmission: (a) Highly seasonal but intense transmission with more or less predictable pattern each year associated with explosive epidemics at five to ten year intervals. (b). Highly seasonal with very little or even no transmission for several years. These areas are also affected at times by dramatic and devastating epidemic which often result from environmental or meteorological changes (WHO, 2003).

2.2.4 The Epidemiology of Malaria in Borno State, North-eastern Nigeria

Malaria in the Sahel fluctuates with a clear seasonal pattern (Pull and Gramiccia, 1976; FMOH, 1991; Molta *et al.,* 1995 and Samdi *et al.*, 2005). The relatively dry northern savanna of Nigeria demonstrates strong seasonality in Malaria transmission with a peak during the wet season. Therefore, the North has unstable hypoendemic or mesoendemic malaria (Molta, 1996). Malaria is considered 'seasonal' when potential transmission occurs 1 to 7 months during the course of one year (Martin and Lefevre, 1995). Malaria prevalence in Maiduguri can be classified as mesoendemic. Earlier works showed that the prevalence of Plasmodium infection among the study communities ranged from 35.2% (95/270) in Maiduguri to 70.2% (433/617) in Damboa both located in Borno State. The overall rate of infection was 50% (1,705/2,885) (Molta *et al.,* 1993). Laboratory studies in Maiduguri indicated mean prevalence of 35.6% as against 57.0% for retrospective studies (Ayanugwo and Kalu 1997). Recent studies have put the malaria prevalence at 27.26% (Samdi *et al.*, 2005) This classification is based on cumulative prevalence obtained for the population under study following the World Health Organisation classification of

malaria endemicity (WHO, 2003). Malaria infection rates fluctuated between 24.8 and 57.7% between 1987 and 1991 while in Damboa at the southern edge of the Sahel there were infection rates of between 66.2 and 75.9% between the years 1988-1995 (Molta *et al.,* 2004). In an area of unstable malaria immunity is short-lived. It might fall several months before the next season of high transmission. So the low immunity plus the increased inoculation rate could result in marked incidence of illness before the rains. All these put together for instance might explain the increase in number of cases due to malaria infection during the dry seasons of 1980 and 1982 as observed by Uwaezuoke (1984). Monthly figures of malaria among in patients in the Sahel show seasonal fluctuations and low values characterise the dry season while high values occur in the rainy season (Molta *et al.,* 1995). Furthermore, Oguche *et al.* (2001) demonstrated this strong seasonality in a study of the pattern of childhood cerebral malaria in the north eastern Nigeria, ninety-five (95) percent of patients presented with the infection between June and November with a peak in October. The seasonal pattern observed in the Sahel is in contrast with the wet forested areas of Nigeria where malaria transmission occurs at high levels all year round and is thus considered 'perennial'. When malaria is potentially transmitted 8 to 12 months a year (Martin and Lefevre, 1995; FMOH 1991), these areas particularly the rural settlement are usually in areas of stable malaria transmission is perennial and little affected by climatic changes and the malaria vectors in this part of Nigeria are highly infective, highly anthropophilic and have high longevity, for instance a survey carried out in Ibeshe coastal region of Lagos state in September 1997, 1068 of the 1118 (96%) female *Anopheles* mosquitoes from the survey which were dissected were positive (Amajoh, 1997). A

similar study was carried out the Sahel about the same period of the year (July-September) which corresponds with the peak malaria transmission period only 2.4% of female anopheles were found to be ELISA positive (Samdi *et al.,* 2006). However vectors in the Sahel incidentally have high number of infective bites per person about 60-100 infective bites per person per year in the savannah ecotype and about 30-60 infective bites per person per year in the forest ecotype. The cumulative entomological inoculation rate in the Sudan savannah reached a maximum of 145 sporozoite positive bites in 1year (out of which 132 were in the wet season) (Amajoh, 1997). Studies in the Garki district in Kano State had estimated that malaria transmission is sustainable when the human population receives about 0.33 infective bites per person i.e. transmission will be maintained as long as each person in the population is infected once every 3 years (Ekanem, 1997). In the Sudan Savanna, there are large seasonal, yearly and local variations in the level of malaria transmission. Molyneux and Gramiccia (1980) further observed during their six years malaria project in Garki, Kano State that, the cumulative prevalence of malaria was very high reaching 100% in the one to eight year age group. This could be ascribed to high levels of transmission resulting from the mosquito biting man at a very high rate infact, the capacity of the mosquitoes to pass malaria from one person to another was about 200- 2000 times greater than the critical value required to maintain malaria as an endemic disease (Molineaux and Gramiccia, 1980).

2.3 THE IMPACT OF CLIMATIC FACTORS ON MALARIA TRANSMISSION

Climate variability, unlike any other epidemiological factor, has the potential to precipitate simultaneously multiple disease epidemics and other types of disasters. Climate change has far reaching consequences that go beyond health and touch on

all life support systems. It is therefore a factor that should be rated high among those that affect human health and survival (Githeko *et al.,* 2000). Climatic conditions such temperature; humidity and rainfall affect the life cycle and behaviour of the vectors as well as sporogonic development of the parasites. Climatic factors also play an important part in species distribution, survival, and vectorial status (WHO, 1982).

The Sahel is considered one of the most sensitive and delicately balanced ecological systems in the world. This zone receives between 700mm and 800mm of rainfall per year (Gadzama, 1991). Generally in areas of low malaria endemicity, the incidence of infection is far more sensitive to climate changes (Martens *et al.* 1995). The variation of the annual rainfall in the Sahel is a very important factor influencing malaria transmission in the Sahel.

Variation in climatic conditions has profound effect on the life of a mosquito and on the development of malaria parasites. Hence its influence on the transmission of the disease and its seasonal incidence. The most important factors are temperature and humidity. Malaria parasites cease to develop in the mosquito when the temperature is below $16^{o}C$. The best conditions for the development of *Plasmodia* in the *Anopheles* and the transmission of infection are when the mean temperature is within a range of $20\text{-}30^{o}c$, while the mean relative humidity is at least 60%. A high relative humidity lengthens the life of the mosquito and enables it to live long enough to transmit the infection to several persons (Bruce-Chwatt, 1986).

In the Sahel, the source of the human malaria parasites that give rise to the cyclic malaria outbreaks or epidemics is maintained by an increased infective reservoir in the population; these susceptible human carriers thus represent a source

of long-term infection. An increase in contact between man and the *Anopheles* vector, greater effectiveness of local *Anopheles* in transmitting the malaria parasite and the sudden increase of the number of vectors, and to greater extent mean longevity of the female *Anopheles* of the species responsible for malaria epidemic. Climatic conditions are usually involved in this phenomenon (Martens *et al.,* 1995, Bruce-Chwatt, 1986, Nurain, 2004). In central and eastern Sudan, for instance, malaria is a disease of the short rainy season. The rest of the year remains dry and almost malaria transmission free. The source of the human malaria parasites that give rise to the cyclic malaria outbreaks in these areas is maintained by clusters of susceptible human carriers who thus represent a source of long-term infection. Longitudinal parasitological surveys have demonstrated that a large proportion of inhabitants who contract malaria during wet season retain chronic sub microscopic asymptomatic infections throughout the dry season. During the dry season of in areas with seasonal malaria transmission, up to 40% of the population harbour sub patent parasitaemia. Of these, 10-12% are gametocyte carriers. The parasites that persist during the dry season most probably seed the next transmission, following annual rain and the resurgence of mosquitoes (Nurain, 2004). According the same author no *Anopheles* mosquitoes are seen during this time of the year.

2.3.1 Temperature

Temperature affects the survival, development and life-cycle of malaria parasites in the *Anopheles* vectors. All species have the shortest development cycle around 27-31^{0}C (Bruce-Chwatt, 1991) from a minimum of 8 days for *P vivax* to a maximum of 15 -21 days for *P. malariae* (Bruce Chwatt, 1991; Dutta and Dutta, 1978; Molineux, 1988) the lower the temperature, the longer the duration of the

cycle (Bruce-Chwatt, 1991). The minimum duration roughly doubles around 20^0C, below 19^0C for *P. falciparum* and 15-16^0C for the 3 other species, the parasites are unlikely to complete their cycle and hence to further propagate the disease. Temperature also modifies the vectorial capacity of the *Anopheles* at around 30-32C vectorial capacity can increase substantially owing to a reduction in the extrinsic incubation period, despite a reduction in the vectors survival rate. Mosquito species such as the *Anopheles gambiae complex, An. funestus, An. darlingi, Culex quinquefasciatus and Aedes aegypti* are responsible for transmission of most vector borne diseases, and are sensitive to temperature changes as immature stages in the aquatic environment and as adults. If water temperature rises, the larvae take a shorter to mature and consequently there is greater capacity to produce more offspring during the transmission period. In warmer climates, adult female mosquitoes digest blood faster and feed more frequently, thus increasing transmission intensity. Similarly, malaria parasites and viruses complete extrinsic incubation within the female mosquito in a shorter time as the temperature rises, thereby increasing the proportion of infective vectors. Warming above 34C generally has negative impact on the survival of vectors and parasites (Githeko *et al.,* 2000) Optimal values of temperature between 25C (Dutta and Dutta*,* 1978). The optimum temperatures lengthen the life span of the mosquitoes and increase the frequency of blood meals taken by the females, to up to one meal every 48 hours (Molineux, 1988). Higher temperature also shortens the aquatic life cycle of mosquitoes (Kondrashin, 1992) and reduces the time between emergences and ovipositions as well as the time between successive ovipositions (Molineux, 1988). There is an optimal range of water temperatures for growth of the immature stages of the

mosquito. This range is lower for species living in temperate than in tropical zones and varies between different species living in the same geographical zone: thus temperature is one of the factors that limits the geographical distribution of a species. Within these optimal ranges, however, there is a largely direct relationship between temperature and growth. For example, mosquitoes breeding in the tropical zone, in water at 23-27oC usually complete their aquatic growth within two weeks (WHO, 1982).

Studies in Maiduguri, by Sara (1990) showed that the Sahel is characterized by a lengthy dry seasons of about eight months, with a mean annual temperature of about 27^0C. The study reported that the average optimum temperature for the development of can be arrested completely at 10^0C or over 40^0C when high mortality may occur. Continuous exposure to high temperature (over 30^0C) reduced the average lifespan of mosquitoes, This was clearly observed between the months of March and June 1988, when there was a rapid decline in the population of mosquitoes collected in all sites (Sara,1990). Mosquito density is affected by both temperature and humidity, which in turn are influenced by rainfall. Furthermore, Sara (1990) observed that the prevalence of anophelines varied greatly from rainy to dry seasons and from site to site. In Maiduguri Anophelines apparently increase with increased rainfall, exception to this pattern includes species such as *An. pharoensis* and *An. squamosis*, which prevail even in drier months Gadzama (1983). The highest population of *Anopheles* was obtained in the months of August, September, January and February 1983. These mosquitoes were rarely present between March and May 1983. The months of April and May recorded complete absence of Anophelines. This observation according to Sara (1990) could be explained by the absence of

favourable breeding sites for this species during that period (Sara, 1990). This finding is also reflected in the parasite densities of malaria patients sampled about that period of the year. It is also noteworthy that the lowest geometric means asexual parasite density in a longitudinal parasitological study was recorded during this period of the year in Maiduguri (Samdi *et al.,* 2005, Molta *et al.*, 2005). Gadzama (1983) also observed the same pattern during vector studies. However, a negative correlation was observed between the number of mosquitoes caught and temperature in the Sahel (Sara, 1990).

2.3.2 Rainfall

Rainfall generally means new opportunistic breeding places, Nonetheless, rainfall can also destroy existing breeding places, heavy rains can change breeding pools into streams impede the development of mosquitoes eggs or larvae or simply flush eggs or larvae out to the pools, Conversely, exceptional drought conditions can turn streams to pools in which would be breed in profusion. The appearance of such opportunistic mosquitoes breeding sites sometimes preceeded epidemics (Bruce-Chwatt, 1991). The latter occurrence was often observed in Northwest zone of Sri Lanka, where during the years of relative drought great breeding of *An. culifacies* took place and was followed by severe epidemics of malaria (Bruce-Chwatt, 1986). It was fortuitous that the time period of the six year Garki Malaria project which spanned the 1973 drought period served as an example thus, it was possible to observed the effect low rainfall on malaria in the Garki District of Kano State (Betterton and Gadzama, 1981). Of the mosquitoes species found in Garki area three were considered to be major malaria vectors. These were *Anopheles funestus* and two sibling species of *An. gambiae*. The two siblings look alike, but one is more able

to tolerate dry conditions of the Sudan savanna than the other and they also differ in aspect of their biology and behaviour (Desowitz, 1980). It was found that the numbers of *An. funestus* were severely depressed during the drought years, which this was not the case for *An. gambiae* (Molineux and Gramicca, 1980). This was probably due to the difference in the breeding habits of the two species; *An. funestus* prefers semi-permanent waters Sahel by vertical aquatic plants such as read, whereas. *An. gambiae* will breed in the small puddles left by animals hoof prints and the like (Desowitz, 1980). In time of drought there would insufficient water to establish *An. funestus* breeding sites, but even a small amount of rain would be sufficient to create sites suitable for *An. gambiae* (Betterton & Gadzama, 1981). Thus, it is erroneous to believe that malaria, as water-related disease is necessarily reduced by a period of drought (Betterton and Gadzama, 1981).

Repeated rains cause severe flooding resulting in temporary flushing out of breeding places. Consequently the breeding of a vector population is greatly reduced, but becomes, re-established when favourable conditions are restored (Gadzama, 1983).

2.3.3 Relative Humidity

The interaction between rainfall evaporation, runoff, and temperature modulates the ambient air humidity, which in turn affects the survival and activity of *Anopheles* mosquitoes. To survive, they need at least 50% relative humidity (Gueye, 1969) or 60% humidity (Bruce-Chwatt, 1991; Dutta and Dutta*.,* 1978; Molineux 1988). Higher levels lengthen the life span of the mosquitoes and enable them to infect more people (Dutta and Dutta, 1978). In the Sahel, Sara (1990) observed that a constant high temperature and low relative humidity led to drastic decline in the

mosquitoes' populations between March and May i.e. the hot dry Season could affect their life-span reproductive and feeding capacities. Most of the breeding grounds dried up during these months, An insect activities are correlated with daily rhythm of temperature and humidity. Such rhythm is most evident in areas hot and relatively dry days (Gadzama, 1977; Sara, 1990). The best conditions for the development of *Plasmodia* in the *Anopheles* and the transmission of infection are when the mean temperature is within a range of 20-30c, while the mean relative is at least 60%.A high relative humidity lengthens the life of the mosquito and enables it to live long enough to transmit the infection to several persons.

2.4 DIAGNOSIS

Conventional diagnosis still uses the skilled but laborious and time-consuming microscopic examination of thin and thick blood films stained with Giemsa stain (WHO, 1996, Phillips 2001). Newly developed tests include the quantitative buffy coat method (Becton Dickinson, Sparks, Md.) for the fluorescent staining of parasites after enrichment step for the infected erythrocyte (reported to be as good as thick films for *P. falciparum* but not for the other species (Baird *et al.*, 1992); the Parasight F (Becton Dickinson) (Schiff *et al.,* (1993) and the Malaquicktests (Kumar *et al.,*1995) (ICT Diagnostics, Sydney, Australia), based on the immunological capture of the *P. falciparum* histidine –rich protein 2 in whole blood; and the Optimal (Flow Laboratories, Portland, Oreg.) assay, which is an antibody based detection of parasite lactate dehydrogenase (Makler *et al.,* 1998). These antibody based dipstick tests are still being evaluated. PCR based diagnostic tests for human malaria have been developed (Morgan *et al.,* 1998), but these are more applicable to large scale

surveys than to clinical diagnosis. PCR has been effective at detecting submicroscopic levels of parasitaemia (Makler *et al.,* 1998, WHO, 2000).

2.5 MALARIA TREATMENT

The first recorded treatment dates back to 1600, when the bitter back of the cinchona tree in Peru used by the native Peruvian Indians to treat fevers came to the attention of Europe through the Jesuits, similarly 2000 years ago the Chinese developed a fever medicine from the wormwood Artemisia annua from which artemisinins which when combined with other medicines are the leading malaria treatment today. They constitute part of Artemisinin Combined Therapies (ACT). This plant is becoming a cash crop in East Africa (Rugemalila *et al.,* 2006). In East Africa for example, during the early 1900s European colonizers took quininine regularly as preventive treatment at the same time they started controlling mosquito breeding in towns where they mainly lived and in mines and farms which supplied goods to European industries (Rugemalila *et al.,* 2006).

The Second World War was a boon to malaria effort; it brought the warring powers into the tropics, where malaria was rife. It was therefore essential to discover, develop and deploy new malaria control tools in order to protect the forces exposed to malaria and other tropical diseases. So it was part of the war effort that led to the introduction of such new malaria control products as DDT and related insecticidal chemicals. Chloroquine and its relatives, which later constituted the mainstay of malaria prevention and control were also part of the war effort. Even Sulphadoxine Pyrimethamine (SP) was developed in order to contain chloroquine resistant malaria during the Vietnam war (Rugemalila *et al.,* 2006).

After World War two both DDT and Chloroquine entered civilian use. These chemicals were so efficacious and effective that they led to their use in community wide malaria control. By the 1950s there was so much confidence in the prowess of these tools, that an all out war to eradicate malaria from the entire war world was declared; it would rely on DDT and chloroquine (Rugemalila *et al.,* 2006).

2.6 VECTOR CONTROL UNDER THE GLOBAL MALARIA CONTROL STRATEGY

One of the four technical elements of the Global Malaria Control Strategy is vector control. Vector control remains the generally most effective measure to prevent malaria transmission (WHO, 2006). As the second basic element of the Global Malaria Control Strategy, selective application of transmission control measures must be considered wherever malaria is endemic. Since no method of active immunization is available and mass chemotherapy has very limited application, the only way of controlling transmission is usually that provided by the various methods of vector control (WHO, 1995). Generally when a vector spreads disease, it is simpler, cheaper and more cost effective to attack the vector rather than the pathogen. Mosquito control is in practice the measure which gives the greatest benefit in the shorter time to the largest number of individuals in a community, for instance, the rate of malaria parasitaemia in 21 countries of the Americas was found to increase with decreased vector control activities (intradomiciliary sprayings) and parasites diagnostic services between 1960 and 1985 (Knell, 1991; Wernsdorfer, 1986). In Zimbabwe also for instance, the emergence of drug resistant *falciparum* strains in an environment of poor economic performance and severe budgetary cuts of the health sectors prompted interest in simple and inexpensive vector control strategies that encouraged active community

participation (Chirevbu *et al.,* 1995). Inspite of the fact that the strength of of malaria lies in the enormous reproductive capacity of the malaria parasite and vector (mosquito): its weakness is the long development time before an infected mosquito becomes infectious to man which takes 7-21days from egg to a new adult Anopheles depending on temperature, therein lies the potentials for vector control (Knell, 1991).

The role of vector control is to augment the impact of early diagnosis and prompt treatment of malaria cases as described below:

Control and/or prevention of malaria epidemic. This will require, if they can carried out before the expected peak of transmission, residual spraying or the treatment of most area. If the epidemic has already started the use of emergency vector control measures, such as space spraying may be considered, if such methods have been effective for the target species in the same ecological setting and if resources are available for their immediate implementation (WHO, 1995).

Elimination of new foci of infection in malaria-free areas. Depending on the extension and number of such foci, space spraying or indoor residual spraying should be considered as emergency measures.

Prevention of seasonal peaks of malaria transmission. These may sometimes have the characteristics of seasonal epidemics. It may then be possible to institute routine seasonal application of indoor residual spraying and/or treatment of mosquito nets. It may also be worth considering environmental management methods, source reduction or larviciding in areas of high population density, such as urban areas or in development projects, in order to reduce the risk of transmission in the future. (WHO, 1995).

Control of transmission in high-risk situations. Such situations exist in labour or refugee camps, where non-immunes and infected people may come together in conditions of high transmission potential. Similar situations exist in outposts (army, police, prospectors, etc.) in endemic areas. If malaria control is taken into account when the high-risk situation is being created (e.g. when refugee or labour camps are being established), it may be possible to consider environmental measures such as site selection or basic sanitation. However, malaria control is usually required when there is little possibility of changing the location of such camps, although minor improvements in sanitation should be always considered. Emergency malaria control is often required and mass treatment of fever (or mass drug administration) may be the only possibility. Vector control is particularly indicated when mass chemotherapy is used, as a reduction of transmission will reduce the spread of resistant parasites selected by the massive use of drugs.(WHO, 1995, 2003)

Reduction of transmission in areas of high drug resistance. Most of these areas will fall into one or more of the categories described above. Drug resistance may be particularly high in areas which have been subjected to high selection pressure following the massive use of anti-malarials where vector control was considered difficult to organize. Perhaps the most suitable methods in these situations will be the use of insecticide-treated materials, although this may pose serious logistic problems in some areas (WHO, 1995).

Control of endemic malaria. The method most likely to produce sustainable control is the use of insecticide treated materials, although indoor spraying has been and still is the most widely used. Again, in areas of high population density, environmental management and larval control should be considered, since it may be

feasible to integrate malaria control with other mosquito-control activities aimed at controlling other vector-borne diseases or even the control of mosquito nuisance (WHO, 1995).

2.7 VECTOR CONTROL METHODS

Vector control is an effective way of reducing malaria transmission .The main vector control include the use of insecticide treated bed nets , indoor residual spraying (IRS) and larval source management which are all components of Integrated Vector Management (IVM) (Betson *et al.*, 2009; FMOH, 2009). Measures designed to reduce man's contact with Anopheline mosquitoes include: the siting, screening and mosquito-proofing of houses, the use of bednets, protective clothing, and insect repellents. Houses should not be sited near anopheline breeding areas and special mosquito gauzes are available for screening and mosquito proofing of houses. Effective repellents include diethyl toluamide and dimethyl phthalate (Rozendaal, 1997).

2.7.1 Measures against Adult Mosquitoes

Indoor residual spraying remains the most applicable method for transmission control and the most effective for obtaining a large scale impact at an affordable cost .Indoor residual spraying as a malaria intervention with total coverage is an important public health intervention for countries striving to achieve Abuja targets and MDG malaria goals and targets to reduce the malaria burden by 50% in 2010 and reverse the trends by 2015 (FMOH, 2008; FMOH, 2009a). Generally, Indoor residual spraying (IRS) and insecticide treated nets (ITNs) are more applicable geographically than more location/ecology specific measures directed towards larvae. Many important vectors of malaria bite indoors at night, and may rest on

indoor surfaces after biting, whereas larval habitats vary markedly among anopheline species (WHO, 2006). The development of pyrethroids with long residual action and very low mammalian toxicity suggested the possibility of treating mosquito nets to add an insecticidal effect to their mechanical protection, as mosquitoes are positively attracted by the odour of the sleeper inside the net, making the ITN like a baited trap. The insecticidal treatment of nets adds a chemical barrier to the often-physical barrier provided by the net and thus improves its effectiveness in personal protection (WHO, 2006).

In addition to personal protection for sleepers under mosquito nets being greatly enhanced by insecticide treatment, there is much evidence that community wide use of ITNs leads to large-scale killing of mosquitoes in areas where vectors are highly anthropophilic. The community wide use of ITNs reduces the vector population and shortens the mean mosquito lifespan. As a result, this will reduce the malaria sporozoite rate because, as with IRS, very few will survive long enough for sporogonic cycle to be completed. Apart from their killing effect, ITNs will also inhibit mosquito feeding, hence reducing the reproductive potential of highly anthropophilic vectors. These characteristics mean that ITNS may be considered also a vector control measure of general applicability. As with IRS, the vector control effects of ITNs become more apparent when household coverage increases (WHO, 2006).

Indoor residual spraying (IRS)

Residual Insecticide Spraying where the vector enters houses and feeds predominantly on man is still the most effective measure for checking transmission of malaria where local vectors are endophilic. The vectors must rest on the sprayed surface long enough to pick up a fatal dose of the insecticide. The residual

insecticides in common use are chlorinated hydrocarbons e.g DDT or BHC, Dieldrin and Aldrin. Resistance has arisen in some vectors in certain parts of the World to one or more of these chlorinated hydrocarbons & insecticides especially DDT are among the most effective, cheapest and safest residual insecticides available for malaria control in rural areas. Replacement insecticides are considerably more expensive e.g. Malathion is 2-3 times more expensive than DDT and needs to be applied 2-4 times more frequently. Propuxur is 16 times more expensive and requires more applications. Mosquitoes and biting flies seek undisturbed resting sites for part of their life. In drier regions, houses are important resting places for mosquitoes and phlebotomine sandflies. In humid forested areas the insects are less dependent on houses and often rest in vegetation outdoors. However, even species that usually rest outdoors may enter houses to feed and may then spend some time resting indoors before and after feeding (Rozendaal, 1997).

When mosquitoes and other insects rest in houses it is possible to kill them by spraying the walls with residual (long lasting) insecticide. Mosquitoes resting on sprayed walls come into contact with insecticide through their feet and are killed. Some insecticides irritate mosquitoes and cause them to leave houses. In dry or windy areas, this may also result in death due to lack of suitable outdoor resting places. Wall-spraying may not prevent biting. Hungry mosquitoes entering a house may bite first and then be killed when resting on a treated wall (Rozendaal, 1997).

As most anopheline vectors of malaria enter houses to bite and rest, malaria control programmes have focused primarily on the indoor application of residual insecticides to the walls and ceilings of houses. House spraying is still an important malaria control method in some tropical countries while in others its importance is

diminishing because of various problems that have arisen. Methods that are less costly and easier to organize, such as community use of impregnated bednets, and that produce long lasting improvement, such as elimination of breeding sites, are now being increasingly considered (Rozendaal, 1997).

Indoor residual spraying is generally not very effective against *Aedes aegypti*, the vector dengue, or against *Culex quinquefasciatus,* the vector of lymphatic filariasis, at least partly because of their habit of resting on unsprayed objects, such as clothes, curtains and other hanging fabrics rather than on walls and ceilings. Moreover, *Culex quinquefasciatus* is resistant to DDT and other chlorinated hydrocarbon insecticides. Other insecticides, with the exception of the residual pyrethroids, would too expensive for sustained control over many years. A practical problem in urban areas is the large number of rooms that would have to be sprayed (Rozendaal, 1997).

Space spraying

Involves the application of pesticides in the form of fog or mist and is mostly aimed at controlling adult mosquitoes. This is mostly aimed at controlling adult mosquitoes. This is a major anti-epidemic measure in mosquito borne diseases. Application can be done using various fogging machines and mist blowers or using the new ULV pesticide dispersion by air or by ground equipment using very small amount of insecticides (e.g. Malathion, Fenthion). Because the insecticidal action does not last long it is usually necessary to repeat procedure several times. Space sprays are usually applied in and around houses in cities or villages and sometimes on outdoor resting places. It has the following advantages: It has an immediate effect on adult populations of insects and is therefore suitable for the control of

disease outbreaks; for a single application, it is less labour intensive and large areas can be treated fairly quickly; less insecticide is required for one application in urban areas; It kills mosquitoes that do not rest in houses (Rozendaal, 1997).

2.7.2 Measures against the Mosquito Larvae

However, the most effective campaign against Africa vectors of malaria is the eradication of accidentally introduced *Anopheles gambiae* from 5400km largely ideal habitat in Northeast Brazil in the 1930s and 1940s. This outstanding success was achieved through an integrated programme but relied over whelming on larval control (Killeen *et al.*, 2002). This programme was soon repeated in Egypt, another larval control programme successfully suppressed malaria for over 20 years around a Zambian copper mine Killeen (2002). Historical evidence points to the conclusion that the only limitations to the effective coverage of larval control are practical rather than fundamental, adult mosquitoes are highly flying insects that can readily defect and avoid many intervention measurements whereas mosquitoes eggs, larvae and pupa are confined within relatively small aquatic habitats and cannot readily escape control measures. Hence, larval control strategies against vectors in sub Sahara Africa could be highly effective, and complementary to adult control (Killeen, 2002).

The advantages of larval control compared to methods like house spraying for instance is that, it has been observed with respect to house spraying against adult mosquitoes that houses occupants commonly welcome the first spraying of their homes, later they resent the inconvenience and perhaps the odours of repeated sprays. Spraying of temples, mosques and other sacred buildings may be prohibited. Refusal leads to reduced overall coverage and impair mosquito control. They are a

type of resistance in the human population and may be just as problematic as resistance in the insect (Knell, 1991).

2.8 MOSQUITOES

Mosquitoes belong to the

Phylum: Arthropoda

Order: Diptera

Family: Culicidae,

Subfamilies: Anophelinae (3genera) *Anopheles, Chagasia, Bironella*

Culicinae (34 genera) In. *Aedes, Culex, Mansonia*, *Toxorhynchites*

Genus (3 genera) *Anopheles, Chagasia and Bironella.*

Mosquitoes belong to the order Diptera, and suborder nematocera. They represent the largest group among the dipterans. There exists around 3,300 species of mosquitoes belonging to different genera: *Aedes, Culex, Anopheles*, etc. The classification of *Anopheles* species dates back to 1818, when the genus *Anopheles* was described by Meigen. Among all mosquitoes only the *Anopheles* are potentially capable of transmitting human malaria.There are known to be 422 species of anophelines in the World, among which 68 have been associated with the transmission of the four forms of human malaria .In Africa and the Indian ocean, only around ten species are concerned. *Anopheles* mosquitoes are absent in New Zealand, New Caledonia, Micronesia, Polynesia where the *Anopheles* mosquitoes are absent and which, for this reason, are malaria free. The genus *Anopheles* is currently subdivided into six sub-genuses *Anopheles* (Theobald), Cellia (Theobald), Kerteszia (Theobald), *Nyssorhynchus* (Blanchard), *Stethomyia* (Theobald), *Lophopodomya* (Antunes) (Sanofi, 2004).

Early entomological studies based on classic morphological approach led to the definition of two main components in the vectorial system, mainly *Anopheles funestus* Giles 1900 and *An. gambiae* Giles 1902 which came to be widely used as operational taxonomic units by malariologists.Other units were later added to the system, such as *An. nili* (Theobald) 1904 and *An. moucheti* Evans (1925) but these vectors were shown to have only local importance (Coluzzi, 1984).

Although the genera *Anopheles* and *Toxorhynchites* are regarded as primitive, they have not yet been found as fossils. It is believed that mosquitoes originated in the Jurassic geological period and that, in the absence of mammals, they fed on reptiles, amphibians or birds, as do some species today; there is little evidence for this, however, and ideas about their origins must remain speculative. From comparative chromosomal study of nematoceran genera, it appears that mosquitoes evolved from the chaoboridae and that the *toxorhynchitinae* is the most primitive of the three culicid subfamilies derived from a Mochlonyx-like ancestor. The Anophelinae and Culicinae evolved along separate lines (Service, 1993).

2.8.1 Biology of Malaria Vector Species

The *Anopheles* vector is the link between man and the malaria parasite. *Anopheles gambiae sensu lato* consists of at least seven species, five of which are vectors of human malaria. Amongst these are *An. gambiae* s.s and *An. arabiensis. An.gambiae* s.s has been divided into five chromosomal forms designated with a non-Linean nomenclature: Bamako, Mopti, Savanna, Forest, and Bissau (Coluzzi *et al.*, 1985, Toure *et al.*, 1994, 1998). (The two major vectors in Africa) (Gillies and De Meillon 1968). *An. merus, An. melas,* and *An. bwambae* (the minor vectors). The other species *An. quadriannulatus* A and B are the non-vector species from southern

Africa, and Ethiopia respectively (Gillies and Coetzee, 1987; Hunt *et al.,* 1998). The *An. funestus* group also consists of nine morphologically indistinguishable species. These include, *A. funestus* sensu stricto*, An. vaneedeni, An. parensis* Gillies, *An. aruni* Sobti*, An. fuscivenosus* Leesoni, *An. confusus* Evans and Leesoni, *An. leesoni* Evans*, An. rivulorum* Leesoni and *An. brucei* (Gillies and De Meillon, 1968; Gillies and Coetzee, 1987). Among which is the discovery of species complexes adds a new dimension to vector control. Species complexes can have species, which vary in their behaviour and vectorial capacity. This creates many problems for programme managers. It is therefore, imperative to determine a sibling species composition, biology and roles in disease transmission before implementing or changing control measures (WHO, 2005).

The most important vectors in the Afro tropical region (Africa south of the Sahara, Madagascar, Seychelles and Mauritius) are the *An. gambiae* complex (which include *An. gambiae, An. arabiensis, An. melas, An. merus, An. bwambae, An. quadriannulatus* and *An. funestus* (Service, 1996). It is now also important in addition to identifying species complex to take closer look at the biology, seasonal abundance, host preference, infection rates, resting habits and biting cycle of a species (or species complex) before introducing control measures; particularly if a large difference in behaviour or response to vector control is noticed. Among the *An. gambiae* complex, *An. gambiae* sensu stricto is the most important malaria vector and it is probably the world's most efficient vector (Service, 1996). It breeds in sunlit pools, puddles, burrow pits and rice fields. It bites humans both indoors (endophagic) and outdoors (exophagic) and rests mainly indoors but may also rest outdoors. The other important species of the *An. gambiae* complex *An. arabiensis*

has similar breeding and biting habits to *An. gambiae ss* except that it tends to occur in drier areas and is more likely to bite cattle and rest outdoors (exophilic). *An. funestus,* the other major vector in the afrotropical zone, prefers shaded habitats and breeds in permanent waters, especially with vegetation, such as marshes edges of streams, rivers and ditches and rice fields with mature plants providing shade. It bites humans predominantly but also domestic animals and is exophagic and endophagic (Service, 1996).

Since the sixties, Service (1961, 1963) provided a distribution map of *Anopheles* mosquitoes of Nigeria and the Cameroons. Prior to this, Smart (1948) studied the distribution of *Anopheles* mosquitoes in West African countries of the Gambia, Sierra Leone, Liberia, Ghana, Nigeria, Guinea, Cameroon, Gabon and Congo.

Other notable works conducted in Northern Nigeria notable studies are (Service, 1965; Rishikesh *et al.,* 1985; Molineux and Gramiccia, 1980; Anyanwu and Iwuala, 1999). Awolola *et al.,* (2002, 2003, 2005) recently conducted several surveys in South-western Nigeria.

Mosquitoes are found all over Nigeria and are not restricted by change in topography across Nigeria. The main vectors of malaria in Nigeria are: *An. gambiae* ss (sensu stricto), which is predominant in humid areas. It is present in high density, anthropophilic and is a very important vector of malaria. In the humid savanna, some of the important *Anopheles* species include *An. gambiae, An. funestus,* and *An. moucheti, An. melas* (Awolola *et al.,* 2002).

While in the Sahel, *Anopheles pharoensis* and *An. squamosus* are available in great numbers in Sahel zones (Gadzama, 1983), (Gad, 1988) recorded twelve *Anopheles* species in Borno State. *Anopheles* population densities and malaria

transmission show marked seasonal fluctuations with peaks during wet months of August and September. Common *Anopheles,* species during the rainy season include: *An. gambiae, An. funestus, An. pharoensis, An. ziemanni* and *An. squamosus* (Gadzama, 1983). It is interesting to note that *An. pharoensis* and *An. squamosus* constituted the greatest proportion of the anophelines caught in the drier zones of Maiduguri and Ngala, except for *An. gambiae* sl and *An. funestus,* According to Gadzama (1983) a state of ignorance prevails as to the vectorial status nor is there basic knowledge of the seasonal contributions of the vectors to the *levels* of malaria. Sara (1990) studied "The bionomics of Mosquitoes" in selected sites of Maiduguri and observed that *Anopheles pharoensis, An. squamosus, An. gambiae* and *An. funestus* were the *Anopheles* species prevalent in the town. The persistence of *An. pharoensis* and *An. squamosus* throughout the dry and wet months of the year was observed.

Baseline data on the variety of insects of medical and veterinary importance collected at various sites in the Hadejia-Nguru wetlands of Northeastern Nigeria during November 1995 to March 1997, revealed the presence of the following Anophiline mosquitoes in decreasing of abundance, *An. gambiae* s.l (30.4%), *An. squamosus* (27.5%), *An. rufipes* (12.3%), *An. pharoensis* (11.9%) *An. ziemanni* (9.6%), *An. maculipalpis* (6.3%) and *An. funestus* (2.0%) (Molta *et al.,* 1999). It is of interest that the study revealed the presence of at least six *Anopheles* species. Unfortunately, the identification procedure of using keys employed in this work is tedious and does not differentiate between closely related species like the sibling species of *An. gambiae* Giles complex (e.g.*An. gambiae* ss and *An. arabiensis*). Studies in Kaduna area by Service (1963) showed various proportions of the A and B

forms. The two species can and often are sympatric (Coluzzi, 1970), and are the main vectors of malaria in West Africa (Lemasson *et al.,* 1997). But *An.* arabiensis appears more adapted to the Sahelian environment (Lemasson *et al.,* 1997) and persists longer the dry season than *An. gambiae* (White, 1974). Both species have the same nocturnal biting cycles and endophagic rates (Lemasson *et al.*, 1997) and are established vectors of malaria and filariasis. *An. arabiensis* is more dominant in the savannah ecotype. It prefers arid environment. It is also more zoophilic and exophilic while *An. melas* is the salt-water species. It is generally more exophagic and *An. funestus* is much more common in fresh water in the dry season because it is a permanent water breeder (Amajoh, 1997).

Table 1: MALARIA VECTORS OF BIOCLIMATIC ZONES OF NIGERIA

Bioclimatic Zone	**Vector Species**	**Sites/Localities**	**Authors**
Northen Guinea Savanna	*An.nili An. coustani, An. garnhami An. macullipennis. An. rufipes An. gambiae*	Federe, Bassa, Foron, Jos, Vom, Bukuru	Anyanwu and Iwuala, 1999
Sahel Savannah	*An. arabiensis, An. gambiae s.s, An. pharoensis, An. Maculiplpis, An, rufipes, An. ziemanni, An. funetus, An. squamosis*	Konduga, Marte, Maiduguri, Damboa	Gadzama, 1983, Sara, 1990, Kalu, 1992,Molta *et al.,*1999;Samdi *et al.,*2006.
Forest	*An. gambiae ss, An. arabiensis, An. funetus, An. rivulorum, An. hancocki*	Ibadan	Awolola *et al.,* 2003.
Sudan Savannah	*An. gambiae, An. arabiensis, An.funetus, An. rivulorum*	Garki, Katsina	Molineaux & Gramiccia, 1980
Savanna Forest	*An. gambiae s.s., An. arabiensis, An. Hargreavesi, An. moucheti, An. nili, An. funestus*	Sapele, Benin, Auchi	Coluzzi *et a.l,* 1979
Mangrove Forest/Swamp	*An. gambiae, An. arabiensis, An.funestus, An. rivulorum, An. melas, An. moucheti*	Akaka, Ilara, Ijesa-Isu	Awolola *et al.,* (2002), Oyewole *et a.,,* (2007)

2.8.2 Malaria Vector Sibling Species Identification

Malaria vector control either by insecticide treated nets, indoor residual spray or genetic control strategies require accurate mosquito identification and information on the behaviour of the vector species (Awolola *et al.,* 2005). Species complexes that vary in behaviour and vectorial capacity present a real problem to malarial control (Onyabe and Conn, 2001). All the vectors belong to species complexes whose members vary widely in their vectorial capacity and competence (Coetzee *et al.,* 2000).

In Nigeria, large numbers of anophelines are involved in transmission, but their distribution and vectorial capacity is not fully understood. Any vector control method must therefore take into account this heterogeneity (Okwa *et al.,* 2006). It is therefore important that studies to confirm the predominant sibling species of *An. gambiae* sensu lato be carried out, if meaningful control strategies are to be formulated against the malaria vectors of the Sahel savannah. It is suspected that *An. arabiensis* may predominate in the Sahel Savannah (Gadzama, 1983). The discovery of species complex adds a new dimension adds a new dimension to vector control. A closer look at the biology at the vector control, abundance, host preference infection rates, resting habits and biting cycle of a species (or species complex) before introducing control measures; particularly of a large difference in behaviour or response to vector control is noticed (WHO, 2005).

It is, therefore, imperative to determine a sibling species composition, biology and roles in disease transmission before implementing or changing control measures

(WHO, 2005). The relevance of cryptic species recognition to efficient control of disease is exemplified in the discovery of *Anopheles maculipennis* as a complex of species and the effective control of malaria that followed in Europe (Bates, 1940; Stegnii and Kabanova, 1978).

The discovery too of the 6 sibling pecies under the name of *An. gambiae* also has had a tremendous impact on the understanding of epidemiology of malaria transmission in different regions of Africa (Coluzzi and Sabatini, 1967; Coluzzi *et al;* 1979; Davidson *et al.,* 1967). Many anopheline mosquito species exhibit considerable variation in malaria transmitting capacity and differences in vectorial capacity is associated with such factors as preference for human Vs animals, indoor Vs outdoor biting and preferred time of night for biting (Maurice and Pearce, 1987). Therefore, if the mosquito species responsible for transmitting the disease in a given can be identified and its habits determined, control measures can be directed against that species, especially when it involves a complex of species (Toure, 1989). *Anopheles gambiae* s.s and *An. arabiensis,* the two most important vectors of the *An. gambiae* complex in sub Saharan Africa, have shown differences not only in geographical distribution but also in their behaviour and population dynamics (Coluzzi *et al.,* 1979; Lanzaro *et al.,* 1998; Petrarca *et al.,* 2000). Separation of the two species is complicated by their confusing flexibility and probable gene flow between them (Besansky *et al.,* 1997). Because these two morphologically identical species are often found in sympatry with other members of the *An. gambiae* complex over much of their geographical range, their relative roles in the transmission of *Plasmodium* spp. And

the ecological conditions, which determine their distributions, are difficult to evaluate. Taxonomic identification of the *An. gambiae* complex has been largely based on adult characters such as mating incompatibility (Davidson, 1964) and differences in chromosome morphology (Coluzzi and Sabatini, 1967), isoenzymes (Miles, 1978) and DNA characteristics (Gale and Crampton, 1987). Some of the taxonomic complexity within *An. gambiae ss* has been elucidated through the analysis of chromosome inversion (Coluzzi *et al.,* 1979, 1985; Petrarca *et al.,* 2000), hydrocarbons (Milligan *et al.,* 1993) and increasingly by the use of molecular methods to identify species, chromosomal forms (i.e. specific karyotypes) and geographical variants (Favia *et al.,* 1997; Donnelly and Townson, 2000; Gentile *et al.,* 2001). *Anopheles gambiae ss* and *An. arabiensis* show complex patterns of inversion polymorphism mainly involving paracentric inversions mainly on the 2R chromosome. The inversion polymorphisms in *An. gambiae ss* are characterized by a correspondence between the frequencies of the different karyotypes and geographical and ecological clines. Twelve such inversion arrangements have so far been commonly found (Black and Lanzaro, 2001). Certain karyotypes intergrade completely, others partially, while other forms there are a total lack of gene flow. This high level of chromosomal polymorphism has led to recognition, today, of five chromosomally distinct forms in West Africa: 'forest', Savanna, Bamako, Mopti and Bissau (Coluzzi *et al.,* 1985; Toure *et al.,* 1989; Favia *et al.,* 1997). In addition, two molecular forms (denoted M and S) have been described and their relationship with chromosomal is currently under investigation (Della Torre *et al.*, 2001, Favia *et al.*, 2001). These forms share similar bionomical characteristics,

with their pattern of spatial and seasonal distribution governed by competitive exclusion (Coluzzi *et al.,* 1979, 1989). The overall picture is one of recent and incipient speciation related to forest recession and agricultural development in West Africa and the resultant adaptation of the vectors (Black and Lanzaro, 2001; Favia *et al.,* 2001; Torre *et al.,* 2001). Little definitive work has been done on the larvae of the *An. gambiae* complex was recognized on the basis of chromosomes of the larvae of *An. gambiae* ss and *An. arabiensis,* by Coluzzi and Sabatini (1967) and Service (1970). Bryan *et al.* (1982) considered that, although the forest and Savanna populations of *An. gambiae* were sympatric in their biting and resting habits, they could have distinct larval and mating sites and that could be crucial in terms of their control. The first successful segregation of individual larvae from colony stock of *An. gambiae* ss and *An. arabiensis* by cuticular hydrocarbon analysis was reported by Anyanwu *et al.* (1994).

2.8.3 Methods of Vector Species Identification

The need for accurate method of identifying vectors is reflected by the fact that the basic taxonomy of the best known vectors has remained contentious and has necessitated reinterpretation of the biology of vectors such as *Anopheles gambiae* complex (Coluzzi *et al.,* 1999; Bryan *et al;* 1982a, 1982b) the *Aedes scutellaris* group (Huang and Hitchcock, 1980; Rai *et al.,* 1982).

Recently emphasis and efforts are being directed to the development and application of new tools for the specific identification of insect-borne disease vectors more so as vector borne diseases today are transmitted by members of a sibling

species complex. Among the most recent and widely used tools for identification of members of siblings species complexes or for other studies on population heterogeneities include: cytotaxonomy of *Anopheles* (Coluzzi *et al.,* 1985) and *Simulium* species (Vajime and Dunbar, 1975); electrophoretic isoenzyme characterization of *An. gambiae* sibling species (Miles, 1978) , Cuticular hydrocarbon analysis of *Anopheles* (Carlson and Service 1979, 1980; Milligan *et al;* 1986) and black flies (Phillips *et al;* 1985) and DNA probes (Gale and Crampton, 1987). Polymerase Chain Reaction (PCR) (Paskewitz *et al;* 1990).

The main techniques used for malaria vectors of the genus *Anopheles* are:

1. **Morphological characters**

Early entomological studies based on the classic morphological approach led to the definition of two main components in the malaria vectorial system, namely *Anopheles funestus* Giles 1900 and *An.gambiae* Giles 1902,which came to be widely used as operational taxonomic units by malariologists .Other units were later added to the system,such as *An. nili* (Theobald) 1904 and *An. moucheti* (Evans) 1925,but these vectors were shown to have only local importance(Coluzzi,1984). This is the basic first line technique; it requires patience and time and utilizes keys. It can easily be carried on the field and in the laboratory with few equipment required such as a stereo microscope. Its major disadvantage is that it is unable to distinguish sibling species. Though generally sibling species are morphological characters can be used to identify sibling species after performing a rigorous statistical analysis on numerous morphological characteristics of the adults, larval and eggs (WHO, 2005)

Morphometrics alone cannot be used as a reliable tool for species identification owing to absence of clear cut and reliable characters and variation in values parameters adopted. For instance, the adult female vector mosquitoes of many species have less specific forms of external genitalia, making them more difficult than males. In case the case of the immature stages, even though they may be more distinctive is some species than the adults, the number of branches on key setae may not be absolutely difference between species (White, 1982a).

2. Crossing of experiments

The reproductive isolation observed in field generally breaks down in laboratory and different species mate and produce hybrid progeny. However, genetic differences between species are expressed in the form of non-viability of hybrid progeny of immature stage, hybrid females can be fertile. Thus, hybrid sterility is used as criterion in designating populations as separate species. Exceptions do exist and certain species that do not mate in nature will cross in the laboratory and produce fertile progeny. These techniques can be used to recognize sibling species but can not be used as a routine diagnostic test in field studies for the identification of sibling species (WHO, 2005).

Such hybridization studies illustrating mating incompatility have been particularly important in the *An.gambiae* complex enabling the discovery of the fresh water species A and B. (Davidson and Jackson, 1962) and species C (Paterson *et al.,* 1963, Davidson 1964a) as well as determining the status of the brackish water forms: *An. melas* and *An. merus* (Davidson, 1964b).

3. Cytogenetic techniques

All anopheles studied so far have three pairs of chromosomes, two pairs of autosomes and one pair of sex chromosomes – xx in females and xy in males. Based on position of the centromere each chromosome is designated as aerocentric, submetacentric, and metacentric. These structural variations of mitotic or metotic chromosomes at metaphase are used to identify sibling species (WHO, 2005).

Using techniques that change the DNA structure in chromosomes, banding patterns of mitotic chromosomes can be visualized. These heterochromatic variations are also used to identify sibling species. Mitotic chromosomes are found best in late III instar or early IV instar brain tissue or and meiotic chromosomes in newly emerged adult gonadal tissue (testes and ovaries) (WHO, 2005). These techniques are simple to do and the cost are low. The collected females cannot be used to observe the chromosomes (WHO, 2005).

Coluzzi (1970) had observed that salivary gland chromosomes may provide important data on polymorphism, phylogenetic relationship, and species differentiation. Use of cytogenetic methods has been quite useful in: providing-information on the geographical and seasonal distribution of the sibling species especially species A and B in East and West Africa where the 2 species are sympatric as well as their bionomics and relation to malaria transmission.

4. Enzyme electrophoretic variations

Enzymes electrophoresis has been extensive used, in the studies of species complexes (Miles, 1978). The technique involves the detection of protein bands of an enzyme system with different mobilities as a function of electric charge and molecular structure. On a gel zymogram of an enzyme system, electrophoretic variations in the form of bands with different mobilities represent proteins coded by different alleles (allozymes). These alleles being codominant behave like paracentric inversions, and the two homozygotes and heterozygotes can be differentiated because of the simplicity of the procedures for the processing and interpretation of data, these techniques permits a large-scale sampling of natural populations and is very useful as a diagnostic tool in the routine identification of species (WHO, 2005)

If a single fully diagnostic enzyme system cannot be identified (i.e. without any polymorphism), several enzyme systems which differ in their frequencies of alleles between populations can be identified and used in association. This will reduce the error in identification. For species complexes with several members, enzyme systems that are diagnostic for different members of the same species complex can be identified and used in the form of a biochemical key: with this technique, larvae, pupae or adults can be used in the identification. The only disadvantage with this technique is that specimen that are collected from the field and are not used immediately with have to be stored at below freezing. In order to retain enzyme activity (loc. cit).

5. Cuticular hydrocarbon analysis

Cuticular hydrocarbon analysis for sibling species identification involves - determining species-specific differences in the hydrocarbons contained in the wax layer of insect cuticle. The wax layer lies beneath the outer most cuticular layer. It should be noted that this technique could be employed to identify variations of members of an already identified species complex but cannot be employed to recognize new complexes. In addition it cannot be used in routine field studies (Hamilton and Service, 1983; WHO 2005). Observations for the larvae of *An. gambiae* complex suggest that quantitative variations in cuticular hydrocarbons like the polytene chromosome rearrangements or isoenzyme pattern analysis can play a crucial role in species discrimination (Anyanwu 1994).

6. Molecular techniques

Advancements in DNA recombinant technology have facilitated the development of simple and rapid molecular tools for the identification of sibling species. The most widely used molecular method is identification by DNA probes. Non-radioactive probe methods remove the hazards of radioisotopes and make the assays simple and usable under field conditions. The advantage of DNA probes, as with isozymes, is that species can be identified at all stages of the mosquito's life cycle. Also, if field kits are developed as has been done for the members of the *An. gambiae* complex, probes can be used with much more ease in field laboratories. Difference in DNA units are being used extensively to develop species specific primers for PCR (Polymerase Chain Reaction) assay (Paskewit *et al;* 1990). Restriction fragment length polymorphic DNA (RAPD-PCR) and single strand confirmation polymorphism (SSCP)

visualization are also being generated as diagnostic tools. To develop molecular tools expertise is required and the techniques are expensive to conduct (WHO, 2005).

7. Polymerase chain reaction

The fundamental principle of applying the PCR (Polymerase Chain Reaction) in diagnostics is to make huge number of copies of a gene. If a given DNA fragment is present, it will be multiplied by, perhaps a million fold through the reaction, yielding so much of the substance that it can be detected easily. This is necessary to have enough starting template for sequencing. The process begins by heating the DNA fragment to separate it into two strands. An enzyme that copies DNA, called DNA polymerase from a hot springs bacterium, Thermus aquaticus which can withstand the denaturing temperatures is then added with primer nucleotide sequences to create two complete copies of the original DNA fragment .By repeating this process, millions of copies of the original DNA fragment can be made in a short time. In about twenty cycles, a million copies of the DNA is made in thirty cycles, a billion copies. (Groth *et al.*, 2001; WHO, 2005) Depending on the availability of primers,any DNA can be amplified in this technique.For example, mitochondrial DNA from the remains of a 40,000-year-old woolly mammoth was amplified using PCR. The Mammoth�mitochondrial DNA was very similar in sequence to that of modern elephant's (Tamarin and Leavitt, 1991).

There are three major steps in PCR, which are repeated for 30 or 40 cycles. This is done on an automated cycler, which can heat and cool the tubes with the reaction. In cases in which very little sample is obtained (e.g., hair or degraded blood) Polymerase Chain Reaction can be used to amplify whatever DNA is present. This technique was originally developed to amplify a region of the DNA between two known sequences such that primers could be created for each strand of the double helical DNA to be amplified. In the technique the DNA sample is heated to separate its strands. Then the primer nucleotide sequences and the ingredients for DNA replication are added, leading to the replication of the two strands. After a few minutes, the mixture is denatured to separate the strands and a new cycle of replication is initiated. The technique is aided by using

Denaturation at 94°C:

During the denaturation, the double strand melts open to single stranded DNA, all enzymatic reactions stop (for example: the extension from a previous cycle).

Annealing at 54°C:

The primers are jiggling around, caused by the Brownian motion. Ionic bonds are constantly formed and broken between the single stranded primer and the single stranded template. The more stable bonds last a little bit longer (primers that fit exactly) and on that little piece of double stranded DNA (template and primer), the polymerase can attach and starts copying the template. Once there are a few bases built in, the ionic bond is so strong between the template and the primer, that it does not break any more (WHO, 2005).

Extension at 72°C:

This is the ideal working temperature for the polymerase. The primers, where there are a few bases built in, already have a stronger ionic attraction to the template than the forces breaking these attractions. Primers that are on positions with no exact match get loose again (because of the higher temperature) and don't give an extension of fragment.

The bases (complementary to the template) are coupled to the primer on the 3´ side (the polymerase adds dNTP's from 5´ to 3´, reading the template from 3' to 5' side; bases are added complementary to the template). Because both strands are copied during PCR, there is exponential increase of the number of copies of the gene. Suppose there is only one copy of the wanted gene before the cycling starts, after one cycle, there will be 2 copies, after two cycles, there will be 4 copies, three cycles will result in 8 copies and so on (WHO, 2005).

2.9 MOSQUITO BIONOMICS

An understanding of mosquito bionomics is of key importance in the planning methods of mosquito control. Bionomics deals with the relationship between a given species and its environment (WHO, 1982).

Water is an essential component of the mosquito environment and whether it is running, standing, sweet or brackish, shaded or sunlit, frequently determines which species of mosquito breeds in it. The environments of the immature stages and adult mosquitoes are interdependent since the adult mosquito must have access to water for egg laying. The adult mosquito environment is, however, largely aerial and

terrestrial, the former environment being necessary for mating and dispersal and the latter providing habitats for feeding, resting and completion of the cycle of ovarian development from bloodmeal to egg laying (WHO, 1982).

2.9.1 Immature Stages of the Mosquito

The environment of the immature stages of the mosquito is aquatic, its mobile stages (i.e. larva and pupa) dependent on atmospheric air for breathing and therefore typically spending much of their lives suspended from the surface film of aquatic environment.

There is an optimal range of water temperatures for growth of the immature stages of the mosquito. This range is lower for species living in temperate than in tropical zones and varies between different species living in the same geographical zone: thus temperature is one of the factors that limits the geographical distribution of a species. Within these optimal ranges, however, there is a largely direct relationship between temperature and growth. For example, mosquitoes breeding n the tropical zone, in water at 23-27°C usually complete their aquatic growth within two weeks. Moderately frequent rainfall usually increases the opportunities for prolific breeding, but repeated and heavy rainfall causes severe flooding resulting in a temporary flushing out of breeding places and reduction in mosquito population. The depth to which light penetrates the water in which the mosquito is breeding is generally not an important factor since the immature stages live largely at the water surface, but the extent to which the breeding place is shaded or exposed to sun determines which species of mosquito inhabit a particular water body. Hedges, planted to give shade

over breeding place or clearing of forests to allow sun to penetrate have been successfully used for environmental control of several malaria vectors (*Anopheles minimus* and *An. balabacensis*). Unless islands of vegetation are present to provide local breeding sites, mosquito larvae are not found on open surfaces of large bodies of deep fresh water (e.g. lakes, ponds, rivers or reservoirs) but are confined to their sheltered shallow edges. The immatures stages of some species (*An. gambiae s.l*) are found throughout the entire surface of swamps and of shallow temporary rainwater pools. Some species (*An. funestus*) breed in clear fresh water with vertical vegetation whereas others are adapted to breeding in brackish water (*An. sundaicus*) or highly polluted water *(Culex quinquefasciatus)*. The aquatic environment of some species is associated with particular plants. For example, Mansonia larvae are linked with the presence of fresh water lettuce (Pistia) and *Aedes* simpsoni with axillary breeding in banana plants. Other species *Ae. aegypti, Eretmapodites* and *Chrysogaster* breed in great numbers in small containers such as old tins, tyres and coconut husks (WHO, 1982).

2.9.2 Activities of Adult Mosquitoes

Anopheles prefers a rural habitat but adult mosquitoes are not usually found more than 2 or 3 kilometres from their breeding places in any large number. However, strong seasonal winds may carry Anopheles up to 30km or more from their main breeding sites and various other factors may greatly influence the average flight range of some species. Normally, females disperse more than males. Males subsist on plant juices and nectar while females, in addition to this, need blood protein for the

maturation of their eggs. Some prefer human blood and are said to be anthropophilic, others prefer animal blood and are zoophilic. Mosquitoes which enter and rest in human house are referred to as endophilic but are exophilic when they prefer to rest outside. All these characters are of great importance in a control programme, the habitats of the vector species must be well understood by teams engaged in malaria control (Amajoh, 1997).

2.9.3 Mating Behaviour

Mating usually occurs within 24-48 hours after emergence. In some species the males form a swarm, frequently located over contrasting or sharply defined points, e.g., the top of a tree, stake or rock, or over the corner of a building. Swarming usually occurs at dawn or in the evening, but may be seen in shaded areas in the middle of the day. Females entering the swarm are seized and the pair drops out of the swarm. After insemination, the spermatozoa are stored in the female mosquito, in an organ called spermatheca, and drawn on for fertilization of all the eggs produced throughout the remainder of its life (monogamy) (WHO, 1982).

2.9.4 Dispersal and Flight Range

The adult mosquito of most species does not fly great distances and the male is a much weaker flyer than the female. Thus the presence of a large number of adult male mosquitoes indicates that breeding places of that particular species are close by. In normal atmospheric circumstances, most individual mosquitoes of tropical species

apparently fly within 3km, although there records of a few species or occasional individual (WHO, 1982).

Biting habits

Many of the habits of adult mosquitoes are linked to their being both cold blooded and physiologically ill-fitted to withstand very dry environments. Flight, host seeking and feeding generally take place in a warm humid environment. Species that are associated with open terrains and sunlit habitats fly and feed between the hours of dusk and dawn when the air is more humid. Many of these species associated with dnse vegetational habitats such as forests or plantations, where daytime humidities are generally higher than in open terrain, fly and feed during the daylight hours (WHO, 1982).

Host preferences

The environment of the adult female mosquito includes a host.If the preferred host is man the mosquito is referred to as anthropophilic; if animal, as zoophilic; and if there is no fixed preference, as an indiscriminate biter. In the absence of the preferred host, some species (e.g. *An. gambiae* s.l) are facultative feeders and will feed readily on another host (WHO, 1982).

Resting habits

Species that enter houses at nght for feeding at night and for resting in the daytime are described as endophagic and endophilic as compared with exophagic and exophilic mosquitoes which feed and rest outdoors (WHO, 1982).

Gonotrophic cycle

Some species rest inside the house for a period of 2-3 hours before feeding indoors on man and then gorged with bloodmeal rest indoors for 24-48 hours until the blood has been digested and the ovaries contain mature eggs, i.e they are gravid. The gravid mosquito typically leaves the houses at dusk in search of a suitable aquatic site for egg laying (WHO, 1982).

2.9.5 Life Cycle of the Mosquito

Malaria is transmitted to humans by female mosquitos of the genus *Anopheles*. Male mosquitos feed on plant juices and nectar, rather than blood, and therefore cannot transmit the disease. Moreover, only certain species of anopheline mosquito are successful vectors of the parasites. Some specicies prefer to take their blood meals from animals and thus transmit malaria to humans very rarely or not at all; some species do not live long enough to allow the parasites to multiply and develop inside them; and in some species the parasites seem to be incapable of development (WHO, 1992a). There are four different stages in the life cycle of the mosquito of which the adult is the flying stage. The adult female anopheline takes a blood meal approximately every two or three days. This is necessary for the development of a batch of eggs, which are normally laid before a further blood meal is taken. The time required for digestion of the blood meal and development of the eggs varies with the temperature and humidity of the air. Eggs are laid on water in batches of about 100-150. The sites for egg laying vary from small amounts of residual water in such places as discarded food tins, coconut husks and hoof-primts, to large areas of water in

streams, canal, rivers ponds and lakes. Each species of mosquitoes prefers a particular kind of water surface on which to lay its eggs (WHO, 1992a).

Female mosquitoes continue to lay eggs throughout their lives. Most lay between one and three batches, although in a few instances as many as 13 batches have been reported. Under the best conditions the average life of female anopheline mosquitos is about two to three weeks (WHO, 1992). After two to three days, the eggs hatch and the mosquito larvae emerge. The larvae generally live just below the surface of the water (since they need to breath air) and take food from the water. If disturbed, most larve swim quickly towards the bottom of the water, returning to the surface soon afterwards in order to breathe; certain species, however, move rapidly sideways just below the surface. There are four larval stages or instars. The time required for the different stages to develop depends on various factors, including water temperature: the development period is shorter in warm water than in cool water. The small larva emerging from the egg is called the first instar. After one to two days it sheds its skin and becomes the second instar; at further intervals of about two days the third and fourth instars appear. At normal tropical water temperatures, the larval stage thus lasts about 8 to 10 days. Finally, the fourth instar develops into a pupa. It is during the pupal stage, which lasts for two or three days at tropical temperature that the major transformation takes place and the aquatic organism changes into an adult mosquito capable of flight. The pupa, shaped like a comma, stays on the surface of the water and swims when disturbed but does not feed. Its skin then splits and the adult insect emerges and eventually flies away. Mating soon

follows; the female, which mates only once, normally takes its first blood meal after mating and then develops its first batch of eggs.

Most anopheline mosquitoes bite during the night, some of sunset, others around midnight. Some enter houses to bite, while others usually bite out doors. After biting, mosquito usually rest for a short period. Indoor-biting mosquitos frequently rest on a wall, under furniture or on hanging cloths. Mosquito that bite outdoors may rest on plants, in dark holes in the ground, or in other cool dark places. Mosquitos may bite both people and animals. Host preferences differ between the species: some mosuitos prefer to take blood from humans, others take only animal blood. Clearly, those that prefer human blood are the more dangerous as they are more likely to transmit diseases notably malaria (WHO, 1992).

2.10 MALARIA PREVALENCE IN NIGERIA

Malaria is a public health problem in Nigeria accounting for 65% of hospital admission, 25% infant mortality, 30%childhood mortality and associated with 11% maternal deaths (FMOH, 2009b). It is endemic throughout the country with more than 90% of the population at risk of stable endemic malaria. At least 50% of the population suffers from at least one episode of malaria each year .The disease is the commonest cause of out patient attendance across all age groups (FMOH, 2004). Some categories of people are, however, at highest risk of infection. These include children aged less than 5years, pregnant women, visitors from non malarious regions and those with sickle cell anaemia. It has been reported that the disease accounts for 20-30% of under five mortality and that in highly malarious areas of the country the

death toll could be as high as 50%. The Federal Ministry of Health has noted that malaria leads to 25% of infant mortality and 30% of childhood mortality (FMOH, loc. cit).

The result of the most comprehensive study of the malaria situation in Nigeria conducted across the six geographical zones in Nigeria had revealed much about the public health importance of malaria. The study confirmed the fact that malaria is a major cause of morbidity and mortality especially among vulnerable groups including women and children aged less than 5 years (FMOH, loc. cit). The incidence of malaria among the under fives across the six geographical zones during the study were as follows: South-south 32.7%, Southwest-36.6%, Southeast-30.7%, North-Central-58.8%, Northeast-55.3% and Northwest-33.6%. The National Survey has shown that doctors classified 48.2% of the ailments experienced by pregnant women as malaria. About 22.3% had malaria in combination with other illnesses. The prevalence of clear cases of malaria in pregnancy across the geo-political zones were reported as follow: South-south-44%, South-West-46%, SouthEast31%, NorthCentral-56.4%, NorthEast-64.5% and NorthWest-46.6%. The total prevalence was found to be 48.2% (FMOH, 2004).

Since the report of Chloroquine resistance in the North-eastern Nigeria by Daniel *et al.*, (1989), there have been evidence of the increasing resistance to Chloroquine and Sulphadoxine which has now been put at of an average of 50% up from 12.7% as at 1993 (Molta *et al.*, 1993; Molta *et al.*, 2004; Watila *et al.*, 2006).

Gametocyte carriage rate too increased from 2.7% to 10% in the Sahel indicating the possibility of a continual increase in anti malarial resistance (Molta *et al.*, 2000; Watila *et al.*, 2006). In a highly malaria endemic such as the Northeast chemoprophylaxis using artemisinin-based combination therapy are strongly advocated. Studies have indicated that artemisinin derivatives reduce transmissibility of *P. falciparum* because of their gametocytocidal. The effect on gametocytes could further reduce the spread of resistant parasites (Targett *et al.*, 2001; Molta *et al.,* 2001; Oguche *et al.*, 2006).

2.10.1 Clinical Features and Pathology of Malaria

Malaria begins as influenza like illness with attacks of fever eight days or more after the bite of an infected mosquito. Cycles of fever, shaking chills, drenching sweats and headaches may develop. The frequency and severity of the fever depends on the malaria species involved but it usually lasts 2-3days. The attacks of fever coincide with waves of parasite multiplication and the destruction of red blood cells. Long lasting infections often result in enlargement of the liver and spleen (Rozendaal, 1997).

Malaria caused by *P. falciparum* does not always show this cyclic pattern. It is the most severe type of malaria and, if untreated, may progress to shock, kidney and liver failure, coma or death. Death is often due to parasitized red blood cells blocking the narrow blood vessels in organ of the body. If the blood vessels of the brain are affected, the condition is called cerebral malaria. Prompt treatment is needed to prevent damage to the brain or any other organ*. P. vivax*, *P. malariae* and *P. ovale* are

generally not life threatening but death may occur in very young children and old and sick people (Rozendaal, 1997). With *P. vivax* and *P. ovale* malaria the interval between attacks of fever is typically two days and for *P. malariae* it is three days. For *P. falciparum* malaria the intervals are irregular, usually about 36-40 hours but shorter intervals are also common. The duration of an untreated first attack may last from a week to a month or longer (Rozendaal, 1997). Attacks of illness after an interval of weeks or more so called relapses do not occur with *P. falciparum* but are common in *P. vivax* and *P. ovale* infections. Relapses may occur at irregular intervals for up to two years with *P. vivax* and for up to five years with *P. ovale*. Infections with *P. malariae* may persist for up to 50 years with periods of fever returning at intervals (Rozendaal, 1997).

2.10.2 Mechanism of Immunity in Malaria

Although protective immunity does develop against *P. falciparum,* such protection is not easily won (Phillips, 1994). In areas of endemicity, the pattern is for babies and infants up to the age of about five years to be at risk of dying from the disease but parasite prevalence, parasite density, and the number of clinical episodes does decline progressively with increasing age (Greenwood *et al.*, 1986, McGregor, 1964, 1986). Even in adulthood, however immunity is never complete (McGregor, 1964, 1986) and during the transmission season, it is not unusual for the occasional clinical episode to occur and for parasites to be detectable in the blood even when the patient is clinically well. It might be that after first infections in adulthood, an effective

immunity develops more quickly (Baird *et al.,* 1991; Baird *et al.,* 1995, Jones *et al.,* 1991). As a result of government policy of economic development in Indonesia, there has been massive movement of people from heavily populated areas, often free of malaria, to the outer islands, where malaria is endemic. Many of these transplanted peoples have set up agricultural schemes involving irrigation creating ideal breeding sites for mosquitoes. Consequently malaria exploded among the people for whom it was observed that among the adults there were no fatalities after two years of residence, suggesting that life saving immunity developed in 2 years in adults compared with 5 years in small children (Baird *et al.*, 1995, Jones *et al.*, 1991). Current thinking is that natural acquired immunity is normally specific for a species of malaria parasite. People who move out of an area of endemicity for an extended period appear to lose their immunity, suggesting that repeated exposure is necessary to maintain resistance (McGregor, 1986).

2.10.3 Acquired Immunity

A brief resume of protective immune mechanisms to the malaria parasite following infection and reinfection is as follows. There is considerable uncertainty about mechanisms in vivo in humans (Phillips, 2001).

Ironically, understanding of the immune mechanisms directed against the pre-erythrocytic stages has come from vaccination experiments, initially with animals and later with humans (Nussenzweig *et al.*, 1989), rather than from observations of natural or experimental infections. Very strong resistance to a challenge with viable sporozoites is induced with irradiated sporozoites of *P. berghei* (Nussenzweig *et al.,*

1967) and *P. yoelii* (Weiss *et al.*, 1988) in mice and of *P. falciparum* (Clyde, 1990) and *P. vivax* (Clyde, 1990) in humans. The irradiated sporozoites are able to invade hepatocytes but do not complete their development in the liver and it is thought that this period, albeit shortened, of metabolic activity is important for mobilizing protective immunity (Phillips, 2001). In these murine systems, the degree of immunogenicity of the irradiated sporozoites varied with the inbred strain of mouse and species of rodent malaria agent (Suhrbier, 1991). The protection mechanisms induced by irradiated sporozoites were investigated, and initially the focus was on the role of protective antibody. Serum from immunized mice passively protected mice against a sporozoite challenge, probably by blocking invasion of the hepatocytes by the sporozoites (Potocnjak *et al.,* 1980). Subsequently, it was found that mice lacking B cells but not T cells (Chen *et al.,* 1997) could be immunized with irradiated *P. berghei* sporozoites, which demonstrated that the antibody –independent (cellular) as well as anti body dependent immune mechanisms, are induced by the irradiated sporozoites. Much work has gone into investigating the nature of the antibody independent responses. It has been found that primarily CD8+T cells mediated the protection, by cytotoxic activity directed at the infected hepatocyte, and by secreting gamma interferon, which, in turn, induces nitric oxide dependent killing of the parasite within the hepatocyte (Mellouk *et al.,* 1991; Nardin *et al.,* 1979).

It is very uncertain to what extent the mechanisms of protection against the preerythrocytic stages induced by vaccination with irradiated sporozoites are induced by natural infection (Phillips, 2001). In areas of endemicity, an antibody to sporozoites

is detectable in residents from about 10 years of age (Nardin *et al.,* 1979), and it is reported that serum containing such antibodies can inhibit invasion of hepatoma cells by sporozoites (Nardin *et al.*, 1993). Similarly, using in vitro systems there is evidence of cytotoxic $CD8^+$ Tcells (Doolan *et al.*, 1997) in the peripheral blood of immune adults.

Immunity to the sexual erythrocytic stages following infection is multifactor. It is uncertain to what extent the wealth of information derived from animal models and in vitro assays can be applied to malaria in people (Phillips, 2001). Numerous experiments with animal models showed that passive protection with serum was possible. (Cohen *et al.,* 1961) and later Druile and colleagues (Bouharoun-Tayoun, 1995, Bouharoun-Tayoun, 1990) controlled a patent *P. falciparum* parasitaemia by passive transfer of immunoglobulin G from immune adults. The question is how is the protective antibody exerting its protective effect? In different host –parasite combinations and at different times after infection or reinfection, the mechanism might be different. Thus, roles reported include blocking invasion of red cells by merozoites, opsonization of merozoites and infected erythrocytes promoting their uptake by phagocytic cells, antibody dependent, cellular cytotoxicity, agglutination of merozoites, preventing sequestration of *P. falciparum,* and neutralizing malaria toxins (Long, 1993; Phillips, 1994). As some of these proposals are based solely on in vitro observations, it cannot be assumed that they have a similar role in vivo (Phillips, 2001).

Cellular antibody independent mechanisms of immunity to the sexual erythrocytic parasites are also reported elsewhere. Cytokines such as tumor necrosis factor alpha and most importantly gamma interferon play a major role (Phillips 2001). In a rodent model, there is a switch from antibody independent immune mechanisms during the acute blood parasitaemia to antibody dependent mechanisms as the infection becomes chronic, but it is not clear if this progression occurs in human infections. Exactly how the parasite is affected by the immune response, either within the red cell or as the merozoite passes from one red cell to another, again is not certain (Phillips, 2001).

Finally, protective immunity to the sexual stages is thought to be induced by infection; again, there is evidence from different host parasite combinations that both antibody dependent and antibody independent mechanisms can play a role (Hoffman, 1996). These anti sexual stage protective factors come into effect in the blood meal in the mosquito midgut and may prevent fertilization from occurring (Phillips, 2001).

2.11 INSECT GROWTH REGULATORS

The newest approach to insect pest control is the use of substances that adversely affect insect growth and development. These substances are classified as Insect Growth Regulators (IGRs) or hormone mimics owing to their effects on certain physiological regulatory processes essential to the normal development of insects or their progeny. They are quite selective in their mode of action and potentially act only on target species (Tunaz *et al.*, 2004). Insect growth regulators mimic the actions of

juvenile hormone or moulting hormone. Methoprene and kinoprene act by suppressing the moulting hormone so that developing larvae produce lethal deformities or by interfering with the formation of the insect's cuticle through the inhibition of chitin synthesis. Moulting hormone mimics are collectively known as precocenes. These compounds accelerate the metamorphosis of immature insects to yield precocious adults with significant and often lethal deformities including sterility (Lale, 1996). IGRs and bacterial insecticides are strictly arthropod specific and environmentally safe. Although, their use in malaria has been rather limited, it is important to note that when IGRs are used, only larvae in the late stages are affected, positive larval collection will not be a sign of treatment failure, and successful impact can be demonstrated by the absence of adult emergence in floating cages in treated breeding sites (Najera and Zaim, 2002). An IGR therefore, does not necessarily have to be toxic to its target, but may lead instead to various abnormalities that impair insect survival (Siddall, 1976). Interestingly however, most of the IGRs that have shown effectiveness against insect pest cause the rapid death of the insect through failure of a key regulatory process to operate or function (Tunaz *et al.,* 2004). Insect Growth Regulators (IGRs) affect the hormonal control of mosquito growth and development. The main effect of IGRs is the inhibition of adult emergence, but reproduction and ecdysteriod production in surviving females are also affected (Fournet *et al.*, 1993, 1995). In general, IGRs have high levels of activity and efficacy against various species of mosquitoes in various habitats (Mulla *et al.*, 1989). Additionally, it has been known that they have shown a good margin of safety to non-target biota including fish

and birds. Residue and non target studies indicated that IGRs have no prolonged residues and are an environmentally safe compound with minimal impact on non-target organisms (Miura and Takahashi 1973, 1975; Mulla *et al.,* 1986). On the basis of these attributes, IGRs are suitable candidate larvicides for mosquito control due to their greater margin of safety for non-target biota and operational properties (Dong-Kyu, 2001). IGRs generally have a good margin of safety for most non target biota including invertebrates, fish, birds and other wild life. They are relatively safe for human beings and domestic animals (Tunaz *et al.,* 2004). IGR compounds such as pyriproxyfen, methoprene, diflubenzuron and triflumuron have been recommended by WHO and have already passed WHOPES for use against mosquito immatures (Batra *et al*., 2005). Insect Growth Regulators such as pyriproxyfen and triflumuron have reported to be specific to insects and safe to humans even when used in close contact (Blegvad, 2000).

The World Health Organization has recommended three (3) Insect Growth Regulators (IGRs) for control of mosquito larvae namely Pyriproxyfen, Diflubenzuron and Methoprene. Pyriproxyfen is one of the most potent of IGRs. Standard Evaluation has shown pyriproxyfen as the potent of the three 2.23 and 21.5 times more toxic than diflubenzuron and methoprene respectively. Insect growth regulators (IGR) comprise of:

2.11.1 Juvenile Hormone Analogues

The discovery of juvenile hormone (JH) as a potential insecticide (Williams, 1956) has contributed to the synthesis of several JH analogues (JHAs). Most of the

conventional insecticides are neurotoxins, but JHAs are characterized for their effect on metamorphosis. For this special mode of action, JHAs have been considered to be advantageous in the selective lethal activity on insects and potential safety for vertebrates due to a lack of a specific target site (Miyamoto *et al.*, 1993). Pyriproxyfen, a newly developed JH analogue, has provided the outdoor stability (Dhadialla *et al.,* 1998). It prevents the development of larvae into pupae or pupae into adult.

2.11.2 Chitin Synthesis Inhibitors

Examples include diflubenzuron and trimufluron. These interfere with larval moulting process killing the larvae when they moult. So, they act faster than juvenile hormones. The mode of application, dosage, and residual efficacy depend on the type of breeding site. It is however important to point out that although they are safe to humans when used in normal concentrations in water could disturb development of a number of aquatic arthropods such as crabs, shrimps, and non target arthropods. Thus it is advisable not to use IGR where such organisms are likely found (Njunwa, 2000).

2.11.3 Pyriproxyfen

Code No: S-31183, USDA A13-29835

Chemical Name: Pyriproxyfen (2-[1 – methyl – 2 – (4 – phen – oxyphenoxy) ethoxy] pyridine

Empirical formula: ($C_{20}H_{29}NO_3$)**.**

Molecular weight: 321.37

Appearance: granules

Colour: pale yellow

Vapor pressure: 2x210.6 mm Hg at 20° C

Pyriproxyfen, 2-(1-methyl-2-(4-phenoxy-phenoxy) ethoxy) pyridine, is also known by the code number S-31183 and by the trademark names Nylar and Sumilarv. It is an innovative insect growth regulator with an excellent safety profile. Recently, the joint FAO/WHO Meeting on Pesticide Residues has considered that, because of extremely low toxicity of pyriproxyfen to mammals, pyriproxyfen can be safely added to drinking water at a rate of 0.01mgAI/litre, for mosquito control (FAO 2001). It does not have the killing effect so it is very safe to non-target organisms such as fishes,birds and human.The World Health Organisation has described it as non carcinogenic,non genotoxic and unlikely to cause any hazard in normal use (WHO, 2007). It is not similar to the natural juvenile hormone in chemical structure. It is a juvenile hormone mimic (JHM) and a relatively stable aromatic compound (Kanda *et al.,* 1990). Though not a conventional insecticide, it functions as an insecticide by overloading the hormonal system of the target insect, ultimately affecting egg production, brood care and other social interactions and inhibiting growth (Glancey *et al.,* 1990).

Pyriproxyfen is reported to have 95% inhibition of emergence for mosquito larvae and its effects on mosquito larvae lasted for two months after application

(Miyamoto *et al.*, 1993). In highly polluted water, however, pyriproxyfen readily adsorbed on to organic matter and its biological activity persisted for two months after an initial application rate of 0.11b/acre. Its persistence in water in the absence of organic matter declined as temperature and sunlight exposure increased (Schaefer *et al.*, 1988).

The World Health Organization has recommended the use of pyriproxyfen for the control of some mosquito species at specified rates in specified habitats. Pyriproxyfen granules may be of benefit to control mosquitoes in floodwater situations because the concentration of these materials has been predicted to be higher in the bottom water than the upper water for along period of time (Kamimura and Arakawa 1991). Granules of pyriproxyfen sink to the bottom and the effective component slowly released; thus, the effective component remained in the substratum for a substantial time, being slowly released in the water. Pyriproxyfen was reported to be adsorbed onto organic matter in an animal waste water lagoon and it was effective on mosquito aquatic stages over a 2-month period (Schaefer *et al.*, 1988; Mulligan and Schaefer, 1990; Schaefer and Miura, 1990).. Kamimura and Arakawa (1991) reported that pyriproxyfen was extremely effective against larvae of *Cx. pipiens pallens* and *Cx. tritaeniorhynchus* which showed high resistance to organophosphorus insecticides. Complete inhibition of adult emergence continued for 3 week or more in open containers and irrigation ditches at a concentration of 0.01ppm,in cesspools at 0.05ppm and in sewers with inflow of house water at 0.1ppm.(AI) (WHO, 2003).

Drainage and sanitation facilities have become inadequate as a result of rapidly

expanding urban cities in Nigeria. Hence, *Culex quinquefasciatus* (The most common nuisance mosquito and sometimes a vector of filariasis) have found suitable breeding sites in water polluted with organic material, as could be found in soakaway pits, septic tanks, drains blocked with sewage and refuse. Also *An. gambiae* sensu stricto (Diptera:Culicidae) the major African malaria vector known to breed in clean and clear water is now reported to be adapting to a wide range of water pollution in urban settings in Nigeria. This could have serious implications on the epidemiology of urban malaria (Rozendaal, 1997; Awolola *et al.,* 2007). To further compound this problem, selection for insecticide resistance in *Anopheles gambiae s.l* in the presence of pollutants like spilled petroleum products in Nigeria has now been demonstrated by Djouaka *et al. (*2007).

In urban areas, larval control is often more cost effective than control of adult mosquitoes, because breeding places are limited, easily recognizable, hence larviciding is significantly used for control of nuisance mosquitoes in urban environments (Rozendaal, 1997; Invest and Lucas, 2008). The newest approach to insect pest control is the use of substances that fufil the criteria for an effective insecticide, these are: low mammalian toxicity, highly specific, outdoor stability, low impact on the environment, broad spectrum of activity against all target species of mosquitoes and a long duration of effect which would reduce the frequency of application.When compared to conventional larvicides, the insect growth regulators are known to be safe for the environment and selective in action (Tunaz, 2004; Sumitomo 2007; Jambulingam *et al.,* 2008; Invest and Lucas, 2008).

The World Health Organization has recommended the use of pyriproxyfen, 2-(1-methyl-2-(4-phenoxy-phenoxy-ethoxy) pyridine, known by the trade name Nylar and Sumilarv for the control of all types of mosquito larvae. Though generally classified as a Juvenile Hormone Mimic (JHM), little is known about its exact mode of action and the molecule bears little resemblance to endogenous insect Juvenile Hormone analog (Hatakoshi *et al.*, 1986; Zufaleto *et al.,* 2000). However, it is known to affect morphogenesis during the larval-pupal transformation resulting in pupal death and emergence failure of adult mosquitoes. Embryogenesis and reproduction of insects are also affected. Adults mosquitoes allowed to contact pyriproxyfen treated surfaces can subsequently transfer it to oviposition sites where it can have a subsequent impact on pupal emergence, causing inhibition of emergence too (Itoh *et al.,* 1994; Sihuincha *et al.*, 2005). The effects of pyripoxyfen go beyond the aquatic stages as the ability of any surviving female to reproduce can also be affected (Invest and Lucas, 2008). The favourable safety profile of Pyriproxyfen permits it to be applied even to drinking water for the purpose of control of mosquito larvae at the rate of 0.01 mg/l as recommended jointly by the joint FAO/WHO meeting on pesticide residues after passing all stages of WHO pesticide evaluation scheme (FAO, 2000; WHO 2007). Its chemical persistence appears to be compatible with the environment with no dangers of long term bioaccumulation in the environment (Schaefer *et al.*, 1988). Pyriproxyfen inhibits the normal development of mosquitoes but does not cause rapid killing of larvae. Therefore its effect cannot be tested by observing larvae density. The number of adult which emerge is the only criterion for measurement of

its impact (Mulla *et al.,* 1974; kawada *et al.,* 1988). It has been tested for efficacy in many areas and in various habitats (Mulla *et al.,* 1986; Mulligan and Schaefer 1990; Ansari *et al*., 1991; Chavasse *et al.,* 1995; Yapabandara *et al*., 2001; Yapabandara and Curtis 2002, 2004; Vythiligam *et al*., 2005 and Jambulingam *et al.,* 2008).

In heavily polluted habitats, pyriproxyfen at 0.01-0.05mg/L has been observed to produce 100 percent Emergence inhibition of adult mosquitoes for 1-6 weeks in a variety of habitats (MRC, 2006). Pyriproxyfen has been found to be very effective in very dirty places like sewages drains, stagnant drains and cesspits with >90 percent inhibition. In field studies in Sri Lanka only 2 applications per year of pyriproxyfen was required compared to 12 applications per year of temephos or oil (Yapabandara and Curtis, 2002). Now that insecticide resistance has become a major concern in vector control, with resistance present in mosquitoes to all major classes of chemical insecticides. This can lead to a rise in disease transmission, Hence there is an urgent need for insecticides with a novel mode of action and no recorded field resistance. To our knowledge, there is no known field resistance to pyriproxyfen (Invest and Lucas, 2008). An organophosphate resistant strain of *Cx. quinquefasciatus* pressurized for 17 generations showed no increased tolerance to pyriproxyfen (Schaefer and Mulligan, 1991). Pyriproxyfen was effective against two mosquito strains, found to be resistant to other pesticides. One was found to be resistant to organophosphates and the other to both pyrethroids and methoprene. Pyriproxyfen showed potent activity against both strains thus demonstrating a different mode of action and lack of cross resistance, even to another insect growth regulator (Yoshiaki Kono *et al*., 1997).

CHAPTER THREE
MATERIALS AND METHODS

3.1 STUDY SITE

The research was carried out in Borno State; one of the five states belonging to the former northeastern state which now has Maiduguri as the state capital (Figure 3). The two study sites were (a) Maiduguri town and (b) Damboa, 85 kilometres from Maiduguri. The two places are 85 kilometres apart. The choice of the two zones is based on epidemiological and practical considerations such as record of malaria high endemicity and relatively high human population (Molta *et al.,* 2004).

3.1.1 Maiduguri Study Site

The study lasting for a period of one year centred at the Emergency Paediatric Unit (EPU) of the University of Maiduguri Teaching Hospital (UMTH). The UMTH serves as a referral centre catering for the needs of the entire northeast region. Maiduguri is located on latitude $11^0$40' N and longitude $13^0$05' E, with mean annual rainfall of 650mm.Maiduguri is considered as the transition zone of the Sudan Sahel Savanna (Sara, 1990). The town is inhabited by about 877, 925 people (1991 census figure). The prevalence of *Plasmodium* infection here has been put at 35.2% (95/270). The *Plasmodium falciparum* is the most common cause of malaria and accounting for about 95-98% of infection, while the other species *P. malariae* and *P. ovale* account for 2-5% of the infection (Molta *et al.*, 1993) in Maiduguri.

3.1.2 Damboa Study Site

Damboa is located at the southern edge of the Sahel within the Sudan savannah (Fig 3). 85 kilometres away from Maiduguri, Borno state along Maiduguri-Biu road highway it lies along the bank of one of the seasonal rivers. It has a marked rainy season that spans the period June/July to September/October, and dry season between October/November and May/June. The climate is semi arid, with malaria transmission of the mesoendemic type (Watila *et al.,* 2006). It is essentially a subsistent agricultural community, although considerable animal husbandry and trading are also undertaken. Several man-made ponds created by construction work and a poor drainage system provide favourable breeding sites for mosquitoes that transmit malaria among other diseases.

Damboa, the headquarters of Damboa Local Government Area has a population of 24, 421 (9.9% of the LGA's Population of 247,904 (Molta *et al.,* 2000) Record also indicated high incidence of malaria in this area. It is endemic for *P. falciparum* infection. (Molta *et al.*, 1992, Molta *et al.*, 2004. BOMOH 1986 - 1988). The prevalence of *Plasmodium* infection has been put at 70% (433/617) (Molta *et al.*, 1993). Damboa is a sentinel site of the National Malaria surveillance network.

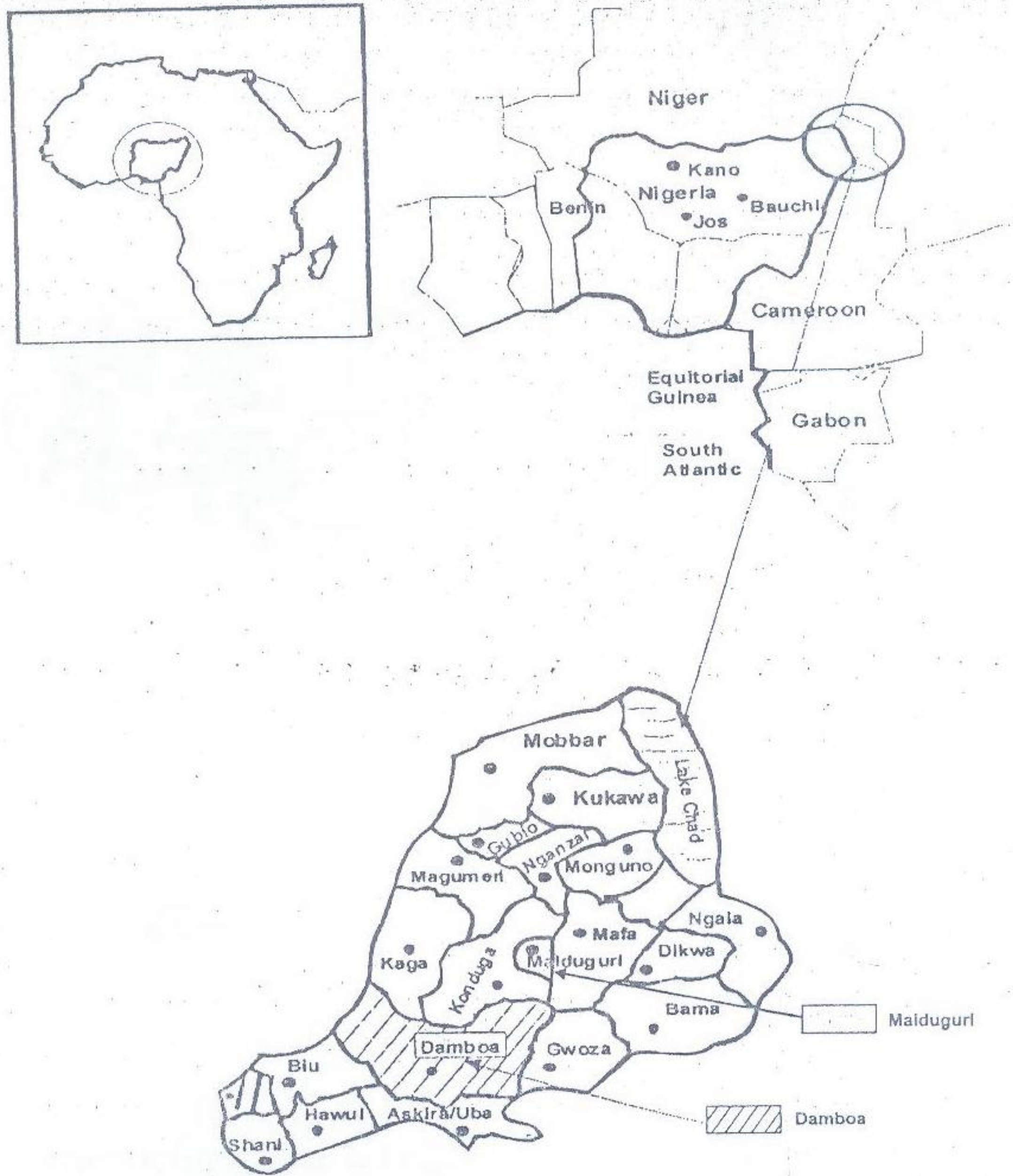


Fig 3: Modified Sketch Map of Borno State Showing the Study
(Source: Borno State Ministry of Lands and Survey 1996)

3.1.3 Climate and Vegetation of Borno State

The climate of Borno state is for the most part semi arid with considerable long period of hot season. The hottest months in the year in most places are in March, April, May and June. For example, the average maximum daily temperature over a period between 1975 and 1980 was 40.75^{o}C for April, 40.06^{o}C for May and 40.02^{o}C for June in Maiduguri. On the other hand the coolest months are in December and January. The mean minimum daily temperature for January was 13.5 ^{O}C for a long period between 1975 and 1980 in Maiduguri (NPC, 1993; Sibina, 1984). Rainfall in Maiduguri is characterised by storms of high intensity and distinct variability from year to year. The wet season starts in April in the Southern parts of the state and lasts for about one hundred and sixty days (although not continous). In the northern parts of the state the rainy seasons starts in May or June and lasts for only ninety days. The mean average annual rainfall in the southern part is 1,000 mm with the highest in July and August, while it is less than 500 milimetres in the northernly part of the state and with a peak in July. From November to March the dry, dusty, north eastern harmattan winds prevail (NPC, 1993). Borno state is characterised by frequent destruction occasioned by terrible windstorms. During the beginning of the rains (May-July), mean wind speed is as high as between 186.77km-200km/hour.

Vegetation

Borno State lies within three belts: the Sahel, the Sudan and the Northern Guinea vegetational zones. The latter occurs in the southern part of the state and

has tall grass woodland savanna vegetation. The Sahel and Sudan vegetational belts are found in the northern parts of the state and are characterised by shrub, thornbush and short grass savanna woodland. These zones have been designated high risk areas for becoming desert. The meteorological data collected by Thiambipillay, Department of Geography, University of Maiduguri-Nigeria, showing the rainfall and the daily maximum an minimum temperature and relative humidity; typical of the entire study area, over a complete year allow the three seasons to be clearly identified (Uwaezuoke, 1984) a short rainy season lasting from June/July to September/October and a long dry season from October/November to May/June. The dry season has 2 parts to it; a cold harmattan period from November/December – January and hot period, February/March – April/May (Molta *et al.,* 1995). Common *Anopheles* species during the rainy season include *An. gambiae* sl and *An. squamosis* (Gadzama, 1983). The *Plasmodium falciparum* is the most common cause of malaria and accounting for about 95-98% of infection, while the other species *P. malariae* and *P. ovale* account for 2-5% of the infection (Molta *et al.*, 1993).

3.1.4 Demography

Borno state has a variety of ethnic groups. The official language is English though kanuri, Babur, Bura, Marghi, Shuwa Ngizim and a sprinkling of Fulani.Hausa is widely spoken throughout the state as a secondary language (NPC, 1993). The town is inhibited by about 877,925 people (projected from 1991 census figure of 629, 486 people.

The majority of the people in Borno State are subsistence farmers, herdsmen or fishermen. They are predominantly Muslim, though there are a few Christians and traditionalists.

3.2 ENTOMOLOGICAL INVESTIGATION

3.2.1 Mosquito Collection, Preservation and Processing

Outdoor and Indoor resting female mosquitoes were collected from human habitations following the methods of Molez *et al.* (1996) in a longitudinal one year study cycle. Indoor mosquito collection was done using spray sheet collection method with pyrethrum spray catches. A method considered most successful in capturing anthropophagic (human biting) and endophilic mosquitoes (Abdoon and Alshahrani, 2003). It is also considered far less ethically objectionable in areas of multidrug resistance (Curtis, 2003).While the outdoor mosquito collection was conducted using modified light traps (Fig 4). This was conducted in 6(six) areas of Damboa using mechanical aspirator with the aid of flashlight (Molta *et al.*, 1999; WHO 2003a). Each mosquito sample was preserved individually in an Eppendorf tube each. The samples are stored dry on silica gel for species-specific molecular assays, PCR and consequent species identification. They were later transferred to the Molecular Entomology and Parasitology Laboratory at the Nigerian Institute of Medical Research, Lagos and unit of Parasitology and Entomology, Department of Zoology, University of Jos for further analysis. Identification of mosquito was carried out by morphological and PCR methods. Adult female mosquitoes were identified as far as possible accordingly using the morphological keys of Gillies and

De Meillon (1968) and Gillies and Coetzee (1987) and stored dry on silica gel for PCR species identification of the *Anopheles gambiae* complex. Molecular assays used were the species specific PCR techniques for confirmatory identification of the species within *An. gambiae* complex. The involvement of each species in malaria transmission was assessed using ELISA tests for *Plasmodium falciparum* sporozoite infection.

3.2.2 Morphological Identification of Mosquito Samples

All mosquitoes collected were identified and sorted out under a stereomicroscope (Leica model NSW series IMNS 210) and Olympus Tokyo VT-II 225329 Entomological microscope. The female *Anopheles* mosquitoes were identified as far as possible using morphological keys of Gillies and De Meillion (1968), Gillies and Coetzee (1987) by sex and whether they were anophelines or culicines. The following key features were considered for the identification of anophelines:

Palps: Smooth with three pale rings including a wide ring at the tip occasionally divided into two .In adult males ,the palps are as long as the proboscis and swollen at both ends while in adult females the palps are as long as proboscis(females have non plumose antennae) (Fig 5 and 6).

Legs: Irregularly speckled (speckling in some specimens is very conspicuous; in others, only just visible).

Tibiae: Narrow pale ring at the tip.

Tarsi: Segments 1-4 pale at tips; segments 2-4 also pale at the base (Fig 5).

Wings: Pale areas very variable in extent (sometimes greatly reduced). Pale scales creamy yellow;pale patches on costa and vein 1 usually long: two pale interruptions at the base of costa;pale spot in 3rd dark area of vein 1; vein 3 pale except at each end;main branch of vein 5 pale except at each end;fringe spots present at tips of veins 2-6 (Fig 5, 6 and 7).

Scutella: Are single lobed

Body size: *Anopheles* is one of the three mosquito genera easily identified from other genera *Anopheles* mosquitoes vary in size ranging from small size such as *An. nili, An. moucheti* and *An. funestus*; through medium size such as *An. squamosus, An. pharoensis, An. maculipalpis, An. gambiae* complex, *An. wellcomei* and *An. rhodesiensis*; to very large size such as *An. implexus* (Gillet, 1972) (Plate 1 and 2).

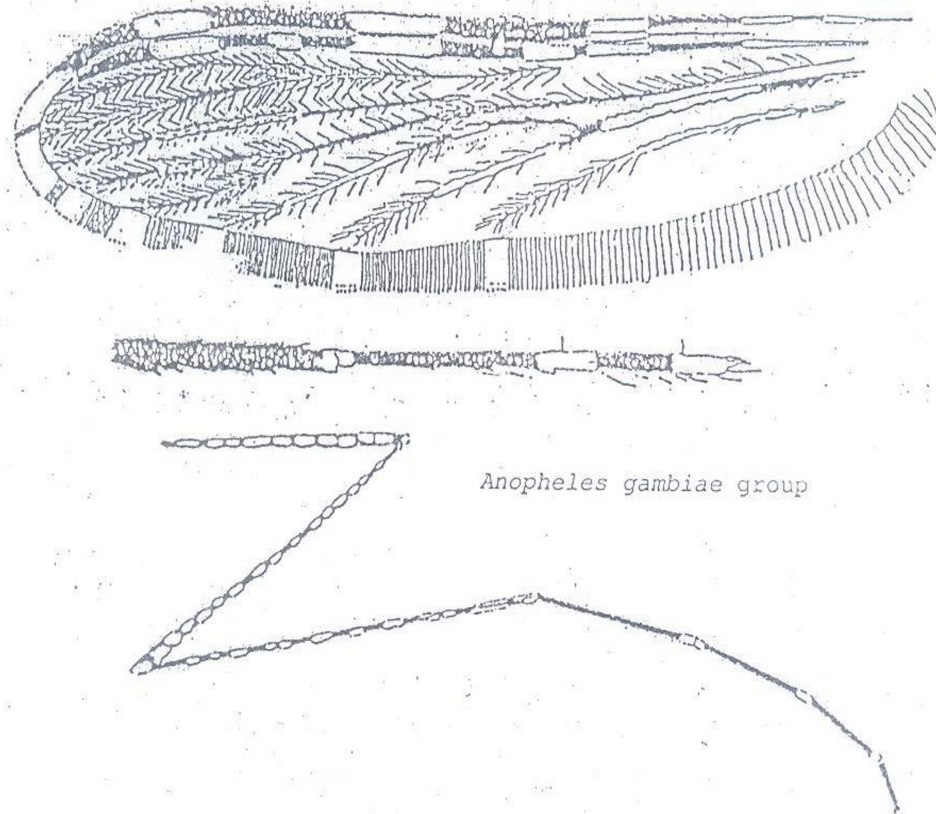


Figure 4: Wings, Palps and Legs of *Anopheles gaubiae* Mosquitoes for Morphological Identification. (Source: Gillies and Coetzee, 1987)

Plate 1: *An. gambiae*. Abdominal pigment (Source Benedict, 2007)

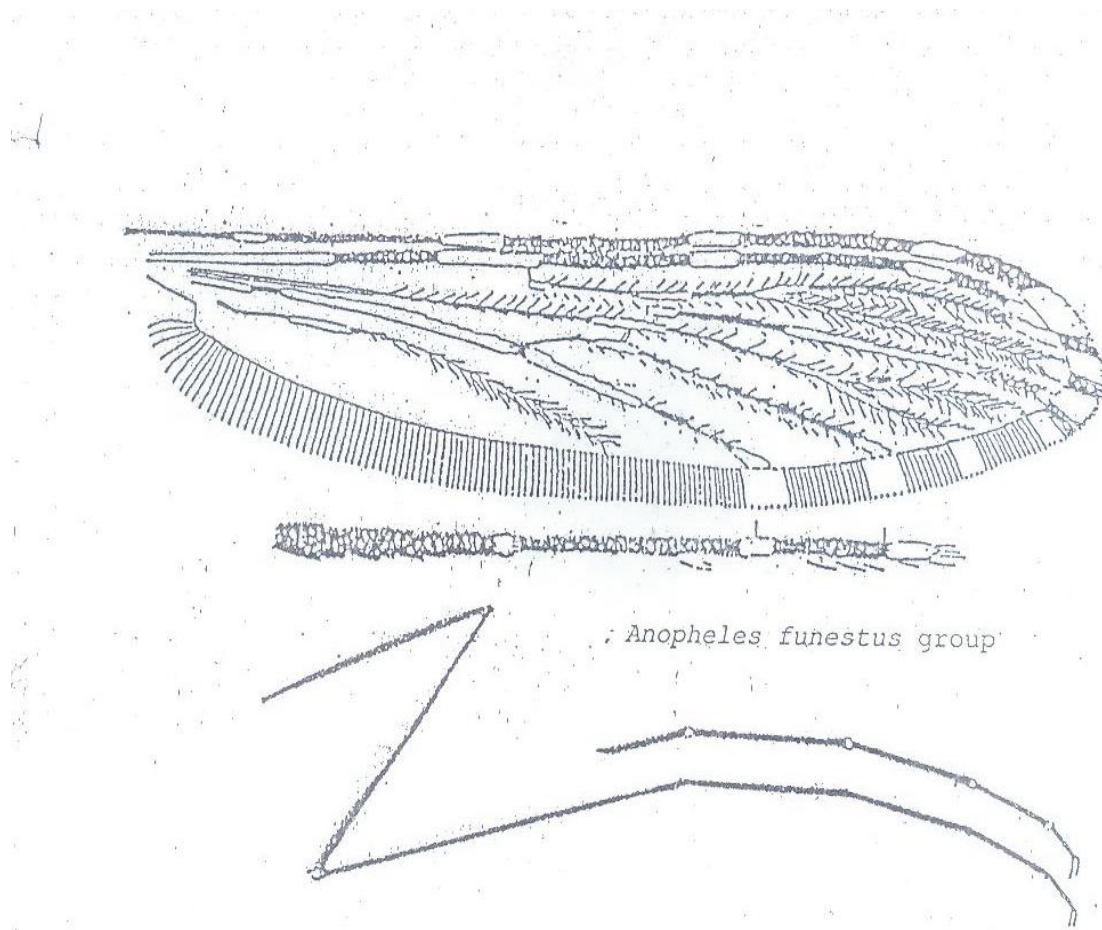


Figure 5: Wings, Palps and Legs of *Anopheles funestus* Mosquitoes for Morphological Identificaiton (Sources: Gillies and Coetzee, 1987)

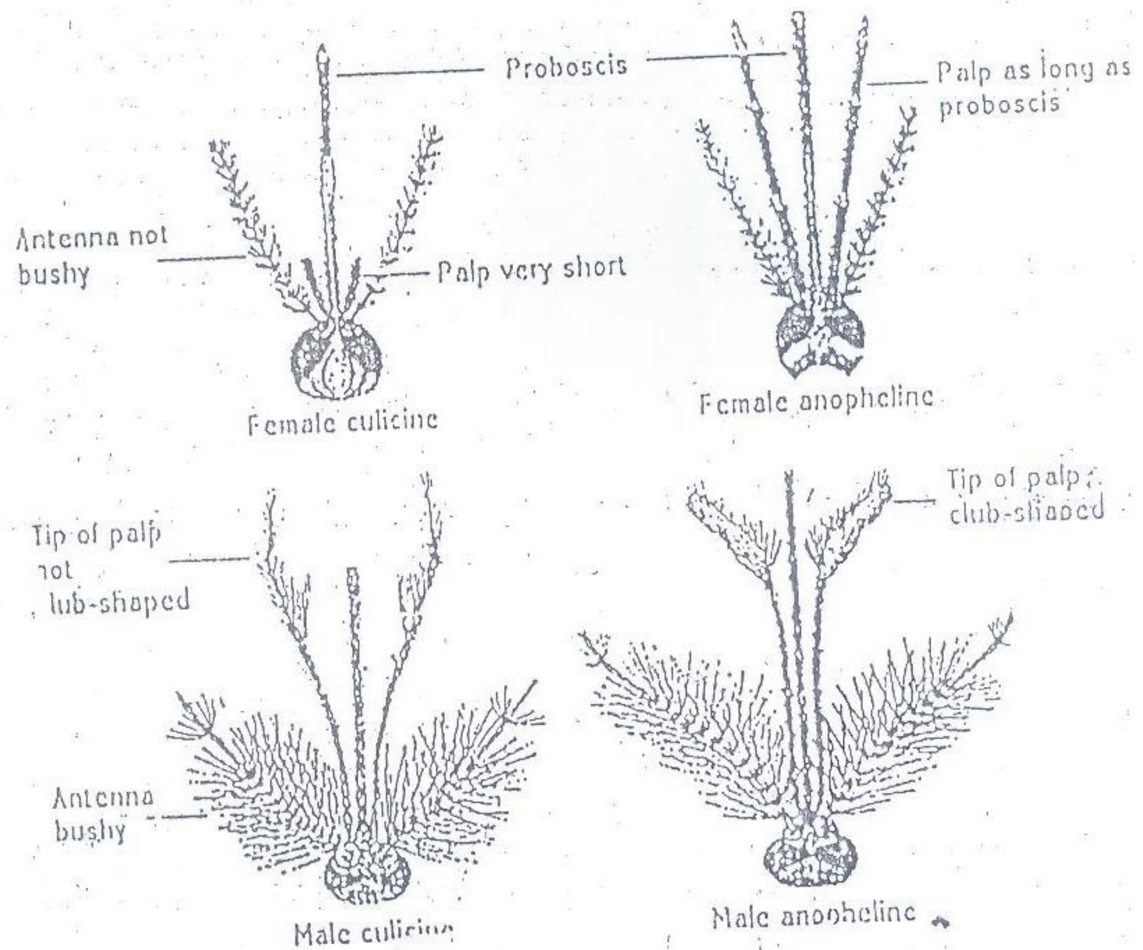


Figure 6: Heads of male and female Anophiline and Culicine Mosquitoes for Morphological Identification. (Source: WHO, 2003)

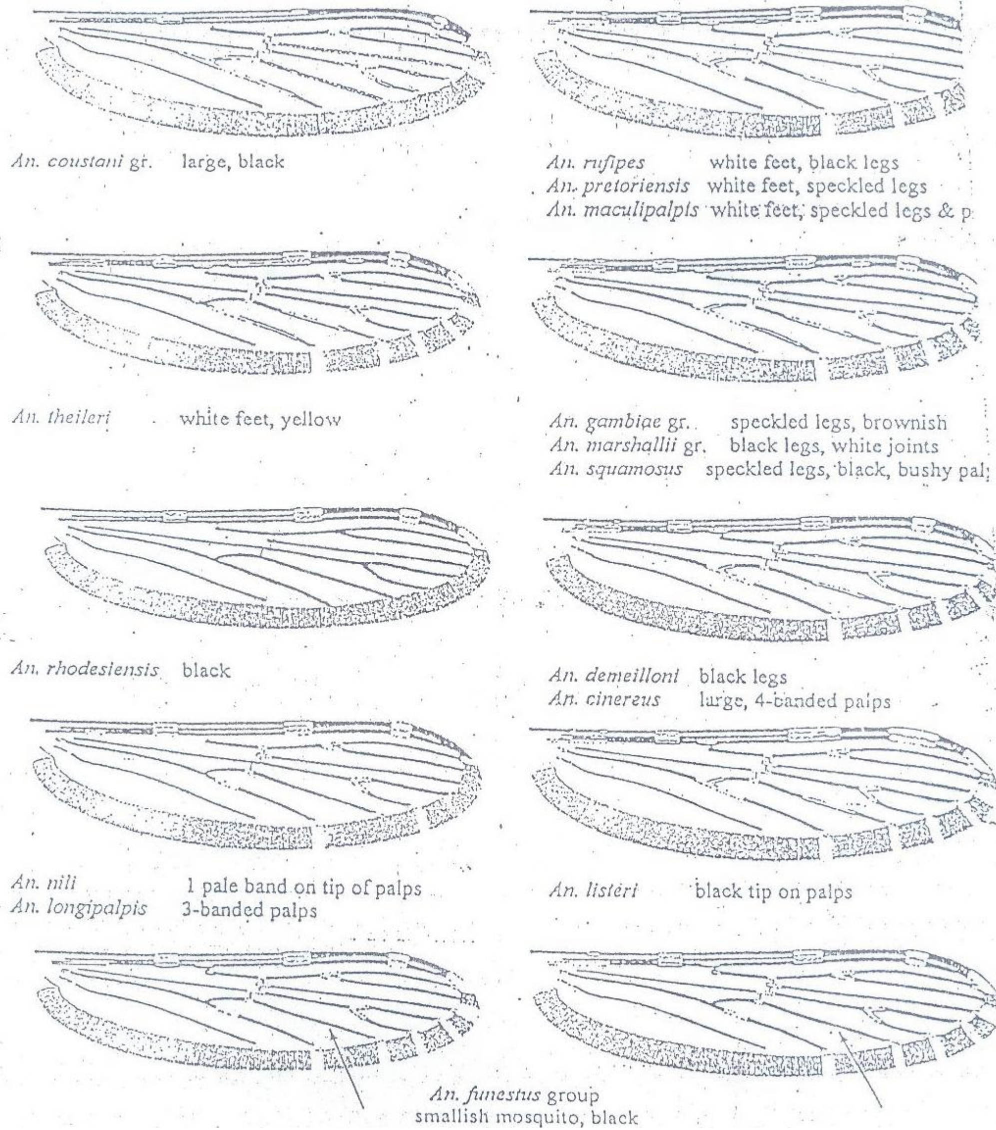


Figure 7: Wings of Prominent Anophiline Mosquitoes for Morphological Identification. (Source Gillies and Coetzee, 1987)

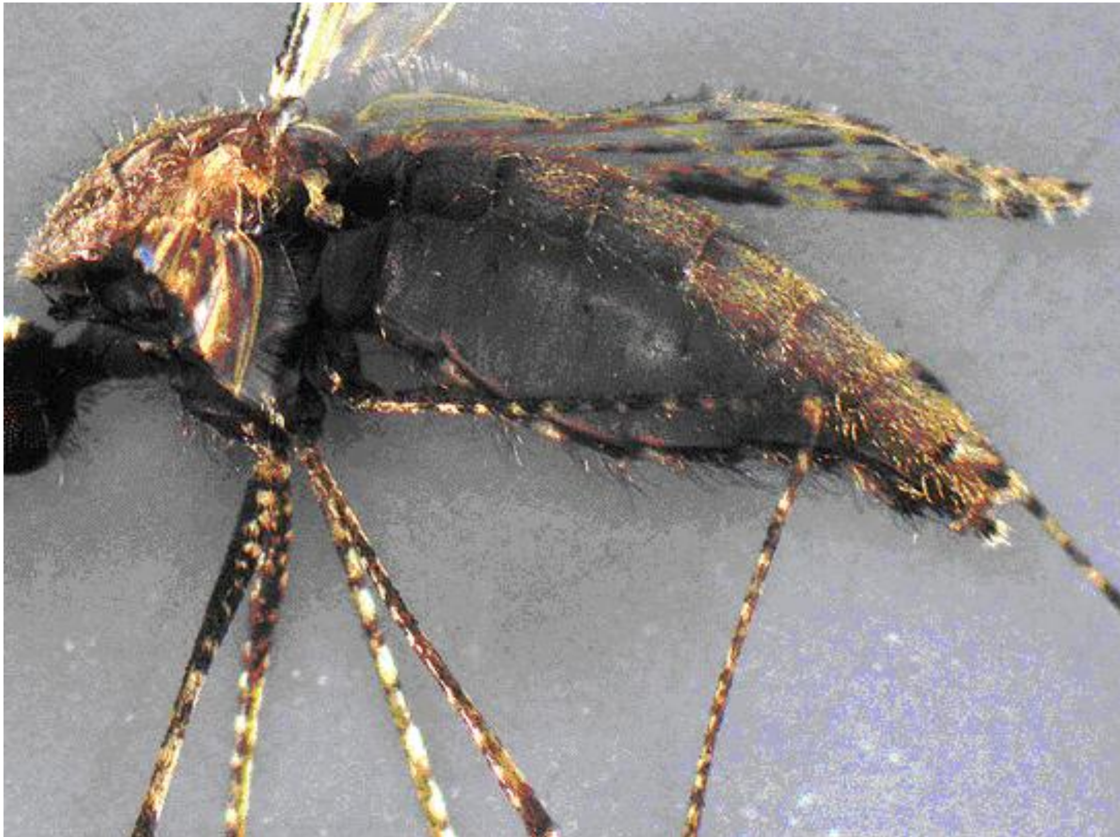


Plate 2: *Anopheles* Mosquito (Source: MR4, 2007)

3.2.3 Dissection of Mosquitoes

All female anophelines collected were dissected transversely at the thorax between the 1st and 3rd pairs of legs using a dissecting microscope. This severed the mosquitoes into two parts for ELISA test. The abdomen of bloodfed anophelines were set aside for blood meal analysis while the head and thorax were used for sporozoite detection. The wings and legs were used for DNA extraction.

3.2.4 PCR Identification of the Members of the *Anopheles gambiae* Sibling Species

Based on the following procedures, two hundred and thirty-three mosquitoes of the *Anopheles gambiae* s.l were selected at random from a pool of equal representation from the 6 six different collection sites with each of the 6 wards having an equal chance of being selected (WHO, 1992) and collection methods and morphologically identified. The PCR was performed with universal and species specific primers for the *An.gambiae* complex .Molecular identification of *An.gambiae* species complex is based on the species specific nucleotide sequences in the ribosomal DNA intergenic spacers (IGS) following the procedure of Scott *et al.* (1993). The amplified DNA was separated on a 2.0% agarose gel stained with ethidium bromide and viewed on a UV transilluminator. Sets of the individual *An. gambiae* s.l selected randomly for polymerase chain reaction (PCR) representing 6 six locations of within Damboa.

Table 2: Polymerase Chain Reaction Protocol

Step 1: Remove chemical from – 20^{0}C freezer and defrost completely.

Step 2: Make Master Mix: (Multiply for number of specimen including 4 standard

Mastermix constituents	X1	X10	X20	X25	X40	X50
10x Reaction buffer	1.25ul	12.5ul	25ul	31.25	50ul	62.5ul
10x dNTPs	1.25ul	12.5ul	25ul	31.25	50ul	62.5ul
Mg C12 solution	0.5ul	5.0ul	10ul	12.5	20ul	25ul
Quad Primer	0.5ul	5.0ul	20ul	25	40ul	50ul
Dei onized water	4.9ul	49.oul	98ul	122.5	196ul	245ul
Rtaq	0.1ul	1.0ul	2ul	2.5	4ul	5ul

Step 3: One leg per specimen was added and placed in Eppie tube

- forceps were wiped between specimens

This was ground in 50µl of deionised water and rinsed with 20ul of same deionised water

This was incubated at 94°C for 10 minutes.

Step 4: The specimen was **c**entrifuged for mins @ 13 k.rpm. to obtain DNA in the supernatant.

Step 5: 12.5µl of mastermix was dispensed into the 0.5ml eppie tubes going into the PCR machine.

Step 6: 0.5 ul of the extracted DNA of each sample was Pipetted into the 0.5ml eppie tubes containing the 12.5ml mastermix

Step 7: The Eppendorf tubes were placed in PCR machine. The PCR machine was programmed for *An.gambiae* complex. The program consisted of one cycle of initial denaturation at 94 o C for 5 minutes, 30 cycles of denaturation at 95°c for 30 seconds, annealing at 50°C for 30 seconds, extension at 72 oC for 30 seconds and final extension at 72 Oc for 7 minutes.

Agarose gel

A 2.5% gel was used for 100 ml: 2.5g agarose (on shelf) and 100ml was added 1 x TAE Buffer.

This was mixed and boiled in microwave until solution is clear.

It was mixed between 1-minute heating. It was ensured that it does not boil over.

This was cooled for 5 minutes. The skin on top was removed and 11µl Ethidium bromide (biohazard) was added and mixed.

Gel was poured into a trough.

Electropheresis: 10µl solution was placed and mixed with small amount of ficol dye in gel. This was run at 100V and not more than 120-150mA.

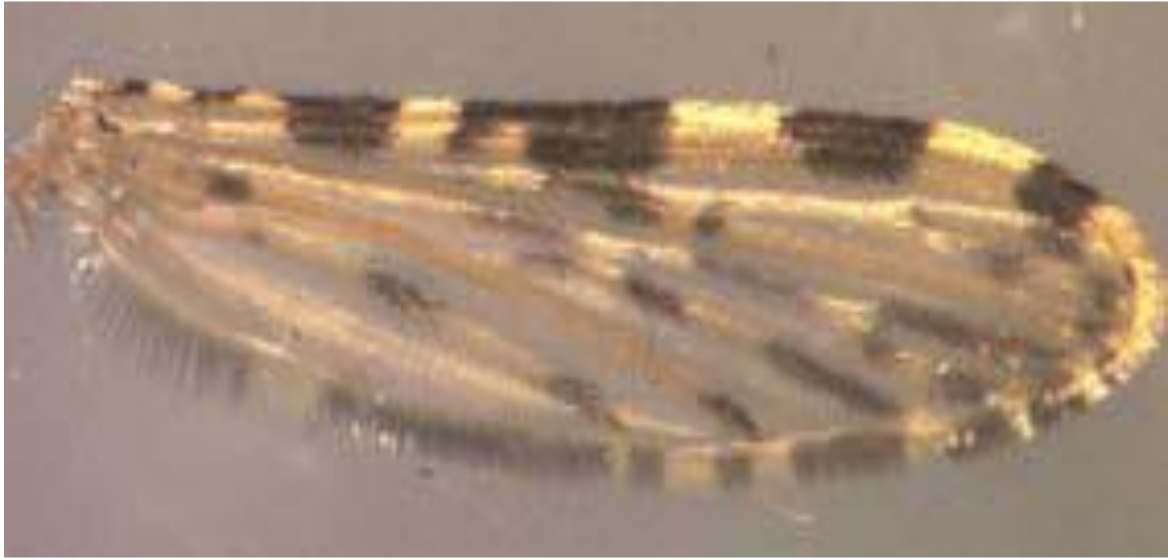


Plate 3: *Anopheles* mosquito Wing and leg (Protarsi) for PCR (Source: MR4, 2007)

Plate 4: *Anopheles* mosquito leg (Protarsi) for PCR (Source: MR4, 2007)

Plate 5: *Anopheles* mosquito head used for *P.falciparum* circumsporozoite Protein determination ((Source: MR4, 2007).

3.2.5 ELISA Detection of *Plasmodium falciparum* Circumsporozoite Protein Determination of Infection Rates in Mosquitoes

The involvement of each species in malaria transmission was assessed using ELISA tests for *Plasmodium falciparum* sporozoite infection. ELISA tests for *Plasmodium falciparum* sporozoite infections were carried out on 289 identified. *An. gambiae* s.l mosquitoes from which samples for PCR were drawn to quantify and give a precise identification of sporozoite species present (i.e. carrying the parasite's circumsporozoite antigen). The head and thorax of each of the mosquito was crushed in PBS (PH7.4) and tested for circumsporozoite antigen using a double antibody ELISA (Burkot *et al.,* 1984). Results were first scored visually and there photometrically at 405 nm using an ELISA plate reader (Anthos 2010, Anthos Labtec GmbH, 5071 Wals/Salzburg, Austria) (Plate 4) 30 min after substrate had been added (Beier *et al.*, 1990).

Plate 6: Blood fed *Anopheles* Mosquito used for Blood meal Analysis (Source: MR4, 2007)

3.2.6 Identification of Blood Meal Source by Direct ELISA

Anophelines were first grouped based on their abdominal conditions before Identification of blood meal sources using direct ELISA. They were generally grouped as unfed, freshly fed, half gravid and gravid (WHO, 2003).

i. **Unfed**-The abdomen is flattened

ii. **Freshly fed**- The abdomen appears bright or dark red from the blood in the midgut. The ovaries occupy only a small area at the tip of the abdomen (Plate 5).

iii. **Half gravid-** The abdomen is dark in colour almost black and occupies three to four segments on the ventral surface and six to seven on the dorsal surface of the abdomen.

iv. **Gravid-**The blood is reduced to a small black patch on the ventral surface or may be completely digested.The ovaries occupy all the rest of the abdomen.Freshly fed (category ii) were used for direct ELISA (Figure 8).

A direct ELISA using anti phosphatase conjugates (Anti human IgG (Fab specific); Anti goat IgG (whole molecule) (Sigma), are to be use to identify Human, Cattle (bovine) and goat (ovine) host respectively, based on the procedures of Beier *et al.* (1988). Mosquitoes with blood meals will be assayed in flat bottomed well plates for each host and absorbance read at 405nm, (Plate 5) 30minutes after the addition of substrate (pNPP).

Sample preparation

Mosquitoes to be tested were preserved dry or frozen.

The abdomen was ground in 50μl PBS (0.10M PBS) pH 7.4

A dilution of 1:50 was made of the mosquito triturate using PBS (pH7.4).

ELISA PROCEDURES:

The microtitre wells were coated with 50ul of mosquito triturate.

The plate was covered and incubated for 3 hrs at room temperature

After incubation, the wells were washed twice with PBS Twenty 20

50μl host specific conjugate was added (antihost IgG conjugated in either peroxidase or phosphates): human IgG 1: 2000, bovine 1:25 dilution in 0.5% boiled casein containing 0.025% Twenty 20. (Peroxidase conjugate for human, phosephate conjugate for bovine).

This was incubated for 1 hr at room temperature.

The wells were washed thrice with PSB-Twenty 20.

1000μl ABTS peroxidase substrate was added (2'2'-azino-di (3-ethylbenzthialine sulfonate)

The absorbance was read at 414mn after 30 minutes.

N.B Samples were considered positive if absorbance value exceeded the mean plus three times the standard deviation of the four negative control.

Positive sample = mean negative control 4 (3 x SD).

Negative control = unfed mosquitoes.

3.2.7 Determination of Human Blood Index (HBI)

Based on the results of the determination of bloodmeal source by direct ELISA method, host feeding preferences are determined by analysis of the sources

of the mosquito blood. The proportion of mosquitoes with human blood referred to as the human blood index, (HBI) in a vector species can be then used as an indication of the degree of anthropophily of that particular species (WHO, 2003). The HBI is calculated using the formula

$$HBI = \frac{\text{Number of mosquitoes with human blood}}{\text{Total number of mosquitoes with blood}}$$

3.3 HUMAN PARASITOLOGICAL INVESTIGATION

3.3.1 Detection /Examination for Parasitaemia

In all, 692 infants and children below 8 year old (6-96 months) presenting at the EPU were consecutively and routinely screened for *Plasmodium* infection.Preparation and staining of the blood slides followed the procedures of the World Health Organization (WHO, 1991, 1996, 2003) were adopted using Giemsa staining at pH 7.2.Two slides are taken: One with a thick film (for rapid staining 10-15 minutes with Giemsa stain, and screening while patient is in attendance), the other with a thick and thin film on the same slide for subsequent standard staining (30-45 minutes with 3% giemsa stain). Parasitaemia is measured by counting the number of asexual parasites against a number of leucocytes in the thick blood film, based on a putative mean count of white blood cells density of 8000 white blood cells per µl. The number of asexual parasites is counted against 200+ leucocytes to the end. Therefore, it is usual that the final blood cell count will be over 200. The parasitaemia per microlitre is calculated by using the formula : Parasitaemia per µl = number of parasites x 8000/ number of leucocytes if 500+ parasites have been

counted without having reached 200 leucocytes,the count is stopped after completing the reading of the last field, and the parasitaemia is calculated according to the formula:

$$\text{Parasitaemia (per µl)} = \frac{\text{Number of parasites x 8000}}{\text{Number of leukocytes}}$$

3.3.2 Blood Examination for Gametocytes (Sexual Parasites)

The presence of gametocytes is evaluated in 1000 leukocytes (approximately 200 fields), multiplied by 8 (Osorio *et al.*, 2002). In order to reveal rounded forms of gametocytes if present, slides were left to dry slowly overnight (WHO, 2003).

3.3.3 Examination of Past Hospital Malaria Parasitology Laboratory Reports

For parasitological data comparison purposes, past hospital malaria parasitology laboratory reports of microscopic examination of asexual forms of *Plasmodium spp* conducted using standard WHO approved methods were examined following the methods of Greenberg *et al.* (1989). In this hospital, standard methods for microscopic examination of asexual forms of *Plasmodium spp* were adopted. Routinely, thick and thin blood smears were obtained on clean glass slides from ethanol-sterilized finger pricks and stained in 4% giemsa solution diluted in phosphate buffer of p H 7.3 The diagnosis of malaria in the hospital is usually confirmed by a parasitological examination (malaria positive smear) following the "plus system" of enumerating parasite density recommended for use by the WHO (1985) This is done when it is not possible to undertake the more accepted parasite count per µl of blood (WHO, 1999).

3.3.4 Statistical Analysis of Data

Chi-square (χ^2) test or fisher's exact test where appropriately used to compare proportions.

3.4 LABORATORY EVALUATION OF PYRIPROXYFEN

3.4.1 Source of Insect

Laboratory reared 4th stage larvae of *Anopheles gambiae* GLO (Gambiae Lagos Outskirts) a pyrethroid susceptible strain colonized in 2007 at the Molecular Entomology Research laboratory of the Nigeria Institute of Medical Research Lagos was used for the bioassays.

3.4.2 Test Chemical

Pyriproxyfen (Sumilarv 0.5% G) batch No 5099 X 53 – W1 product of Sumitomochemical (U.K) Plc procured through Sample Rite Limited London U.K.

3.4.3 Manufacturers Note

This product is normally recommended for application rates of 0.01-0.05mg(a.i)/l active ingredient or 0.01-0.05ppm. The doses rates per hectare can be inaccurate as the depth of the water will affect final concentration of the active ingredient (WHO, 2001).

3.4.4 Experimental Design

Evaluations were done following the methods of Nayar *et al.* (2002) and Yapabandara *et al*. (2002). Pyriproxyfen granules (Sumilarv 0.5G) was dissolved in distilled water to make stock solutions of the following concentrations of 0.01, 0.05, 0. mg(a.i)/l and 0.5 mg(a.i)/l . 250ml of each concentration was dispensed into

500ml beakers. Each concentration and the controls were replicated four times. Twenty 4th instar larvae were introduced into each treatment weekly for four weeks. The beakers were continuously covered with white nylon netting to prevent the escape of emerging adults. The treatment emergence inhibition/mortality and survivorship were recorded daily. The ambient laboratory temperatures were also recorded.

3.4.5 Data analysis

Data analysis was done following the methods of WHO (2005). The data from all replicates of each concentration combined total or mean emergence inhibition calculated based on the number of 4th stage larvae exposed. The efficacy of the formulation through time was assessed as Emergence Inhibition (IE%) in treatment containers adjusted for any pupal mortality in the controls (Mulla *et al.*, 1974; Nayar *et al.,* 2002) since pyriproxyfen inhibits the normal development of mosquitoes but does not cause a rapid killing of larvae, its effect cannot be tested by observing larval density. The number of adults which emerge is the only criterion for measurement of its impact (Mulla *et al.,* 1974; Kawada *et al.,* 1988) .Emergence inhibition (IE) was calculated according to the formula:

% IE = 100 – (T-100)

C

Where T = percentage survival or emergence in treated batches and C= percentage survival or emergence in control.

IE values obtained at each concentration were subjected to probit regression analysis to determine IE_{50} and IE_{90} using the SPSS 16.0 for windows statistical software package (SPSS, 2007) and Epi Info 6.04d*.* Empirical probits were also determined using Finney's table for transformation of percentages to probits (Finney, 1952). Where appropriate Chi-square (χ^2) test or Fishers exact test was used to compare data sets generated, Students-*t*-test, ANOVA, Pearsons correlations and graphs were also used to analyze the data.

CHAPTER FOUR
RESULTS

4.1 ENTOMOLOGICAL INVESTIGATION

4.1.1 Relative Abundance and Species Composition

A total of 16 houses were sampled across the 6 different wards of Damboa .The mean vector population density was approximately two persons per house in the other places. Overall a total of 1030 adult female *Anopheles* mosquitoes were collected 979 (95%) were caught indoors. Mosquitos caught outdoors accounted for 51(5%) of the total collected (n=1030) (Table 3). Significant differences was observed between mosquitoes caught indoors and those caught outdoors (P<0.01).

4.1.2 Species Composition

A total of 1030 adult female mosquitoes were collected, 979(95%) were caught indoors.The outdoor samples accounted for 51(5%) of the collected (n=1030) All species collected fall into four major groups of *Anopheles* mosquitoes: *An. gambiae* s.l 1026 (99.6%) *An. pharoensis* 2(0.19%), *An. squamosus* 1(0.09%) *An. rhodesiensis* 1(0.09%). The indoor collections was statistically significant compared to the outdoor collection (P<0.01) (Table 4).

4.1.3 Seasonal Population Dynamics of Female *Anopheles* Mosquitoes

The density of *An. gambiae* complex resting indoors increased rapidly following the beginning of the rains in the Month of May during which (n=55) 5.3% percent of the total anophilines were caught. This number reached a peak of (n=226) 22 % in the months of September, and fell to (n=214) 20.7 percent in

October and (n=159) 15.4 percent in November. It then began to decline as the dry season began. The number of Anophilines came down to 0 percent in January. No Anophilines were caught for the four dry season months of January, February, March and April (Figure 9).

4.1.4 Resting Preference of Anopheline Mosquitoes

Indoor collection was significantly higher than the outdoor collection with a significant preference of human dwellings (P<0.01) (Table 5)

4.1.5 Examination of Abdominal State of Anophiline

Anopheline were grouped based on their abdominal conditions before identification of blood meal sources using direct ELISA. They were generally grouped as engorged and unengorged. Ninety four percent 970 (94%) of *Anopheles* caught were bloodfed while only 60 (6%) percent were unengored. The proportion of engorged mosquitoes was significantly higher than the proportion of unengorged mosquitoes (P<0.01) (Table 6).

4.1.6 Feeding Preferences of the Anophiline Mosquitoes in Damboa

Results of the ELISA on bloodmeal analysis as shown on Table 7 indicate that 94 (98%) of the Anopheles gambiae s. 1 had taken human blood and only 2 (2%) took bovine blood. The Human Blood Index (HBI) was 0.98 indicating high anthropophilic behaviour as shown on figure 10.

4.1.7 PCR Identification of Two Sibling Species within the *Anopheles gambiae* Complex

Of two hundred and thirty three (233) mosquitoes randomly taken from a total of one thousand and thirty (1030) morphologically identified *An. gambiae* s.l were identified further using PCR, the predominant sibling species (95%) were *An. arabiensis* Patton (n=221) and *An. gambiae* s.s. 5% (n=12). Results on Plate 6 showed that the proportion of *Anopheles arabiensis* was significantly higher than that of *Anopheles gambiae* s.s (χ^2= 8.56, df=1, $p<0.05$).

4.1.8 ELISA Test for *Plasmodium falciparum* Circumsporozoite Protein

Mosquito infection by malaria (*Plasmodium*) parasites was determined using the ELISA method for the detection of *Plasmodium falciparum* sporozoites. Out of 289 randomly selected *Anopheles* mosquitoes, 7(2.4%) were positive for *P. falciparum* circumsporozoite antigen. All seven were *An. arabiensis.*The samples whose optical densities (OD) were highlighted on (Table 8) row 1,2,3 and 6 and (Table 9) row 11 all tested positive.

4.1.9 Determination of Mosquito Bloodmeal Source Using ELISA (HBI)

Mosquito blood feeding preferences based on human blood index (HBI) which is an indication of the degree of anthropophily (preference for human blood) was determined from the proportion of mosquitoes with human blood using direct ELISA. The proportion of mosquitoes with human blood was calculated using the formula. Ninety eight (98%) percent of *An. arabiensis* tested had fed on human blood (Table 10). Only two percent (2%) tested positive for bovine (Table 11).

$$HBI = \frac{\text{Number of mosquitoes with human blood}}{\text{Total number of mosquitoes with blood}}$$

Table 3: Relative Abundance/Numbers of Anophelines sampled in Damboa, Borno State

Wards	***Anopheles* in door**	**Out door**	**No. of Houses**	**No. of persons**
G.G.T.C	389	10	8	364
(DGT)			(Dormitories)	
Bakin Tasha	114	11	2	8
(DBT)				
Gen. Hospital Qtrs	150	4	3	7
(DH)	95	7	1	4
Akaliri (DK)	111	10	1	6
Dispensary	120	9	1	4
Ajari				
Total (%)	**979(95%)**	**51(5%)**	**16**	**393**

Table 4: Species Composition of *Anopheles* Species Caught at Damboa

Species	No	Percentage
Anopheles gambiae s.l	1026	99.6
Anopheles pharoensis	2	0.19
Anopheles squamosus	1	0.09
Anopheles rhodesiensis	1	0.09
Total	**1030**	**100%**

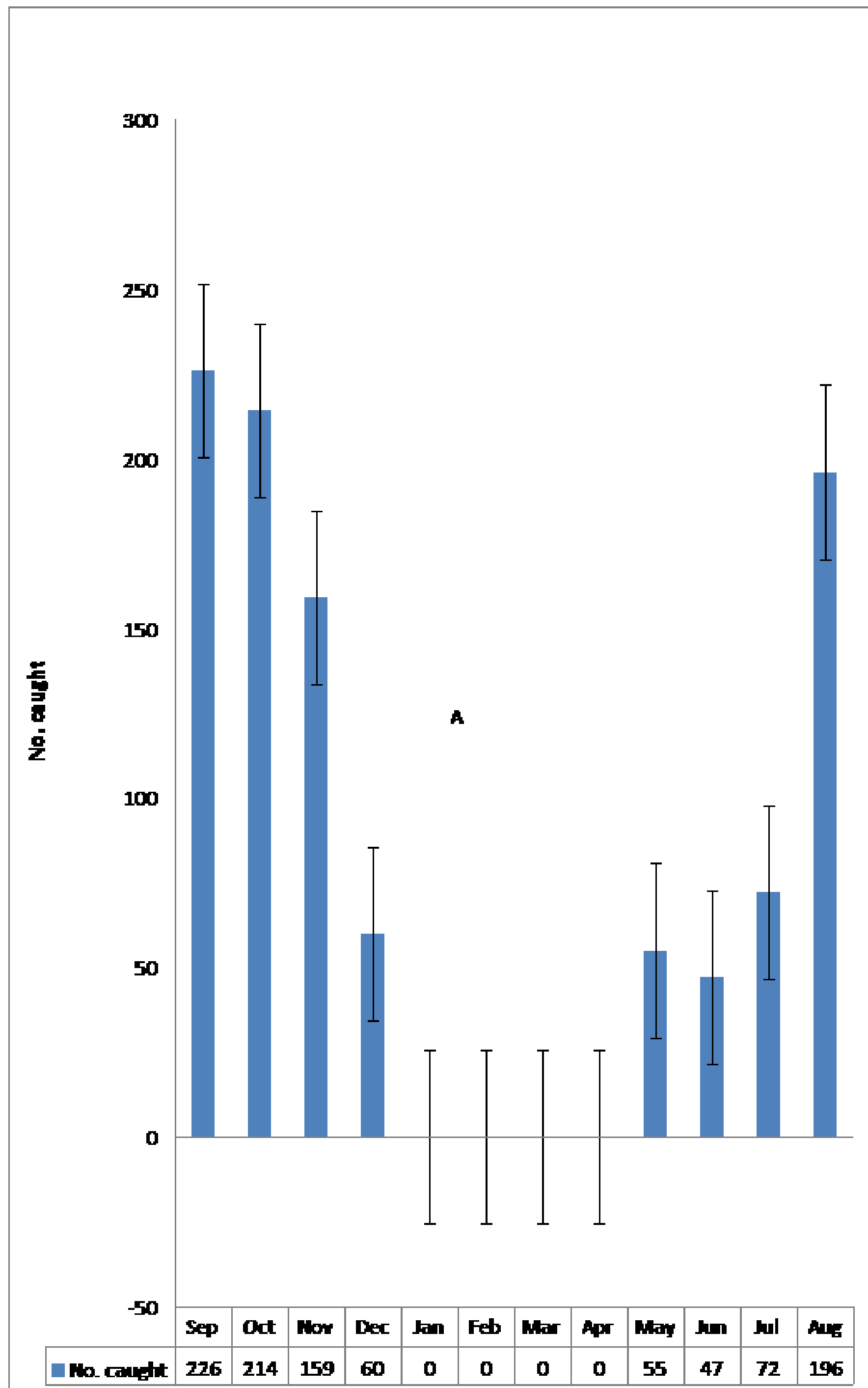


Figure 9: Monthly relative abundance of female Anopheles Mosquiteos 2004-2005

Table 5: Resting Preference of Anopheline Mosquitoes Captured Using Modified Light Traps and Spray Sheet

Species	**Indoors**	**Outdoors**	**Total**
An. gambiae s.l	979 (95)	47(4.80)	1026(99.6)
An. squamosis (0.09)	-	1 (0.09)	1
An. pharoensis (0.19)	-	2 (0.19)	2
An. rhodesensis	-	1 (0.09)	1 (0.09)
Total 1030(100%)	**979 (95)**	**51 (5%)**	

Figures in parenthesis indicate percentage *Z = 40. 97, P< 0.01*

Table 6: Abdominal State of *Anopheles* Species Caught across 6 Wards of Damboa

Anophelines	Blood fed (engorged)	Unengorged
An. gambiae S.I	968	58
An. Pharoensis	0	2
An. Squamosus	1	0
An. Rhodesiensis	1	0
Total	**970 (94%)**	**60(6%)**

Agarose gel Identification: (Form 2)

Name……BORNO……Date……1-9-2005

Locality (Damboa: Barkin Tasha……DK-Alkaliri…)

Name of PCR…….. (Gambiae)

lane	No of tube	id	lane	No of tube	id
1		1kb DNA ladder	**19**	DK 227	Arabiensis
2		Gambiae s.s +ve control	**20**	DK 211	Arabiensis
3		Arabiensis +ve control	**21**	DK 220	Arabiensis
4		Melas +ve control	**22**	DK 216	Arabiensis
5		-VE control	**23**	DK 209	Arabiensis
6	DBT 273	Arabiensis	**24**	DK 221	Arabiensis
7	DBT 278	Arabiensis	**25**		
8	DBT 283	Arabiensis	**26**		1kb DNA ladder
9	DBT 228	Arabiensis	**27**		
10	DBT 271	Arabiensis	**28**		
11	DBT 272	Arabiensis	**29**		
12	DBT 275	Gambiae s.s	**30**		
13	DBT 251	Arabiensis	**31**		
14	DBT 280	Arabiensis	**32**		
15	DBT 277	Arabiensis	**33**		
16	DBT 229	Arabiensis	**34**		
17	DBT 281	Arabiensis	**35**		
18	DBT 279	Arabiensis	**36**		

Photo:

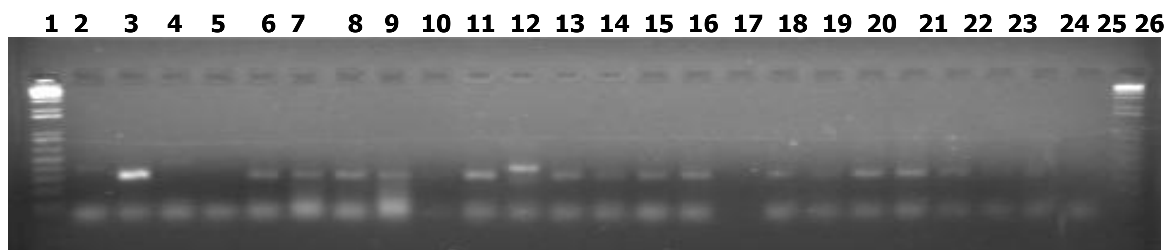


Plate 7: PCR Identification of *An.gambice S.l*

Agarose gel Identification: (Form 2)

Name........BORNO...........**Date**.........1-9-2005.........

Locality……DH (Damboa Hospital) …DD Dispensary…

DGT…Government Technical…

Name of PCR (Gambiae…)

lane	No of tube	id	lane	No of tube	id
1		1kb DNA ladder	**19**	DD 191	Arabiensis
2		Gambiae s.s +ve control	**20**	DD 188	Arabiensis
3		Arabiensis +ve control	**21**	DD 206	Arabiensis
4		Melas +ve control	**22**	DD 198	Arabiensis
5		-ve control	**23**	DD 180	Gambiae s.s
6	DH 270	Arabiensis	**24**	DH 177	Arabiensis
7	DH 282	Arabiensis	**25**	DGT 97	Arabiensis
8	DH 263	Arabiensis	**26**		1kb DNA ladder
9	DH 267	Arabiensis	**27**		
10	DH 281	Arabiensis	**28**		
11	DH 262	Arabiensis	**29**		
12	DH 201	Arabiensis	**30**		
13	DH 179	Arabiensis	**31**		
14	DH 269	Arabiensis	**32**		
15	DH 276	Arabiensis	**33**		
16	DH 258	Arabiensis	**34**		
17	DH 268	Arabiensis	**35**		
18		Arabiensis	**36**		

Photo:

1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26

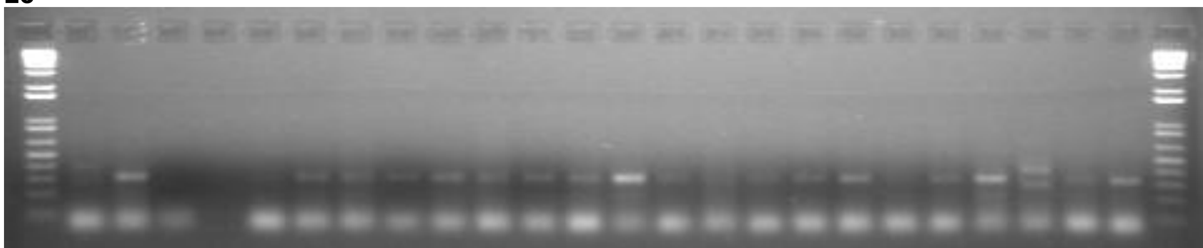


Plate 8: PCR Identification of *An.gambiae S.l*

Agarose gel identification: (Form 2)

Name.... BORNO ....Date 30/08/2005................................

Locality Damboa...............

Name of PCR (Gambiae)

lane	No of tube	id	lane	No of tube	id
1		1kb DNA ladder	**19**	DA 244	Arabiensis
2		Gambiae s.s +ve control	**20**	DA 252	Arabiensis
3		Arabiensis +ve control	**21**	DA 243	Arabiensis
4		Melas +ve control	**22**	DA 253	Gambiae s.s
5		-ve control	**23**	DA 240	Arabiensis
6	DA 241	Arabiensis	**24**	DA 248	Arabiensis
7	DA230	Arabiensis	**25**	DA 231	Arabiensis
8	DA 234	Arabiensis	**26**		1kb DNA ladder
9	DA 245	Arabiensis	**27**		
10	DA 238	Arabiensis	**28**		
11	DA 233	Arabiensis	**29**		
12	DA 242	Arabiensis	**30**		
13	DA 246	Arabiensis	**31**		
14	DA 249	Arabiensis	**32**		
15	DA 237	Arabiensis	**33**		
16	DA 250	Arabiensis	**34**		
17	DA 239		**35**		
18	DA 255	Arabiensis	**36**		

Photo:

1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26

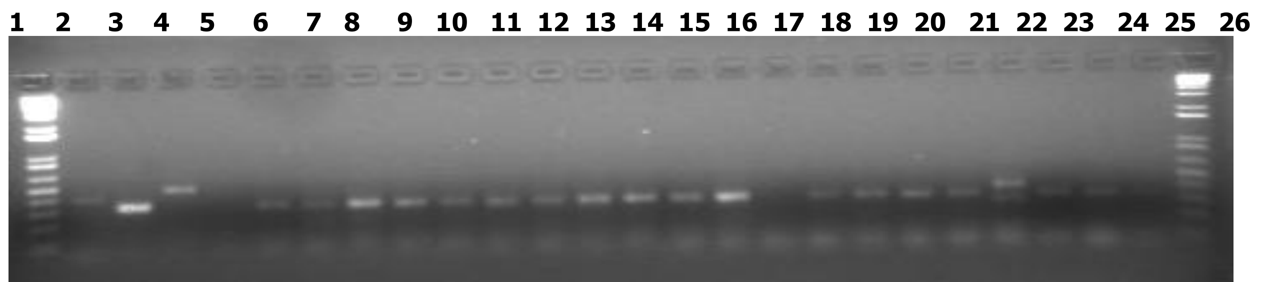


Plate 9: PCR Identification of *An.gambiae S.l*

Table7: Direct ELISA Identification of Feeding Preference/Blood Meals of Indoor and Outdoor Resting *Anopheles arabiensis* Collected from Damboa, Northeastern Nigeria

Origin	Total No.	No for ELISA	Bloodmeal		
			Human	Bovine	HBI
Indoors	979	90	90 (94)[a]	-	
Outdoor	51	6	4 (4)[a]	2(2)[a]	
Total	**1030**	**96**	**94 (98)**	**2 (2)**	**0.98**

[a] *Percentages shown are calculated in relation to the total number of random samples taken for identification by direct ELISA.*

Table 8: ELISA Test for *Plasmodium falciparum* Circumsporozoite Protein.

8/30/2005 8:10:11 PM ID-P20050830-1

1	2	3	4	5	6	7	8	9	10	11	12
0.339	0.196	0.199	0.206	0.167	0.222	0.191	0.150	0.133	0.150	0.128	0.114
0.130	0.192	0.187	0.181	0.176	0.184	0.185	0.134	0.129	0.125	0.106	0.103
0.147	0.108	0.172	0.142	0.117	0.191	0.189	0.105	0.125	0.112	0.084	0.112
0.192	0.198	0.146	0.142	0.144	0.209	0.160	0.141	0.112	0.114	0.088	0.128
0.177	0.157	0.112	0.125	0.115	0.191	0.178	0.144	0.138	0.121	0.106	0.131
0.197	0.110	0.194	0.215	0.101	0.256	0.160	0.102	0.176	0.114	0.088	0.119
0.214	0.210	0.216	0.245	0.206	**0.266**	0.172	0.133	0.151	0.149	0.129	0.146
0.238	0.252	0.230	0.250	0.250	0.258	0.166	0.148	0.129	0.115	0.122	2.434
0.132	0.148	0.194	0.147	0.137	0.144	0.189	0.187	0.253	0.117	2.913	
0.217	0.185	0.182	0.170	0.144	0.104	0.124	0.186	0.150	0.125	0.130	0.371
0.342	0.178	0.122	0.174	0.100	0.126	0.126	0.127	0.197	0.165	0.132	0.270
0.757	0.341	0.187	0.157	0.164	0.109	0.200	0.146	0.167	0.140	0.116	0.222
0.143	0.127	0.128	0.168	0.200	0.179	0.071	0.193	0.154	0.145	0.142	9.999
0.154	0.119	0.145	0.149	0.155	0.139	0.128	0.168	0.193	0.152	0.166	0.196
0.164	**1.245**	**0.451**	0.155	0.118	0.126	0.116	0.107	0.193	0.173	0.129	0.191
0.167	0.137	0.172	0.156	0.167	0.135	0.115	0.142	0.191	0.150	0.191	0.182
0.171	0.200	0.155	0.145	0.229	0.136	0.208	0.172	0.177	0.169	0.196	0.202
2	1	1	1	-	-	-	-	-	-	-	-

Table 9: ELISA Test for *Plasmodium falciparum* Circumsporozoite Protein

1	2	3	4	5	6	7	8	9	10	11	12
0.213	0.153	0.196	0.193	0.142	0.152	0.163	0.295	0.275	0.261	0.233	0.221
0.202	0.157	0.207	0.198	0.184	0.162	0.202	0.293	0.323	0.292	0.234	0.233
0.180	0.193	0.204	0.208	0.182	0.136	0.156	0.233	0.342	0.344	0.289	0.222
0.135	0.189	0.193	0.217	0.166	0.230	0.191	0.164	0.163	0.288	**0.327**	9.999
0.156	0.167	0.169	0.179	0.173	0.170	0.187	0.150	0.133	0.150	**0.336**	0.165
0.176	0.103	0.172	0.147	0.123	0.173	0.104	0.111	0.146	0.128	0.182	0.188
0.182	0.164	0.157	0.145	0.137	0.188	0.160	0.147	0.126	0.117	0.105	0.152
0.178	0.167	0.115	0.151	0.127	0.211	0.227	0.153	0.136	0.135	0.138	0.154
0.188	0.110	0.201	0.224	0.130	0.242	0.154	0.108	0.178	0.135	0.111	0.171
0.185	0.193	0.205	0.253	0.226	0.245	0.161	0.171	0.170	0.166	0.147	0.176
0.188	0.187	0.208	0.234	0.231	0.212	0.186	0.171	0.140	0.170	0.159	0.153
Total	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**2**

Table 10: ELISA Determination of the Mosquito Blood Feeding Source (Preference).

	1	2	3	4	5	6	7	8	9	10	11	12
	0.110	0.136	0.112	0.146	0.188	0.150	0.185	0.168	0.181	0.164	0.259	0.253
	0.100	0.147	0.109	0.117	0.142	0.143	0.130	0.135	0.164	0.184	0.117	0.146
	0.110	0.091	0.113	0.169	0.139	0.180	0.225	0.105	0.168	0.172	0.126	0.154
	0.112	0.161	0.149	0.177	0.190	0.132	0.128	0.187	0.237	0.260	0.157	0.134
	0.106	0.137	0.122	0.172	0.279	0.133	0.142	0.172	0.125	0.147	0.111	0.239
	0.112	0.095	0.145	0.129	0.178	0.163	0.142	0.171	0.264	0.204	0.128	0.152
	0.111	0.196	0.147	0.141	0.299	0.147	0.279	0.188	0.114	0.109	0.141	0.238
	0.143	0.244	0.172	0.158	0.229	0.141	0.179	0.142	0.132	0.123	0.236	0.162
Total	8	6	8	8	8	8	8	8	8	8	8	8

Table 11: ELISA Determination of The Human Blood Index (HBI) Based on Anti Bovine IgG.

1	2	3	4	5	6	7	8	9	10	11	12
0.095	0.084	0.090	0.088	0.100	0.088	0.091	0.076	0.080	0.099	0.102	0.102
0.130	0.119	0.075	0.085	0.084	0.080	0.086	0.066	0.082	0.108	0.091	0.099
0.111	0.086	0.091	0.070	0.071	0.081	0.090	0.058	0.079	0.085	0.094	0.091
0.141	**0.165**	0.072	0.072	0.077	0.074	0.074	0.073	0.085	0.095	0.085	0.094
0.168	0.089	0.072	0.080	0.077	0.084	0.081	0.079	0.074	0.093	0.085	0.089
0.134	0.065	0.106	0.090	0.062	0.077	0.078	0.063	0.080	0.085	0.083	0.098
0.128	0.089	0.089	0.102	0.088	0.090	0.085	0.072	0.086	0.082	0.094	0.095
0.115	0.076	0.101	0.108	0.089	0.092	0.087	0.071	0.080	0.101	0.110	0.102
Total	1	1	-	-	-	-	-	-	-	-	

4.2 HUMAN PARASITOLOGICAL EXAMINATION

4.2.1 Blood Examination for Asexual Parasites.

Of a total of 692 children consecutively screened over a period of one year, 169(24%) ch were positive for malaria parasite; 114(67.46%) of whom were males and 55(32.54%) females. Significant difference ($p<0.05$) in infection rates was observed between the males and the females (Table 12). The levels of asexual parasite density were significantly related to age ($p<0.05$). The majority of infected children (68.0%) were aged between 12-60 months and their asexual parasite density was between 1000-5000 of whole blood (Table 13, 14, 15 and 16). The month of September recorded the highest geometric mean parasite density (GMPD) of 13,655 (Figure 11)while the lowest parasite densities were observed during the dry season months of march, April, and May having GMPD of 0.98, 7.17 and 4.69 respectively as shown on Table 16

4.2.2 Blood Examination for Gametocytes

Gametocytes were detected in 108 (26.3%) of the 411 patients studied over the two main seasons. A higher percentage of males (58.0%) than females (42.0%) were found to be gametocytaemic, but the difference between these rates did not reach statistical significance($p>0.05$; χ^2=0.00028,df=1)(Table 17) Also, a slightly higher number of patients had gametocytes(44%) .The distribution of gametocytes was not significantly affected by age of the patients. (χ^2 = 0.40, df = 2; $P>0.05$) (Table 18). In relation to season, there were 65(42.5%) positive cases of gametocytaemia in the rainy season and 116 (45.0%) during the cool dry

season. The corresponding numbers of negative cases were 88(57.5%) and 142 (55.0%) during the rainy and dry seasons, respectively. Differences between these seasons were significant ($\chi^2=0.239$, df=1; $p<0.05$) (Table 19).

4.2.3 Seasonal variation of Gametocyte Density

In the present study, a high geometric mean gametocyte density was observed at the end of rainy seasons shortly after peak transmission season .The lowest geometric mean gametocyte (GMGD) was recorded in the cool dry season month of November .Gametocytes were detected in 108 (26.3%) of the 411 patients studied over the two main seasons. A higher percentage of males (58.0%) than females (42.0%) were found to be gametocytaemic, but the difference between these rates did not reach statistical significance ($p>0.05$; $\chi^2=0.00028$, df=1) (Table 17) Also, a slightly higher number of patients had gametocytes (44%). The distribution of gametocytes was not significantly affected by age of the patients ($\chi^2 = 0.40$, df = 2; $P>0.05$) (Table 18). In relation to season, there were 65(42.5%) positive cases of gametocytaemia in the rainy season and 116 (45.0%) during the cool dry season. The corresponding numbers of negative cases were 88(57.5%) and 142 (55.0%) during the rainy and dry seasons, respectively. Differences between these seasons were significant ($\chi^2=0.239$, df=1; $p<0.05$) (Table 19).

Table 12: ***Plasmodium falciparum*** **(Asexual Parasites) infection in relation to sex of Children**

Sex	**Positive**		Negative		Total	
	No	%	No	%	No	%
Male	114	67.5	283	54.1	397	57.4
Female	55	32.5	240	45.9	295	42.6
Total	**169**	**100.0**	**523**	**100.0**	**692**	**100.0**

χ^2=2.96; df=1;$p<0.05$ (Significant)

Table 13: Malaria Parasite Density in relation to Age in Children

Age (months)	Frequency of patients in density classes			Positive Freq	%	Negative Freq	%	Total Freq	%
	<1000	1000-5000	>5000						
6-11	19	3	0	22	13.01	20	3.82	42	6.07
12-60	116	7	6	129	76.33	342	65.39	471	68.06
61-96	18	0	0	18	10.65	161	30.78	179	25.87
Total	**153**	**10**	**6**	**169**	**100**	**523**	**100**	**692**	**100**

$\chi^2 = 7.34$ df=2 $P<0.05$

* Classification of age according to F M O H (1991) guidelines for sentinel surveys taking into consideration immunological changes.

Table 14: Parasitological Data on Asexual Stages of Malaria Parasites in 692 Children of Ages 1-8 Years of the Emergency Pediatric Unit University of Maiduguri Teaching Hospital in the Months during the Rainy Season

Season	Months	No Examined.	No. Positive for malaria parasites (%)	No negative for malaria parasite (%)	Geometric mean asexual Parasite Density Asexual (GMPD) parasite/ul	Parasite Density range (Asexual parasite/ul)	Frequency of patients in density classes Asexual parasite/ul		
							<1000	1000-5000	>5000
Rainy	June	30	16(53.3)	14 (46.7)	64	16-400	16	0	0
Season	July	19	9 (47.4)	10 (52.6)	107	16-1,613	8	1	0
	August	68	14 (20.6)	54 (79.4)	353	32-12,280	9	4	1
	Sept	36	10 (27.8)	26 (72.2)	13,655	16-111,111	5	2	3
	Total	153	49 (32.1)	104 (67.9)			38	7	4

Table15: Parasitological Data on Asexual Stages of Malaria Parasites in 692 Children of Ages 1-8 Years of the Emergency Pediatric Unit University of Maiduguri Teaching Hospital during the Cold Dry Season

Season	Months	No. Examined	No. Positive for malaria parasites (%)	No. negative for malaria parasite	Geometric Mean Asexual Parasite Density (GMPD) per/ul	Parasite density range (per/ul)	Frequency of patients in density classes (Asexual parasite/ul)		
							<1000	1000-5000	>5000
Cold Dry Season	Oct	55	14(25.5)	41(74.5)	4916	16-250,000	11	0	3
	Nov	62	10 (83.9)	52 (83.9)	1,695	48-102,564	9	0	1
	Dec	89	32(64.1)	57(64.2)	655	16-1,600	31	1	0
	Jan	52	7 (13.5)	45(86.5)	2,720	16-176,000	6	0	1
Total	**4 months**	**258**	**63 (24.4)**	**195(75.6)**			**57**	**1**	**5**

Table 16: Parasitological Data on Asexual Stages of Malaria Parasites in 692 Severely-Ill Children of Ages 1-8 Years at Emergency Paediatric Unit, UMTH, Maiduguri, Nigeria during the Hot Warm Season.

Season	Months	No. Examined	No. Positive for malaria parasites (%)	No. Negative for malaria parasite (%)	Geometric mean asexual parasite density/(Asexual parasite/μl)	Parasite density range (Asexual parasite/μl)	Frequency of patients in density classes (Asexual parasite/μl)		
							<1000	1000–5000	> 5000
Hot/ warm season	Feb	90	18 (20.0)	73 (80.0)	28.98	16 – 1600	18	0	0
	Mar	65	4 (6.5)	61 (93.85	0.98	0 – 64	4	0	0
	Apr	29	8 (38.0)	21 (62.0)	7.17	16 – 48	8	0	0
	May	58	9 (15.5)	49 (84.6)	4.69	16 – 94	9	0	0
	Jun	49	16 (32.7)	33 (67.3)	16.98	16 – 400	16	0	0
	Total	**281**	**54 (19.3)**	**227 (80.7)**			**54**	**0**	**0**

Table 17: Distribution of Gametocytaemia in Children in Relation to Sex

Sex	Number examined	Number Positive (%)	Number Negative (%)
Male	237	105 (25.5)	132 (32)
Female	174	76 (18.5)	98 (24)
Total	**411**	**181 (44)**	**230 (56)**

$\chi^2 = 0.00028$; df = 1 $p > 0.05$ (NS)

Table 18: Age Related Distribution of Gametocytes in Children

Age (Months)	Number examined	Number Positive (%)	Number Negative (%)
< 12	189	33 (8)	156 (38)
12-60	131	100 (24.3)	31 (7.5)
61-96	91	48 (11.7)	43 (10.5)
Total	**411**	**181 (44)**	**230 (56)**

Chi square $\chi^2 = 0.40$; df = 2 $p > 0.05$ (NS)

Table 19: Relationship between Gametocytaemia and Season

Season	Number examined	Number Positive (%)	Number Negative (%)
Rainy	153	65 (15.8)	88 (21.4)
Dry	258	116 (28.2)	142 (34.5)
Total	**411**	**181**	**230**

Chi square $\chi^2 = 0.239$ df = 1 $P > 0.05$ NS (not significant).

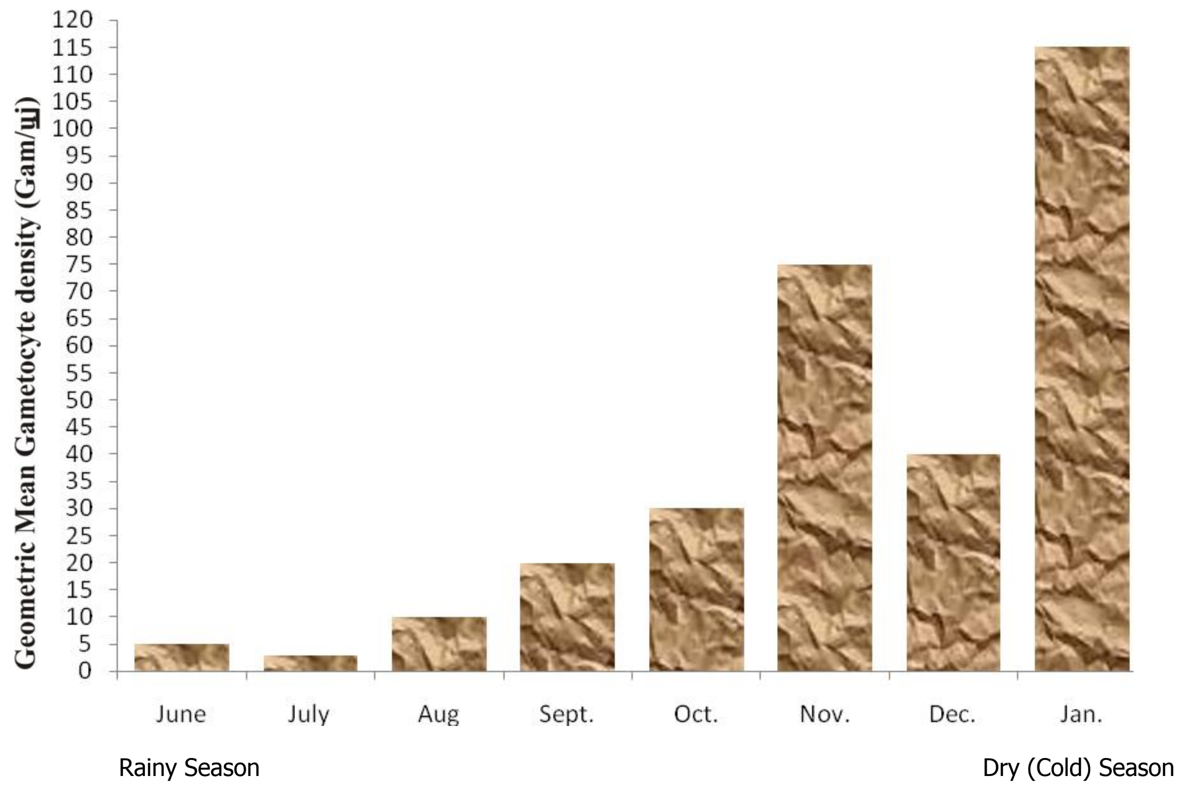


Figure 12: Seasonal Variation of Gametocyte Density

4.2.4 Results of Past Hospital Malaria Parasitology Laboratory Reports

During the period between 2001 and 2004, a total of 13,901 patients attended the outpatient clinic. Two thousand eight hundred and eighteen (2,818) were admitted and 342 died in Damboa General Hospital. Detailed yearly outpatient clinic attendance, hospital admissions and deaths recorded for 2001, 2002, 2003 and 2004 are shown on Figure 13.

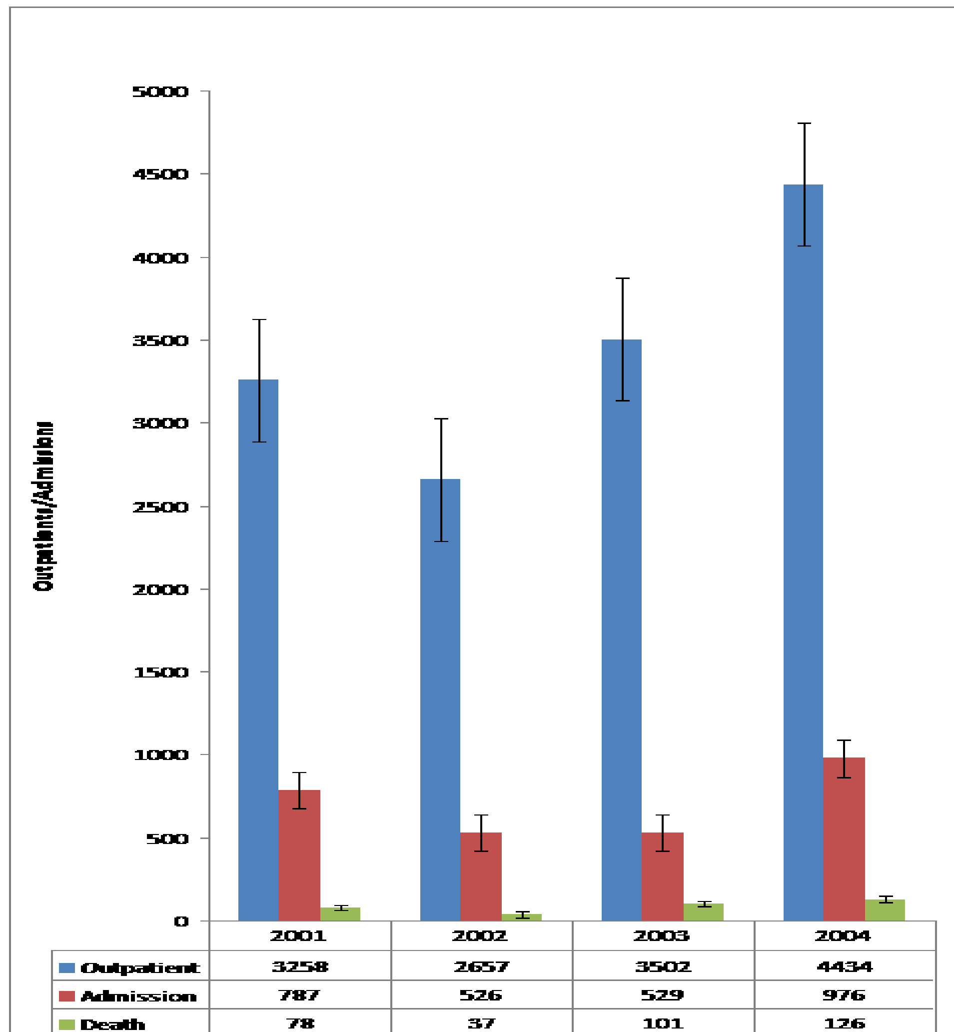
A total of 4,929 children aged 0-4 years attended the Damboa General Hospital from 2001 to 2004 .Three hundred and twenty (33%) children aged 0-4 years had malaria, of this number 188(58.75%) were male and 132(41.25%) female as shown on Table 20.

Malaria accounted for 6.5%, 6.7%, 4.8% and 10% of total outpatient attendance in the four years respectively as shown on Figure 14.

The yearly malaria percentage of all admissions were 27.4%, 32%, 31.4% and 43.4% respectively as shown on Figure 15.

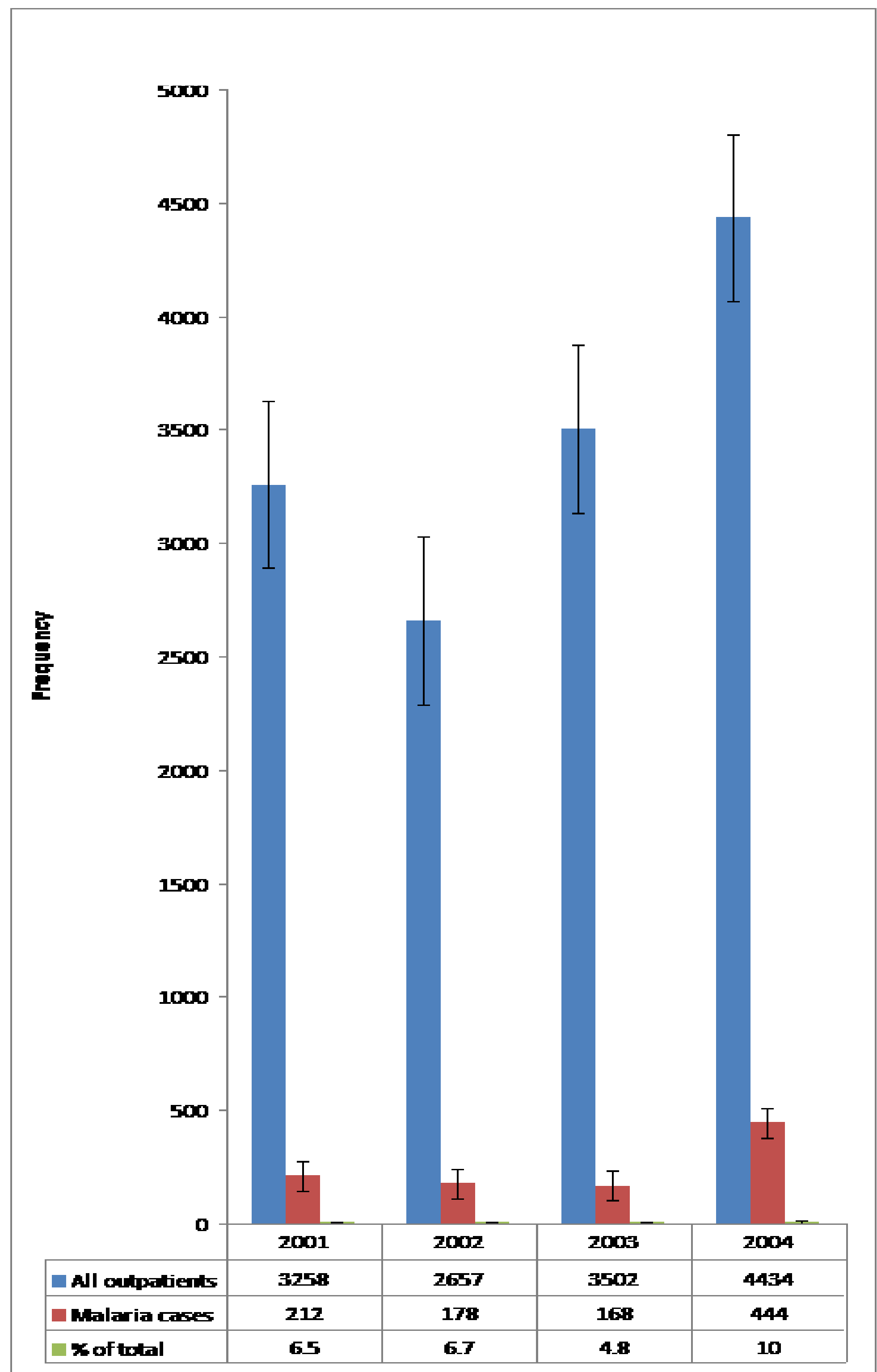
Malaria was responsible. 15.4%, 24.3%, 12.9% and 20.6% mortality for 2001 to. 2004 in this health facility (Figure 16).

The monthly malaria cases, admissions and deaths show seasonal fluctuations with peak incidence of malaria cases in June 2001, October 2002 and 2003 and August 2004.The dry season is characterized by lower numbers of cases compared with those of rainy season (figure 17).



	2001	2002	2003	2004
Outpatient	3258	2657	3502	4434
Admission	787	526	529	976
Death	78	37	101	126

Figure 13: Outpatients' Attendance, Admissions and Deaths at Damboa General Hospital during 2001 – 2004.



	2001	2002	2003	2004
All outpatients	3258	2657	3502	4434
Malaria cases	212	178	168	444
% of total	6.5	6.7	4.8	10

Figure 14: Outpatient Cases and the Proportion due to Malaria in Damboa General Hospital during 2001 – 2004

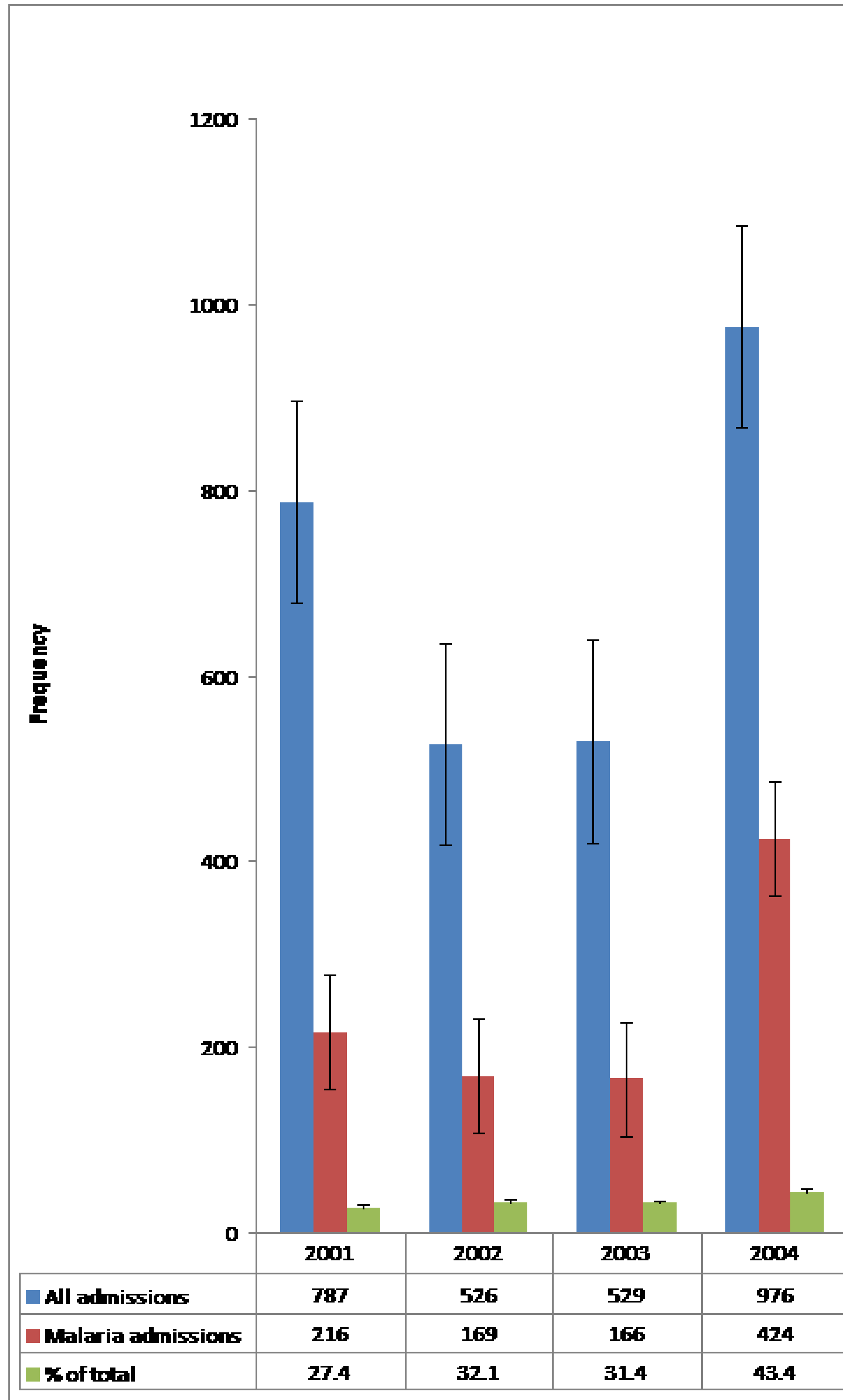
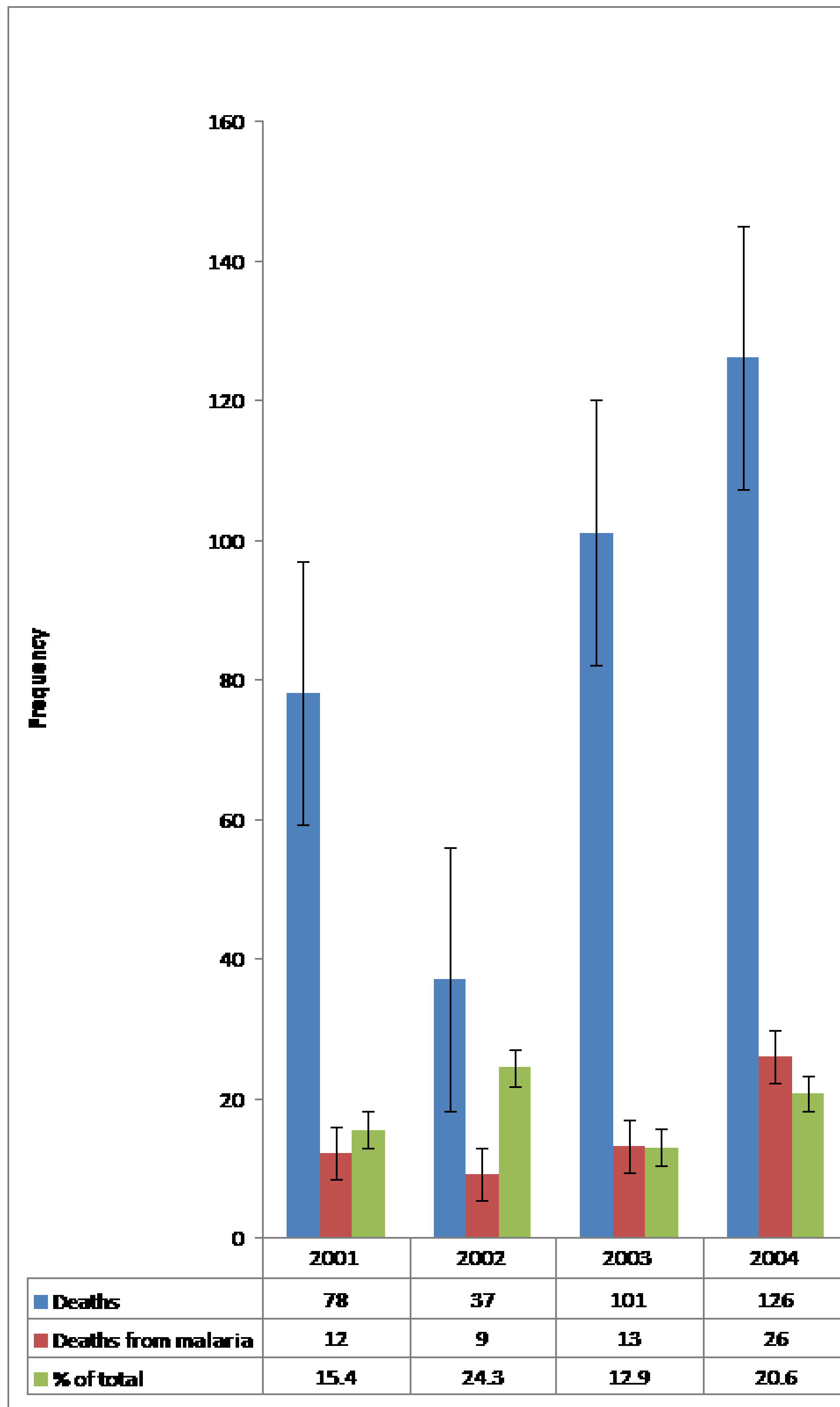


Figure 15: Admissions in Damboa General Hospital and the Proportion due to Malaria during 2001 – 2004.



	2001	2002	2003	2004
Deaths	78	37	101	126
Deaths from malaria	12	9	13	26
% of total	15.4	24.3	12.9	20.6

Figure 16: Mortality in Damboa General Hospital and the Proportion due to Malaria during 2001 - 2004

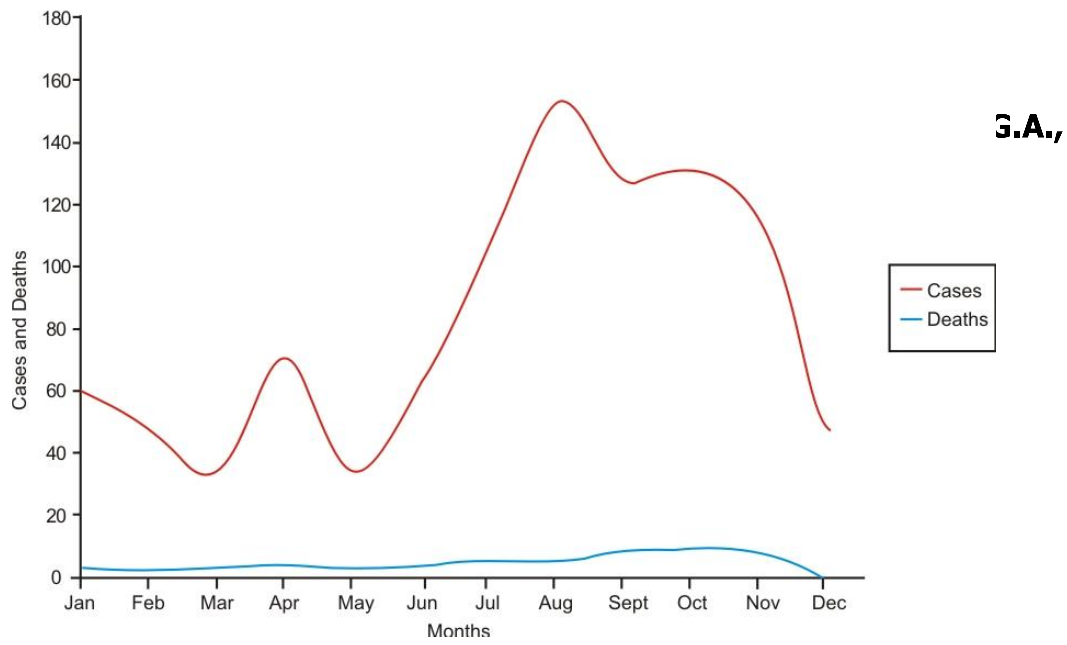


Figure 17: Trend of Monthly Cases and Deaths in Damboa L.G.A., 2001 – 2004

4.3 LABORATORY EVALUATION OF PYRIPROXYFEN

4.3.1 Mean Inhibition of Emergence

Mean Inhibition of Emergence of between 0.1 and 0.5 mg(a.i)/l tration were not significantly different ($p>0.05$) by students -*t*- test (Table 3 and 4). Post-Treatment percentage Emergence Inhibition(EI) of adult emergence of *Anopheles gambiae s.s.* (GLO) exposed to pyriproxyfen at 0.01, 0.05, 0.1 and 0.5 mg(a.i)/l. Inhibition o f Emergence (IE) rates were 86% for the first week (0-7days) then peak to 100% during the second week(8-14days) declined to 73% (15-21days) on the third week and to 36% on the fourth week(22-28day) . Emergence Inhibition (EI) as a result of pyriproxyfen's activity was also noted to be dose dependent ($r=1$) Correlation is significant at the 0.01 level. Comparisons of means of different concentrations against Emergence Inhibition. ANOVA $F= 0.01$,$P= 0.9983$ (Tables 22-25) indicated no significant difference between the different concentrations and the different Emergence Inhibitions.The IE_{50} was 0.00073 mg(a.i)/l. While the LC_{90} 0.211267 mg(a.i)/l (Table 26).

4.3.2 Evaluation of residual effect on Emergence Inhibition (EI) Weeks 1-4

The Emergence Inhibition (EI) increased significantly ($P<0.05$) with increasing concentrations of pyriproxyfen reaching a peak during the second week, it declined from the third week (Figures 1- 4).

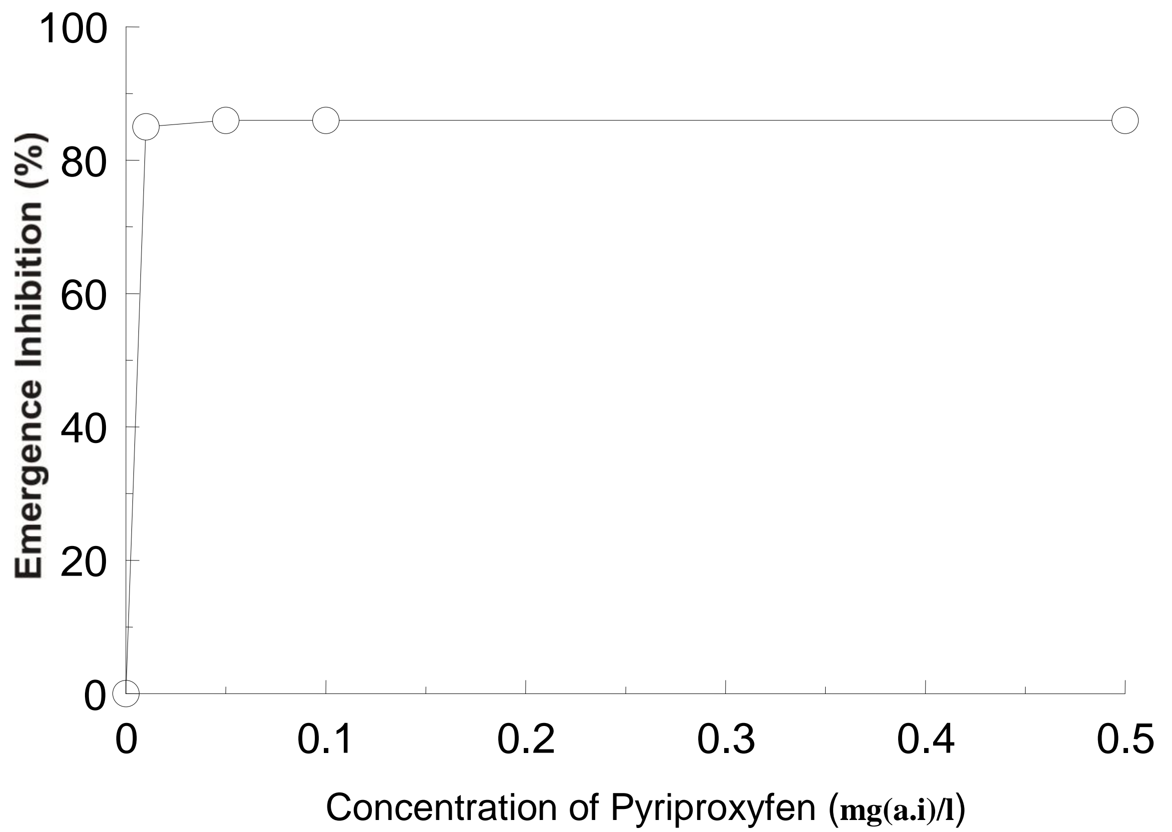


Fig.18: Average Emergence Inhibition of Pyriproxyfen against larvae of *Anopheles gambiae* across all concentrations week1

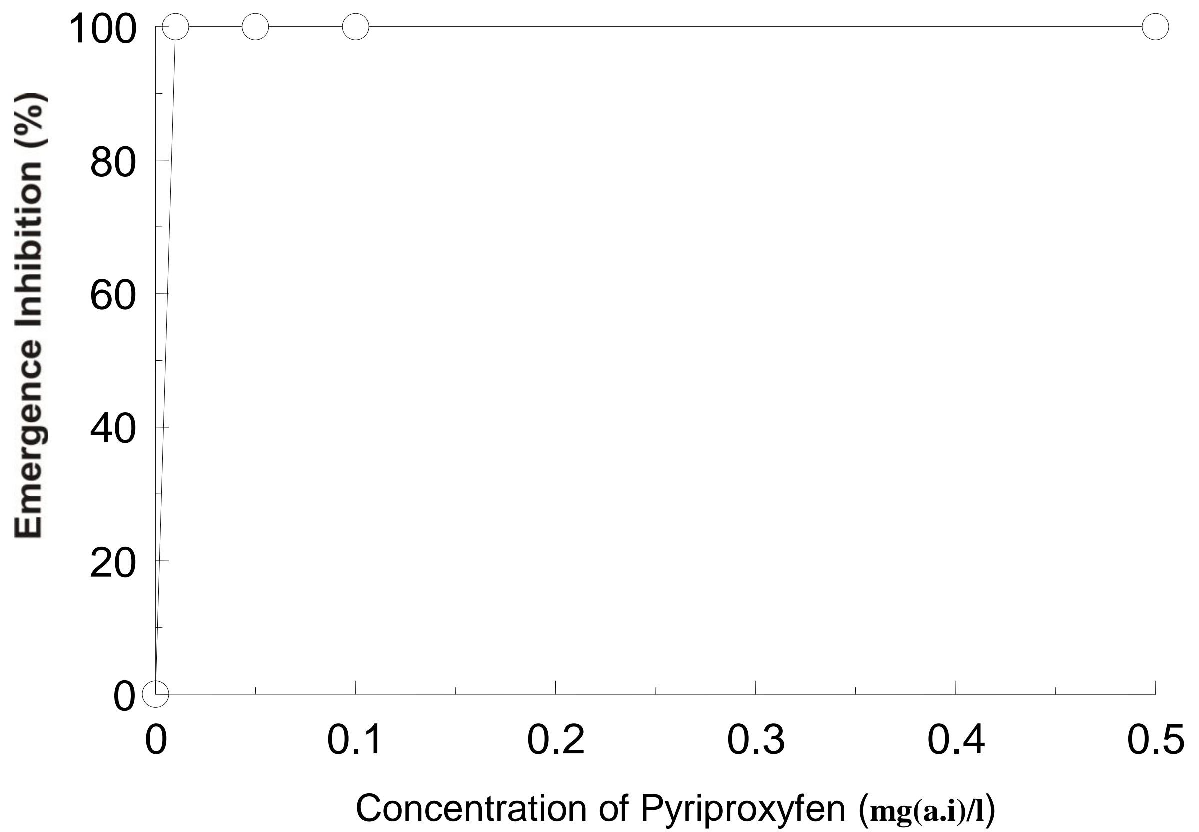


Fig.19. Average Emergence Inhibition of Pyriproxyfen against larvae of Anopheles gambiae(GLO) across all concentrations (GLO) week 2.

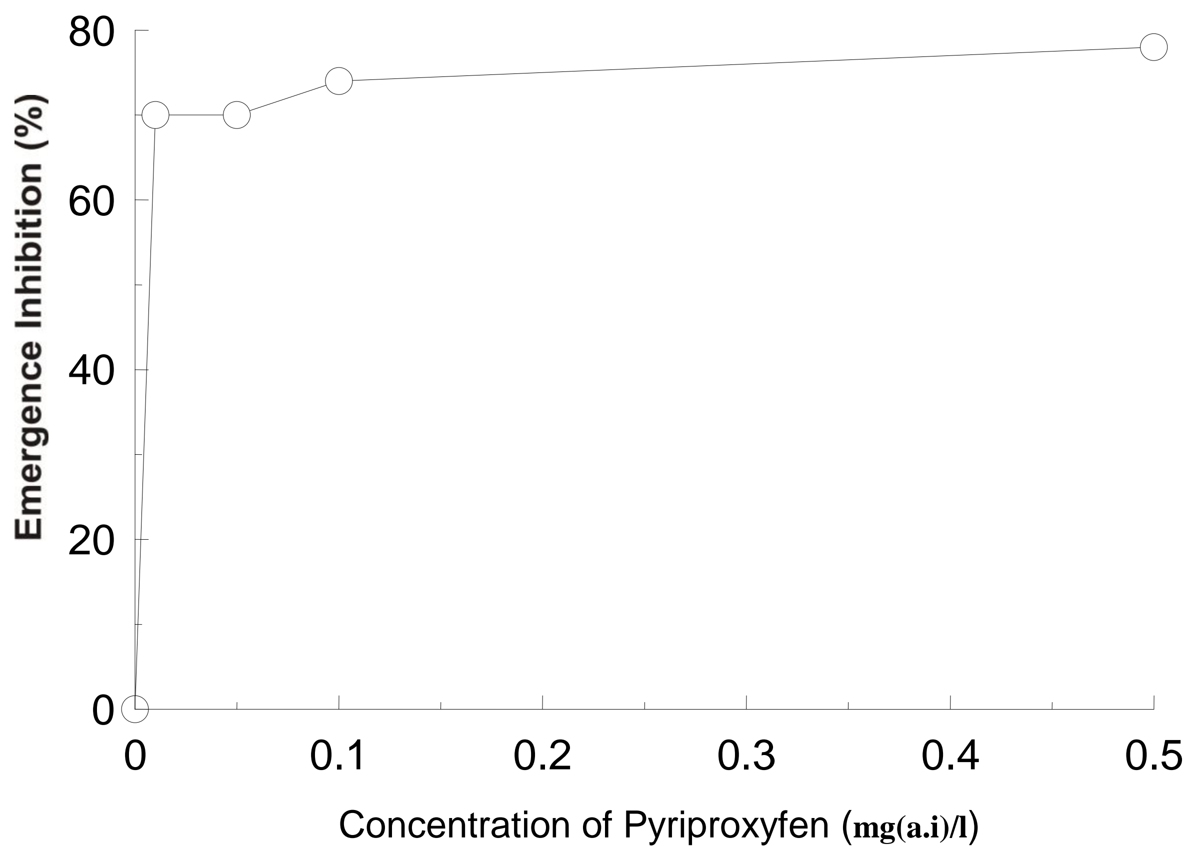


Fig.20. Average Emergence Inhibition of Pyriproxyfen against larvae of Anopheles gambiae (GLO) across all concentrations week 3

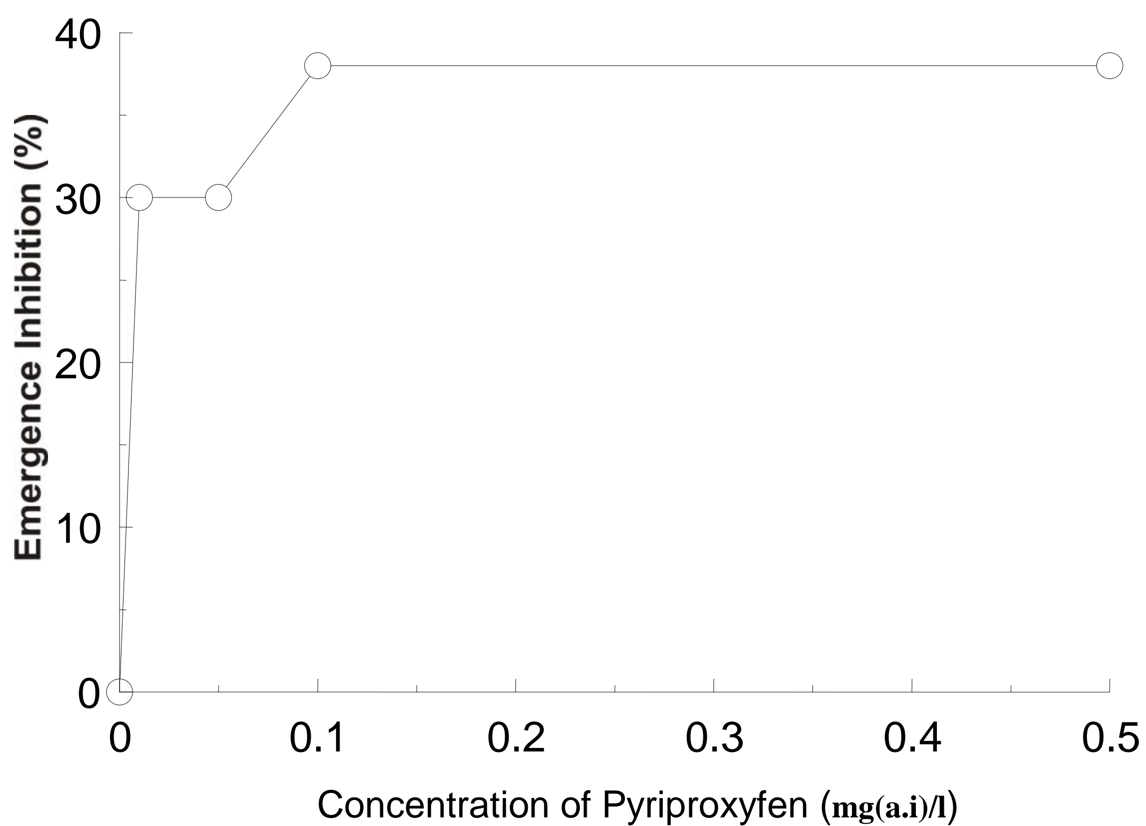


Fig. 21. Average Emergence Inhibition of Pyriproxyfen against larvae of *Anopheles gambiae* (GLO) across concentrations during week 4

CHAPTER FIVE
DISCUSSION

5.1 PCR ANALYSIS OF *ANOPHELES* SPECIES

The present investigation was prompted by the scarcity of information on the precise identity of sibling species of the *Anopheles gambiae* complex in the semi arid Sudano-sahelian area of Borno State and the need to ascertain the vectorial status of these *Anopheles* species using high precision biomolecular tools, including the Polymerase Chain Reaction (PCR) and Enzyme-Linked Immunosorbent Assay (ELISA) (Gadzama, 1983; Molta *et al.*, 1999).

Polymerase Chain Reaction (PCR) and ELISA showed that *An. arabiensis* was the predominant member of the *Anopheles gambiae* complex and the major vector of malaria in Damboa northeastern Nigeria (Samdi *et al.*, 2006).This is agreement with the findings of Hinzoumbe *et al.*(2009). *An. arabiensis* is associated with more arid habitats (Coetzee *et al.,* 2000; Davidson 1967; Omer and Cloudsley-Thompson 1970;El Rayah and Abu Groun 1983; Hamad *et al.,* 2002). The density of *An. arabiensis* resting indoors increased rapidly following the beginning of the rains a situation consistent with the observation of Molta *et al.*(1995)that annual increase was dependent on rainfall . The direct influence of rainfall on the density of *An. arabiensis* is in agreement with the study done in Tanzania by Charlwood *et al.*(1995).

Furthermore findings from this study accords well with Gadzama (1983) and Coetzee *et. al.,* (2000) who found that *An. arabiensis* was associated more with

arid habitats. Earlier Fontenille *et al.*, (1999) had observed that in areas of unstable malaria, *An.arabiensis* was the predominant species of the *An.gambiae* complex. Similarly, Himeidan *et al.* (2004) found that *An. arabiensis* was the main vector (99.9%) in Semi-arid eastern Sudan and *An.pharoensis* was only 0.1%. The same results have been observed in areas of low malaria endemicity in Tanzania where entomological monitoring showed that 99% of malaria vectors caught were *An. Arabiensis* (Mwerinde *et al.*, 2005). From this study it was observed that malaria vector populations of the Sahel, Northeastern Nigeria clearly differs significantly with those of Southern Nigeria, a Polymerase Chain Reaction based test in southern Nigeria identified only 6.3% as *An.arabiensis* while in this work 95% percent observed were *An. Arabiensis* (Awolola *et al.*, 2002).

5.2 ELISA RESULTS OF CIRCUMSPOROZOITE PROTEIN (CSP) ANALYSIS

Mosquito infection by malaria (*Plasmodium*) parasites was determined using the ELISA method for the detection of *Plasmodium falciparum* sporozoite protein.Out of 289 randomly selected *Anopheles* mosquitoes 7(2.4%) were positive for *P. falciparum* circumsporozoite antigen.All seven were *An. arabiensis*. ELISA results of circumsporozoite protein (CSP) rate of 2.4% shows the susceptibility of *An. arabiensis* to local strains of the malaria parasite *Plasmodium falciparum* and its epidemiological importance in malaria transmission in the Sahel (Samdi *et al.*, 2006) . Findings from this work compared to Southwestern Nigeria showed a contrast Awolola *et al.,* (2002) found 0% of *An. arabiensis* infected with *P. falciparum.* However in oyher parts of Southern Nigeria, *An. arabiensis* has been

found to breed extensively in standing water and blocked storm water drains in cities in Nigeria (e.g Sapele) and has been incriminated as vector of urban malaria (WHO, 1988).

Generally there has been an extension in the range of *An. arabiensis* in Nigeria prevailing in arid zones but also in some forest zones. *An. gambiae* s.s and *An. arabiensis* coexists over much of their range (Onyabe and Conn, 2001). However, it is important to note that even in other parts of the southwest such as Ibadan *An. Arabiensis* is an important malaria vector (Awolola *et al.*, 2003). And also the fact that wherever *An. arabiensis* occur in the rainforest,it is associated with extensive land clearance (Coetzee *et al.*, 2000).

Recent investigations in New Bussa by Awolola *et al.* (2006) using ELISA based test have revealed both *An. gambiae ss* and *An. arabiensis* as the main malaria vectors with an overall *Plasmodium falciparum* infection rates of 4.6 and 6.7% respectively as well as showing the importance of *An .arabiensis* as a malaria vector in northern Nigeria compared to Southern Nigeria (Awolola *et al.,* 2006). Earlier works have indicated puzzling shifts in species composition of *An. arabiensis* and *An. gambiae* s.s in Nigeria (Coluzzi *et al.*, 1979).

Though low, a Circumsporozoite (CSP) rate in this study is indeed quite significant as combination of a high human biting rate and a large biting density can result in people receiving several infective bites at night. This may be the case in the sahel which has a high baseline vectorial capacity (contact rate between persons through the vector 2000 times greater than the critical value) (Molineux *et*

al., 1980). *An. arabiensis* females can survive periods of very high temperature and low humidity. This explains the fast reappearance of mosquitoes immediately after the start of the rainy season. (Molineaux *et al.*, 1980; Sanofi, 2002-2004). Lemasson *et al.* (1997) working in Barkedji, a Sahelian area of Senegal observed that *An. arabiensis* represented 79% of the mosquitoes captured and remained in the study area longer than *An. gambiae* after the rains terminated.Lemasson *et al.* (loc.cit.) observed *Plasmodium falciparum* circumsporozoite protein (CSP) rate of only 1.3% but because of the combination of a high human biting rate and a low CSP rate, *An. arabiensis* accounted for 63% of transmission in Senegal. Fontenille *et al.*(1997) also made a similar observation in a study of the transmission of seasonal malaria.In this study *An. arabiensis* with a sporozoite of only 3.7%, 66% of malaria was linked to *An. arabiensis* in Senegal.

5.3 HUMAN BLOOD INDEX (HBI)

Interruption of vector-human contact is of priority in breaking the transmission chain of malaria parasites (Kweka *et al.,* 2009). An effective and a better vector species must have a marked preference for human blood rather than for animal blood with a high human blood index (Bruce-Chwatt, 1986). In this study, ninety eight (98%) percent of *An. arabiensis* mosquitoes tested had fed on human blood/show preference for human blood. Only two (2%) percent tested positive for bovine blood with a Human Blood Index (HBI) of 0.98. indicating a high human-vector contact this could be a contributing factor to the high malaria transmission resulting in 66-76 infection rate (Molta *et al.*, 2004). 0.98 Human

blood index is also in agreement with findings of earlier attempts at mosquito bloodmeal identification on outdoor collection of *Anopheles gambiae* s.l in Northern Nigeria Service (1963) found a high human blood index of 0.89 and 0.97 respectively (Service, 1963). Generally 95 % of the *An. arabiensis* were caught indoors.This finding showed a tendency to feed on man in preference to other animals (anthropophily as opposed to zoophily). This finding is also in agreement with that of workers in the Sahel, northeastern Nigeria. Slides obtained from squashed blood-fed mosquitoes showed that 97% of the blood-fed adults of *An. gambiae* s.l had ingested human blood (Sara,1990).This finding is also in agreement with recent findings for *An.arabiensis* populations in western Africa, the proportion of bloodmeals from humans (human blood index, HBI) is now 80-100% indoors, and most feeding and resting occurs indoors (Molineux and Gramiccia, 1980; Fontenille *et al.*, 1997; Bogh *et al.,* 2001) Recent observations by has shown that *An. arabiensis* was now substantially more anthropophilic with a high human blood index of 0.923. It is now the primary vector responsible for malaria transmission in Zambia (Kent *et al.*, 2007). Though the host-choice behavior of *An. arabiensis* is less rigidly directed to man than *An. gambiae* s.s. and shows different degrees of zoophagic behavior, with higher rates of zoophagy when many large mammals are available, apparently more so in East than West Africa, (Coluzzi 1984, White *et al.*1972, Takken *et al.* 1996, Githeko *et al.* 1994, Waka *et al.*, 2005). An important index in designing control strategy is the proportion of blood meals taken on man followed by resting indoors (WHO, 2003).

Since 95 % of the *An. arabiensis* were caught indoors and 98 percent had fed on man. Indoor-based methods of control, such as the use of insecticide impregnated bednets and house spraying with residual insecticide should be highly effective in this area. Environmental management and other supplementary integrated malaria control strategies deserve attention too.

5.4 LONGITUDINAL PARASITOLOGICAL STUDY

5.4.1 Asexual Parasites

This study has further confirmed that malaria parasitaemia in the Sahel fluctuates with a clear seasonal pattern in agreement with Pull and Gramiccia (1976) that the relatively dry northern Savannah of the country demonstrates strong seasonality in malaria transmission(Oguche *et al.,* 2001) further demonstrated this strong seasonality in a study of the pattern cerebral malaria in northeastern Nigeria.Ninety five percent of the patients presented between June and November with a peak in October.

In this study, a total of 692 children consecutively screened over a period of one year, 169(24%) children were positive for malaria parasite; 114(67.46%) of whom were males and 55(32.54%) females. Significant difference (χ^2 =2.96; df=1; $p<0.05$) in infection rates was observed between the males and the females. The levels of asexual parasitaemia were significantly related to age ($p<0.05$). The majority of infected children (68.0%) were aged between 12-60 months and their asexual parasite density was between 1000-5000 of whole blood. The month of September recorded the highest geometric mean parasite density

(GMPD) of 13,655 while the lowest parasite densities were observed during the dry season months of March, April, and May having GMPD of 0.98,7.17 and 4.69 respectively (Samdi *et al.*,2006).

The data obtained during this (longitudinal parasitological) study further attests to the seasonality of malaria in this zone with an early and a late peak transmission earlier reported by Borno State Ministry of Health (1986-1988). The relatively dry northern savanna of Nigeria demonstrates strong seasonality in malaria transmission. The north has unstable (hypoendemic or mesoendemic) malaria (Oguche *et al.,* 2001). Monthly records of malaria among in-patients by Molta *et al.* (1995) suggests that malaria in the Sahel showed seasonal fluctuations with low values were characteristic of the dry season and high value of the rainy season. This is in contrast with the situation in the wet forested areas of Southern Nigeria where malaria transmission was high all year round (FMOH, 1991). Enosolease and Awodu (2003) in Benin Southern Nigeria also reported malaria parasitaemia fluctuation throughout the years without any clear pattern and devoid of seasonality.

In the present study there was a gradual build up of parasites observed during the rainy season with a sharp peak in September and a gradual tailing off as the dry season advanced. As observed by Molyneaux and Gramiccia (1980) while monitoring malaria in the population in this region there were seasonal patterns of acquisition and loss of infection but very little variation between years and only a slight reduction during the drought years. The highest Geometric Mean

asexual Parasite Densities (GMPD) of 13,655 was observed in the month of September (Samdi *et al.*, 2005a). This could be attributed to repeated inoculation of parasites (sporozoites) with increasing *Anopheles's* biting density as breeding conditions became more favourable.

This longitudinal parasitological study has further confirmed earlier findings of the seasonal fluctuation of malaria parasitaemia in the Sahel. (Pull and Gramiccia, 1976, Molta *et al.*, 1995; FMOH, 1991 and Nurain, 2004). Nurain (2004) observed in the Sahel of central and eastern Sudan, that malaria was a disease of the short rainy season while the rest of the year remained dry and almost malaria free. Oguche *et al.* (2001) noted that since the study area lies in a zone of unstable malaria transmission with a short transmission season immunity in the populace is unable to reach high levels hence the possibility of severe malaria could be very high (Oguche *et al.*, 2001).

The finding on the seasonality of malaria in the Sahel is strategic to planning vector control operations in the study area, since the choice of vector control will depend on the magnitude of the malaria burden and the feasibility of timely and correct application (WHO, 1995).

5.4.2 Sexual Parasites (Gametocytes)

In the present study a high percentage of gametocyte carriers were observed after the rainy season. Molez *et al.* (1996) observed that the highest gametocyte indices are often seen at the end of rainy seasons that is shortly after peak transmission season (Molez *et al.*, 1996).

In this study, gametocytes found were all of *P. falciparum* and only children less than 5years constituted parasite reservoirs (Samdi *et al*., 2005b). When malaria is endemic a proportion of the population are usually carriers of gametocytes and this may be particularly the case among young children(Bruce-Chwatt, 1986).The prevalence of gametocytaemia decreases with rising age until it reaches low level in the adults. Earlier investigations in the study area had indicated that Gametocyte carriage rate increased from 2.7% in 1993 to 10% in 2006 (Molta *et al*., 2000; Watila *et al.,* 2006) suggesting the possibility of a continual increase in antimalarial resistance. This increase in the number of gametocyte carriers can also enhance the infection rate of mosquitoes in the study area (Molta *et al*., 2000).

Other possible reasons for the high percentage of gametocytes in this study could be drug-induced increase in the frequency of gametocyte carriage through self-medication using sub-optimal doses of antimalarial drugs. An earlier study by Oguche *et al.* (2001) had shown that 62.5% of patients in the northeast zone had attempted home treatment with chloroquine before visiting health facilities. Sutherland *et al.* (2002) found that after treatment with chloroquine gametocytes underwent selection for resistance associated alleles at the pfmdrl-86 and pfcrt-76 and are infective to mosquitoes.

In a preliminary assessment of mefloquine/sulphadoxine pyrimethamine combination in thetreatment of uncomplicated malaria in children in Northeastern Nigeria, Molta *et al.* (2001) observed that there was a gradual increase in

gametocytes following mefloquine/sulphadoxine pyrimethamine therapy. The observation showed that mefloquine/sulphadoxine pyrimethamine has no gametocytocidal activity against *Plasmodium*. Targett *et al.* (2001) also observed that the prevalence of mosquito infection was significantly higher in mosquitoes fed on blood samples from children treated with chloroquine. Chloroquine has a stimulating effect on the infectivity of gametocytes (Robert, 2001). These findings are relevant when considered against the background of a spread of resistance to antimalarial drugs in northeastern Nigeria.

There are obvious benefits to be derived from the use of antimalarial drugs that have gametocytocidal activity, including Dihydroarte-misinin recently tested in the Maiduguri area by Molta *et al.* (2001). Such drugs are expected to produce fewer gametocyte loads. It is reasonable to expect that lower gametocyte prevalence and infectivity should result in less transmission, fewer re-infections and a decrease in the number of treated malaria episodes as suggested by Targett *et al.* (2001). Nurain (2004) working in Sahelian Sudan also similarly observed that targeting the gametocyte reservoir by administering gametocytocidal drugs during pre-transmission season has a significant impact in reducing malaria burden in short seasonal transmission. Other interventions such as use of antimalarial drugs or combinations should be evaluated in order to devise the most cost effective intervention for control of the gametocyte reservoir and hence malaria transmission in these areas (Nurain, 2004).

Vector control should be employed in an area of increasing antimalarial drug resistance alongside other intervention strategies e.g. use of insecticide treated nets, behavioural changes, elimination of mosquito breeding breeding sites and larviciding used (Curtis, 1991).

5.4.3 Review of Past Hospital Malaria Parasitology Laboratory Reports

The yearly malaria percentage of all admissions of 27.4%, 32.1%, 31.4% and 43.4% for 2001, 2002, 2003 and 2004 respectively is quite high indicating that malaria is a common disease in Damboa. This is in agreement with the findings of Molta *et al*. (1995, 2004). These figures are much higher than the 8% and 7% recorded as the malaria percentage of all admissions in other places in the Northeast (Molta *et al.*, 1995). Three hundred and twenty infants and children representing 33% of all malaria patients seen at Damboa General Hospital during 2001-2004 were under 4 years of age. This is in agreement with the age distribution of 48,874 malaria cases compiled from all sentinel surveillance sites in Nigeria (FMOH, 1991) indicating that a large percentage (at least more than one third) of children under five years of age are at a special risk from malaria (FMOH, 1991).The monthly figures of malaria cases for the study period show seasonal variations, low values are characteristic of the dry season and high values of rainy season. Graphic representation shows that the peak incidence of malaria occurred in June 2001, then in October (2002, 2003) and August 2004. This is in agreement with the unpublished data of Borno State Ministry of Health; 1986-1988 (BOMOH, 1988). That in this state, transmission of the disease occurs all year round with

peaks during the middle and late rainy season. Malaria alone was responsible for 15.4%, 27%, 17% and 19% mortality in 2001-2004 respectively. Thirty six (57%) out of 60 malaria deaths in Damboa in this study were in the 0-4years category. This finding supports the conclusion of the studies by the Federal Ministry of Health that malaria is one of the five major causes of childhood mortality. It is noteworthy that the highest case fatality ratio of 4:3 across the four sentinel sites in Nigeria was observed in this zone (FMOH, 1991).

5.5 EVALUATION OF S-31183 PYRIPROXYFEN (SUMILARV 0.5GR)

Pyriproxyfen was effective against the larvae of *Anopheles gambiae*. The residual activity and Emergence Inhibition (EI) increased steadily post treatment. Average Emergence Inhibition (EI) rates ranged from 86 to 100 percent during the first seven days of application, though a slight decline to 96 percent was observed in the second week a peak across all doses and the EI declined to 74 percent in the third and finally 34 percent in the fourth week respectively. This study has shown the effect of pyriproxyfen's activity on Mortality and Emergence Inhibition (EI) respectively within a short period of only 28days in an environment completely devoid of organic mattter. Its activity was noted to be dose dependent as no significant difference was observed between treatment mortality at 0.1 and 0.5 mg(a.i).Therefore under test conditions increasing the dose beyond 0.1 mg(a.i) has no significant effect on mortality (Yapabandara and Curtis, 2002). Doses of between 0.01 and 0.1mg(a.i) was observed to give complete Emergence inhibition in both the laboratory and the field (Yapabandara and Curtis 2002).

Numerous works have indicated clearly that the doses as used for the laboratory evaluation would be expected to give far higher kill and much greater Emergence Inhibition for much longer periods under field conditions in the presence of organic matter, therefore increasing the dose would also make the treatment last longer (Nayar *et al.,* 2002; Vythilingam *et al.,* 2005). Generally a decline in activity after just 28 days was observed by several earlier workers in laboratory biossays of Pyripoxyfen in the absence of organic matter. For instance, Yapabandara and Curtis (2002) observed that pyriproxyfen in laboratory trial showed much shorter persistence in the prevention of adult emergence than bioassays in floating cages in the field. Schaefer *et al.*(1988) further observed that the persistence of pyriproxyfen in water in the absence of organic matter declined as temperature and sunlight increased. In the field Pyriproxyen granules is reported to be absorbed onto organic matter and the effective component rests in the substratum for a long period before being slowly released in the water over a substantial period thus lengthening its period of persistence over a long period, no such organic matter was used during this laboratory trials (Kamimura and Arakawa, 1991; Lee, 2001). Though laboratory evaluation is important, only field studies can give a more reliable indication of how long the effect of pyriproxyfen would persist. Studies have shown that though pyriproxyfen inhibits the normal development of mosquitoes it does not cause rapid killing of larvae. So its effect cannot be tested by observing larvae density but the number of adults which emerge is the only criterion for measurement of its impact (Mulla *et al.,* 1974;

kawada *et al.,* 1988). Though the major interventions for diseases like malaria are indoor residual sprays and the use of long lasting insecticidal treated nets. These do not provide complete control especially in the urban area where recent data indicates that urban malaria is being associated with the adaptation of *An.gambiae s.s* to a wide range of polluted water and temporary breeding sites. The addition of an effective larviciding program would further increase the impact on vectors and their associated disease agents. This is in view of the several studies conducted in Africa which have shown 80% of Anopheline breeding sites to be man made and close to human habitation (Awolola *et al.*, 2007; Invest and Lucas, 2008).

5.6 CONCLUSION

In conclusion, Polymerase Chain Reaction (PCR) showed that *An. arabiensis* is the predominant *Anopheles* in the study area,the vector is anthropophilic (preference for humans)and endophilic(bites indoors). This may help narrow down vector control measures in the sahel to a selective, targeted, site specific, ecologically sound and cost effective (malaria) vector control strategy suited to local environmental and epidemiological conditions of the northeastern Nigeria but the same is not easily applicable to South western Nigeria where PCR based test identified a more diverse mosquito fauna.This may have implications on control measures targeting a single species, which will have little impact on malaria infection associated with either *An. funestus* or *An. arabiensis*. In the South, the contribution of the three most important afrotropical malaria vectors may account

for the perennial malaria transmission, compared to other parts of Nigeria like the Sahel where one vector species predominates and transmission is seasonal.

The high number of *An. arabiensis* caught indoors and the positive sporozoite rate indicate their epidemiological impotance in malaria transmission in this part of the Sahel.

Mosquito infection by malaria (*Plasmodium*) parasites determined using the ELISA method for the detection of *Plasmodium falciparum* sporozoite protein showed that out of 289 randomly selected *Anopheles* mosquitoes 7(2.4%) were positive for *P. falciparum* circumsporozoite antigen. All seven were *An. arabiensis* indicating susceptibility of *An. arabiensis* to local strains of the malaria parasite *Plasmodium falciparum* and its epidemiological importance in malaria transmission in the Sahel.

Mosquito blood feeding preferences was determined by direct ELISA using the Human Blood Index (HBI) which is an indication of the degree of anthropophily showed that the proportion of *An.arabiensis* with human blood was ninety eight (98%) percent and only two (2%) percent tested positive for bovine blood indicating a tendency to feed on man in preference to other animals (anthropophily as opposed to zoophily). Mosquitoes that prefer human blood to other animals are more dangerous as they more likely to transmit diseases notably malaria. Findings from the longitudinal parasitological study indicated the peak period of malaria transmission as September with the highest Geometric Mean Asexual Parasite Densities (GMPD) of 13,655 asexual parasites per microlitre of

blood. This finding is strategic to timely planning and correct application of vector control operations such as Indoor Residual Spraying (IRS) in the study area to prevent seasonal peaks of malaria transmission. The high prevalence of gametocytes amongst the vector age group reservoir (children aged 1-8years). screened during the study indicates the possibility of a continual increase in antimalarial drug resistance in the Sahel.

Though the doses of pyriproxyfen used in this work under field conditions have been shown to produce extended residual activity and much higher inhibition rates but the persistence of pyriproxyfen in water in the absence of organic matter and increased temperature and sunlight declines as was observed in this case. However the important larvicidal potentials of pyriproxyfen at low dose has been demonstrated by this preliminary study. It deserves further consideration as a candidate larvicide for integrated vector management (IVM) in the urban areas of Nigeria. The major interventions for diseases like malaria are indoor residual sprays and the use of long lasting insecticidal treated nets. These do not provide complete control especially in the urban area where recent data indicates that urban m alaria is being associated with the adaptation of *An.gambiae s.s* to a wide range of polluted water and temporary breeding sites. The addition of an effective larviciding program with S-31183 pyriproxyfen would further increase the impact on vectors and their associated disease agents. This is in view of the several studies conducted in Africa which have shown 80% of Anopheline breeding sites to be man made and close to human habitation (Awolola *et al.*, 2007; Invest and Lucas, 2008).

5.7 RECOMMENDATIONS

1. The Polymerase Chain Reaction (PCR) results showed that 95% of the members of *An. gambiae* complex in the study area are *An.arabiensis*. They are endophilic and anthropophilic can help narrow down vector control options.

2. Interruption of vector-human contact is of priority in breaking the transmission chain of malaria parasites .The human blood index (HBI) of 98 percent shows a very high human-vector contact. Reducing this contact is advocated. This could be through screening of houses in urban quarters and environmental.

3. The timing of residual insecticide spraying is crucial in obtaining maximum benefit. Results from the longitudinal parasitological study indicated that the peak period of malaria transmission is September with the highest Geometric Mean asexual Parasite Densities (GMPD) of 13,655 asexual parasites per microlitre of blood. This finding is strategic to timely planning and correct application of vector control operations such as Indoor Residual Spraying (IRS) in the study area to prevent seasonal peaks of malaria transmission.

4. Malaria Early Warning Systems (MEWS) which employs the use of Remote sensing (RS) and Geographic information Sensing (GIS) are important meteorological data based surveillance tools should be put in place. Findings from this work showed that malaria transmission intensity varies

according to rainfall in the semi arid Sudano Sahel, under such situations Seasonal epidemics (type 11) of malaria are a major threat.

5. The high prevalence of gametocytes amongst the children screened during the study indicates the possibility of a continual increase in antimalarial drug resistance in the Sahel. Therefore, this study recommends reduction in the number of gametocyte carriers in the population by the timely use of gametocytocidal anti-malarial drugs such as the dihydroartemisinins in line with World Health Organization recommendation that the use of artemisinin–based combinations (ACTs) as the gold standard antimalarial for managing uncomplicated malaria cases. It has an impact on transmission as well.

6. Both the longitudinal entomological and parasitological studies clearly show that vector control activities should target the period starting from June(when the vector buildup begins) up to (December ,covering both the early and late peak transmission for maximum impact.

5.8 SUGGESTIONS FOR FUTURE RESEARCH

1. Extensive studies must be carried on Entomological Inoculation Rates (EIR) of malaria vectors of the study area to assess the epidemiological impact of vector control activities.

2. Studies on Man Biting Rate (MBR) rate of the Anopheline fauna in the study area is recommended for the future.

3. A baseline determination of Susceptibility of malaria vectors to WHOPES-approved insecticides for key vector control measures such Indoor Residual Spraying (IRS) is recommended for the future.

4. A baseline study determination of the susceptibility of malaria vectors of the study area to all pyrethroids used in insecticide treated nets widely distributed by the Federal Ministry of Health must be done in the future.

5. Field trials of environmentally friendly larvicides with wide safety margins such as pyriproxyfen (s-31183) on discrete malaria vector breeding places and polluted places in urban areas is needed in the future.

6. A circumsporozoite analysis of *An. pharoensis* a secondary malaria vector of importance in the Sahel is recommended in the future.

5.9 SUMMARY OF RESULTS

1. A total of 1030 female *Anopheles* mosquitoes were caught consisting of five species. Namely, 1026 (99.6%) of *Anopheles gambiae* complex while 2 (0.19%) were *An. pharoensis*; 1 (0.09%) *An. squamosis* while 1 (0.09%) was *An. rhodesiensis.*

2. Two hundred and thirty three (233) mosquitoes randomly taken from the one thousand and thirty (1030) morphologically identified *An.gambiae s.l* subjected further to PCR analysis which showed that the predominant sibling species were *An. arabiensis* Patton 95%(n=221) and *An. gambiae* s.s. 5% (n=12). Three other species of *Anopheles* mosquitoes collected were morphologically identified to be *An. pharoensis*, *An. squamosus* and

An. rhodesiense. Results obtained showed that the population of *Anopheles arabiensis* was significantly higher than that of *Anopheles gambiae* ss (χ^2 = 8.56 df=1, $p<0.05$).

3. Mosquito infection by malaria (*Plasmodium*) parasites was determined using the ELISA method for the detection of *Plasmodium falciparum* sporozoites.out of 289 randomly selected *Anopheles* mosquitoes 7 (2.4%) were positive for *P. falciparum* circumsporozoite antigen.All seven were *An. arabiensis*.

4 Mosquito bloodmeal source was determined using DIRECT ELISA and the Human Blood index (HBI) gave a proportion of mosquitoes with human blood and hence an indication of anthropophily. Ninety eight (98%) percent of *An. arabiensis* mosquitoes tested had fed on human blood. Only two (2%) percent tested positive for bovine blood.

5. Of a total of 692 children consecutively screened over a period of one year, 169(24%) were positive for malaria parasite; 114(67.46%) of whom were males and 55 (32.54%) females. Significant difference ($p<0.05$) in infection rates was observed between the males and the females. The levels of parasitaemia asexual parasite were significantly related to age ($p<0.05$). The majority of infected children (68.0%) were aged between 12-60 months and their asexual parasite density was between 1000-5000 of whole blood. The month of September recorded the highest geometric mean parasite density (GMPD) of 13,655 while the lowest parasite densities were

observed during the dry season months of March, April, and May with GMPD values of 0.98, 7.17 and 4.69 respectively.

6. Gametocytaemia was not significantly affected by the age of neither the patients nor the season (χ^2 =0.04; df=2 p>0.05).

7. During the period between 2001 and 2004, a total of 13,901 patients attended the outpatient clinic. Two thousand eight hundred and eighteen (2,818) were admitted and 342 died in Damboa General Hospital. Detailed yearly outpatient clinic attendance, hospital admissions and deaths recorded for 2001, 2002, 2003 and 2004.

8. A total of 4,929 children aged 0-4 years attended the Damboa General Hospital from 2001 to 2004 .Three hundred and twenty (33%) children aged 0-4 years had malaria, of this number 188(58.75%) were male and 132(41.25%) female.

9. The monthly malaria cases,admissions and deaths show seasonal fluctuations with peak incidence of malaria cases in June 2001, October 2002 and 2003 and August 2004.The dry season is characterized by lower numbers of cases compared with those of rainy season.

10. Pyriproxyfen was effective against the larvae of *Anopheles gambiae*. The residual activity and Emergence Inhibition (EI) increased steadily post treatment.

11. Average Emergence Inhibition (EI) rates ranged from 86 to 100 percent during the first seven days of application, though a slight decline to 96

percent was observed in the second week a peak across all doses and the EI declined to 74 percent in the third and finally 34 percent in the fourth week respectively.

12. A significant correlation was observed between dose and Emergence Inhibition ($r=1$, $P<0.01$), while means of mortality between 0.1mg/L and 0.5mg/L treatments were not significant different ($P> 0.05$).

13. Means of percentage adult emergence inhibition (EI) was found to be statistically significant at all the application rates ($\chi^2 = 3.49$; df = 3; $p < 0.05$.)

14. The means of Emergence Inhibition (EI) between 0.1ppm and 0.5ppm treatments were not significantly different ($P> 0.05$). Emergence Inhibition (EI) rates ranged between 86-100 percent during the first seven days of application, 48-96 percent during the second week, 43-74percent during the third and subsequently 18-64 percent by the fourth week.

15. Means of percentage adult emergence inhibition (EI) was found to be statistically significant at all the application rates ($\chi^2 = 3.49$; df = 3; $p < 0.05$.).

The data generated from all entomological and parasitological studies will provide a useful basis for the purpose of designing a suitable malaria control strategy in line with integrated vector management (IVM) now advocated by the Federal Ministry of Health.

5.10 CONTRIBUTIONS TO KNOWLEDGE

This study has made 6 major contributions to knowledge by providing key baseline data for malaria vector control activities the Federal Ministry of Health desires to scale up. This is in line with the principles that, the control of malaria requires a break in the epidemiological chain: either by acting on the human parasite reservoir or by reducing man- vector contact.

1. This is one of the works selected and cited by the World Health Organization (WHO) for the document entitled: Malaria Entomological profile for Nigeria (1919-2007) available online at http://www.afro.who.int/vbc/reports/entomological_nigeria.pdf the report represents an analysis of 86 selected baseline research works on malaria vectors in Nigeria over the last nine decades(1919-2007).
2. This is the first documented attempt in this part of the Sudan Savanna at the southern edge of Sahelian part of Borno state Northeastern Nigeria at the use of high precision biomolecular tools technique of Polymerase Chain Reaction (PCR) for the identification of the *Anopheles gambiae* complex and *An. arabiensis.*
3. This is the first documented attempt at the use of ELISA tests to show that *An. arabiensis* is the vector of *Plasmodium falciparum* in the study area and to determine sporozoite rate by a quantification of the number of the sporozoites present based on the detection on a specific antigen on the

sporozoite surface,the circumsporozoite protein(or CSP1) and give a precise identification of the sporozoites as those of *Plasmodium falciparum.*

4. This is the first documented attempt at the use of molecular methods employing direct ELISA to show that *An.arabiensis* which is known to predominantly feed on animals does not feed on animals in the study area but on mainly on human beings (human blood) thus conclusively incriminating *An.arabiensis* as the predominant malaria vector in the study area

5. This work determined the degree of man-vector contact between (*An. arabiensis* and man (human blood index of 0.98) (98% human biter) This is critical figure for the evaluation of the outcome of the success of the major malaria vector aimed at decreasing Human – Vector Contact. interventions (ITNS and IRS) currently being scaled up in Nigeria.

6. This work documented proof to show that the resting preference of *An. arabiensis* at the study area is not exophilic but endophilic.This is crucial to the recommendation and success of Indoor Residual Spray (IRS) and the promotion of Insecticide treated nets (ITNS) as a malaria vector control strategy in the study area.

7. Where malaria transmission is seasonal, optimal timing of vector control activities is crucial to its success.This is the first documented attempt at a longitudinal human parasitological investigation to provide baseline

information to further show the period within a season for timing vector control activities in the Sudano-Sahel.

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APPENDIX I: VECTORS SELECTED RANDOMLY FROM EACH OF THE SIX SITES FOR PCR

SET 1

Bakin Tasha (Code: DBT)

238	271	273
251	228	281
229	279	278
283	275	284
272	280	277

SET 2

Ajari Damboa (Code: DA)

248	234	242	235	252
231	245	249	239	243
241	238	237	255	253
240	233	250	244	

SET 3

Alkaliri Damboa (Code: DK)

211	221	209	217
215	216	227	213

212	224	220
226	214	210

SET 4

Government Girls Technical College (Code: DGT)

86	153	149	116	124
121	161	164	118	122
142	126	97	169	93
140	160	103	89	113

SET 5

General Hospital Quarters (Code: DH)

264	270	284	259
269	281	257	263
266	268	274	276
282	258	262	265

SET6

Dispensary area (Code: DD)

201	178	173
184	192	195
199		

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APPENDIX II: CONTROL OF MOSQUITO LARVAE

Insecticide	Formulation	Dosage of active ingredient
Oils		
Fuel oil	Solution	142-190L/ha
Fuel oil + spreading agent	Solution	19-47L/ha
Organophosphates		
Chlopyriphos	EC	11-25g/ha
Fenthion	EC	22-112g/ha
Primiphos-Methyl	EC	50-500g/ha
Temephos	EC_1 GR	56-112g/ha
Insect growth regulators		
Diflubenzuron	GR	25-100g/ha
Methoprene	EC	20-40g/ha
Pyriproxyfen	GR	5-10g/ha
Microbial insecticides		
B. Thurigiensis israelensis	Slow-release formulations	(b)
B. Sphaericus	Slow-release formulations	(b)
a EC= emulsifiable concentrate : GR= granule b. The dosage will depend on the formulation used.		