THE TRANSMISSION ATTRIBUTES OF PERI-URBAN MALARIA IN LUSAKA, ZAMBIA

by

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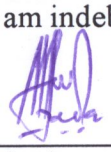
ACRONYMS

BCC	:	Behavioural Change for Communication
Bti	:	*Bacillus thuringensis* var. *israelensis*
DALYS	:	Disability Adjusted Life Years
DDT	:	Dichlorodiphenyltrichloroethane
DNA	:	Deoxyribose Nucleic Acid
EDTA	:	Ethylamine tetra-acetic acid
GPS	:	Geographical Positioning System
IGS	:	Intergenic Spacers
ITS	:	Internal Transcribed Spacer
IRS	:	Indoor Residual Spraying
ITN	:	Insecticide Treated Nets
IVM	:	Integrated Vector Management
KAP	:	Knowledge, Attitudes and Practices
PCR	:	Polymerase Chain Reaction
RBM	:	Roll Back Malaria
rDNA	:	ribosomal DNA
Tris	:	2-amino-2(hydroxymethyl)-propane-1, 3-diol
TE	:	Tris and EDTA
WHO	:	World Health Organization

DECLARATION

I, Emmanuel Chanda, hereby declare that the work presented in this dissertation is entirely a product of my own and unaided investigation and, in submitting it for the Master of Science Degree in the School of Medicine of the University of Zambia, further attest that it has not been submitted before in part or wholly for any other degree programme or examination in any other University. The various resources to which I am indebted are acknowledged.



(Signature of Candidate)

8th day of May 2007

We have read this dissertation and we approve it for examination

Supervisor Dr. C. J. Shinondo

Signed

Date 4/06/07

Co- supervisor.....

Signed.....

Date.....

CERTIFICATE OF APPROVAL

The dissertation of Emmanuel Chanda has been approved as fulfilling the requirements for the award of Master of Science degree in Medical Parasitology by the University of Zambia

Examiner I

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Examiner II

Signed ..... Date 31 May 2007

Examiner III

Signed ..... Date .....

ABSTRACT

Malaria is a disease of great public health importance in Zambia. It is a major cause of morbidity and mortality and is endemic in all nine provinces. The malaria transmission paradigms may be higher in urban populations than in peri-urban and rural population and peri-urban areas tend to become less malarias as they become better established. *Anopheles gambiae s.s*, *Anopheles arabiensis* and *Anopheles funestus* are the most important malaria vectors in Zambia but the knowledge of their actual distribution and the resultant malaria cases in peri-urban Lusaka is fragmented. This study assessed malaria transmission factors and attributes in peri-urban Lusaka by determining *Anopheles* species prevalence and bionomics, parasite rates, residents KAP levels and provide the evidence of local transmission as well as recommending appropriate vector control strategies.

This study was conducted in four locations of peri-urban Lusaka, namely Kabanana, Chazanga, Chipata and Kalikiliki from May to October. Data was collected through entomological and parasitological surveys, Questionnaire and PCR methods. Written consent was sought from participating subjects for inclusion in the study. The study was empirical in nature with appropriate scientific methods and ideal sample sizes and data was analyzed by Epi-Info 3.2.2.

This study identified three kinds of Anopheline mosquito breeding habitats in peri-urban Lusaka, namely Transient, Semi-permanent and Permanent. The semi-permanent were the most prevalent and most preferred breeding sites for *Anopheles* species.

Anopheles gambiae s.l constituted 10% of the indoor pyrethrum collected mosquitoes. Molecular speciation showed that Eighteen (95%) *Anopheles* specimens amplified for *Anopheles gambiae s.s* at 390 bp and only one (5%) specimen amplified for *Anopheles arabiensis* 315 bp.

Parasite rates in peri-urban Lusaka were at 25.6% and were completely due to *Plasmodium falciparum* mono infections with 98.7% trophozoites and 1.3% gametocytaemia rates. The 0-4 years group had the highest infection rate (31.8%) with the 5-15 years and above 15 year age groups supporting the highest and lowest parasitaemia densities respectively.

There were appreciably high levels of knowledge on malaria as regards the disease among peri-urban Lusaka residents but low knowledge of control and prevention, which explains the high infection rates. It was also established that migration does not contribute significantly towards transmission. The congestion in households has probably contributed to the high transmission in peri-urban Lusaka.

Local transmission of malaria in peri-urban Lusaka was evident in that 31.8% of febrile children under the age of five had malaria confirmed by microscopy. Furthermore, the presence of children under five years (78%) who lived in peri-urban Lusaka, with no history of travel, the presence of gametocyte bearers and the vector in the community perpetuate the transmission cycle. Local transmission is also strongly supported by the proximity of ideal breeding habitats and the presence of efficient *Anopheles gambiae* complex species (*Anopheles gambiae and Anopheles arabiensis)* in the houses.

The study concluded that there is local transmission of malaria in peri-urban Lusaka. The presence of both *Anopheles gambiae s.s and Anopheles arabiensis* merits the implementation of an Integrated Vector Management criterion in peri-urban Lusaka based on this transmission paradigm.

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CHAPTER 1.0: INTRODUCTION

Malaria is a febrile disease condition due to an infection with Protozoa of the genus *Plasmodium*, which are pigment producing amoeboid intracellular parasites of hepatocytes and red blood cells. This fatal and devastating disease, transmitted by female anopheline mosquito vectors, is extremely debilitating and exacts a high price from the affected communities. The name malaria is derived from an Italian word "Mal", "aria" that literally means "bad air" and a Latin word paludisme that derives from "palus" or marshy grounds (Manson-Bahr and Bell, 1991). Although human malaria parasites were first seen in 1880, their development both in the Anopheline mosquito and in human blood stream was only well understood by 1900 by Ronald Ross and the entire life cycle was not elucidated until more recently (Markel and Voges, 1999).

The geographical distribution of the global malarial burden is uneven but concentrated in more than 100 countries found in warmer regions of the world in tropical and subtropical countries. However, indigenous malaria has been recorded as far north as 64°N latitude (Archangel in the USSR) and as far south as 32°S latitude (Cordoba in Argentina). This follows the 16 degrees Celsius summer isotherm limit. In terms of vertical distribution, the disease has occurred in the Dead Sea area at 400m below sea level, and at Londiani (Kenya) at 2591m above sea level. Within these latitude and altitude limits, there are large areas that are free of malaria. Malaria is essentially a focal disease, since the transmission of malaria depends greatly on local environmental and other conditions (Bruce–Chwatt, 1985). Although the global pattern of malaria transmission is confined to the tropics and sub-tropical areas, it also extends to the temperate regions such as USSR (Sachs and Malaney, 2002).

The disease continues to be an increasingly important health concern in many endemic areas where it remains a major contributor to childhood morbidity and mortality (Mc Clean et al, 2002). The disease accounts for an estimated loss of 46.5 million disability adjusted life years (DALYS) with almost 90% currently

concentrated in Sub-Saharan Africa (Keiser et al, 2005). More than 40% of the world population is at risk and every year, close to 10% of the earth's population suffers from malaria.

The morbidity rates in more than 100 countries, principally those in the developing world, stand between 300 to 500 million people per year and mortality rates of over 2 million in children and pregnant women, with 2.4 billion people vulnerable to infection (Hentschel, 2002). A death from malaria, therefore, occurs every 30 seconds resulting in a daily loss of more than 2000 young lives the world over (Weiss, 2002). The gravity of the problem is enormously high in tropical Africa where at least 85 to 90% of deaths from malaria occur (Hoffman, 2002). In sub-Saharan Africa, the disease kills one child in 20 before the age of 5. These estimates, therefore, render malaria the most prominent parasitic disease and one of the top 3 killers among the communicable diseases (Greenwood and Mutabingwa, 2002).

In many developing countries, malaria control programmes are fragmented or non-existent. The cost of malaria control increases and the efficacy of treatment is reduced leading to delays in eradicating parasitaemia, which increases the risk of complication. In areas of high transmission rates, young children suffer repeated episodes of symptomatic malaria during the first few years of life, contributing to high child mortality, malnutrition, anaemia and stunted growth. (Mc Clean et al, 2002).

Although the disease burden due to malaria is on the increase globally, the malarious countries of sub Saharan Africa are worst affected and this is evidenced by the resurgence of malaria in areas where it was initially controlled (Mc Clean et al, 2002). In most of these sub Saharan countries the problem is exacerbated either by the fact that malaria control programmes have virtually been scaled down prior to the Roll Back Malaria (RBM) initiative or do not exist at all. Compounding factors such as presence of *Plasmodium falciparum*, and the emergence of resistance to existing affordable drugs further aggravates this

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situation. In Africa for example, Chloroquine resistance is wide spread and resistance to Sulphadoxine/Pyrimethamine is being detected frequently (Robert, 2002).

Furthermore, the presence of mosquito vectors that are refractile to insecticides has also contributed significantly to the resurgence of malaria. For instance, the emergency of mosquitoes resistant to pyrethroid insecticides in South Africa now threatens insecticide-treated bed-net programmes (Greenwood and Mutabingwa, 2002). Environmental changes as a result of irrigation agriculture and construction have led to an increase in malaria transmission (Keiser *et al*, 2005). In West Africa, Malaria vectors (*Anopheles gambiae* complex) have adapted to breeding in polluted water (Gomes *et al*, 1998) therefore posing another challenge to vector control.

Increased population and Human migration has contributed to malaria resurgence. During the past two decades the population of many malaria endemic countries has doubled, thus greatly increasing the absolute numbers of those at risk. In malaria endemic countries, travel of non-immunes from malaria-free to malaria endemic areas for work is probably an increasingly important and largely unrecognized cause of severe malaria (Martens and Hall, 2000).

Poverty levels are also worsened by the morbidity due to malaria infections. Those people who cannot afford the most effective medicines will be regularly incapacitated from bouts of malaria fever (Lindblade *et al,* 1999). Floods associated with increased EL nino rains have precipitated epidemics of malaria in Africa (Brown *et al*, 1998). However, a report by Hay (2002) on malaria resurgence in the East Africa highlands indicates that climatic change is not responsible for the resurgence of malaria.

1.1 Historical Perspective of Malaria Control in Zambia

Malaria prevention and control in the copper mining towns in Zambia has a long history. Utzinger et al., 2002 cites early economic payoffs of malaria control activities between 1930-1950 that were carried out on the copper belt communities in Zambia. Control of *Anopheles gambiae* s.l. through environmental control has succeeded in several parts of the world. Early malaria prevention and control efforts in the copper belt towns had a stronger emphasis on environmental management, combined with diagnosis and treatment of malaria cases (National Malaria Control Centre, 2000). These source reduction activities in Zambia seven decades ago reduced malaria incidence by 50% (Utzinger et al, 2001). Additionally, the statutory instrument referred to as the Mosquito Extermination ACT (CAP 312) of 1944 (Mosquito Extermination Act, 1944), whose goal was primarily to improve hygienic measures was introduced. However, amendment of the ACT (CAP 537) in 1964 obliged each household to stop mosquito breeding in their immediate environment by requiring mining companies, irrigation and water supply works to take specific measures (Mosquito Extermination Act, 1964).

By the 1950s national health authorities in Zambia adopted Indoor Residual Spraying (IRS) in urban communities, including the Copper mining areas. However, the decline in copper price together with the ban of DDT for agricultural use led to unavailability of affordable and effective insecticides and negatively affected the Zambia's national malaria control programme. Thus, by 1980, the IRS coverage was reduced markedly and finally stopped altogether.

1.2 Re-appraisal of Malaria in Zambia

Malaria is one of the major causes of morbidity and mortality, especially in children under the age of five in Zambia (National Malaria Control Centre, 2001). The disease is hyper-endemic in hot riverine valleys with perennial transmission,

meso- to hypo-endemic on plateaus, and hypo-endemic in urban areas (Watts et al., 1990). Malaria is endemic in all nine provinces and communities in many parts of Zambia have been found to rate malaria as their number one health problem (Chimumbwa, 1998). Malaria causes anaemia in children and are unable to concentrate at school (Premji *et al*, 1995 and Shiff *et al*, 1996), and consequently the society as a whole is debilitated. The National Malaria Control Centre (2001) report shows that malaria accounts for the greatest number of Disability Adjusted Life Years (DALY) lost (6.8 million) followed by the Acute Respiratory Infections (5.4 million) and HIV/AIDS (3.2 million). In this case, malaria creates a significant barrier to economic development.

Malaria in Zambia has been on the increase with an annual incidence rate of 7.3% and rising mortality rates of 5.2% and 7.7% in children and adults respectively (Ministry of Health, 1998). The disease is meso- to hyperendemic in rural areas, and hypoendemic in urban areas of Zambia (Watts et al., 1990). Malaria control activities that were in effect since 1964 have slowly and steadily grounded to a halt. Noticeably, so that prevalence and incidence of malaria increased in the late 1970's. This upsurge of malaria prevalence and incidence was further aggravated by the development of chloroquine resistance in 1995 (National Malaria Control Centre, 1995). By 2002 in Zambia overwhelming evidence showed that chloroquine was failing to cure over 50% of patients by day 14 (National Malaria Control Centre, 2000; Hamer et al, 2003; Bijl et al, 2000) and by 2003, pyrimethamine with sulphadoxine was failing in 8-33% of patients (Bijl et al, 2000; Chanda et al, 2004).

To illustrate this, public health sector records show upward trends in malaria morbidity and mortality rates. From 1976 to 1999, the malaria incidence rate per 1000 population nearly tripled from 121.5 to 308.4. Malaria case fatality rates show a similar pattern. The proportion of health centre and hospital admissions for malaria that ended in death rose from 10.6 per 1000 in 1976 to 27.4 in 1989.

From 1990 to 1994, the proportion of hospital admissions for malaria that ended in death rose from 38.8 to 51.3 per 1000 (National Malaria Control Centre, 2001).

Children under five years accounted for 45.7% of admissions and 48.6% of deaths in 1993. The same group accounted for 45.5% of admissions and 46.1% of malaria deaths in 1994. Malaria deaths were slightly more common in this group among children 0-1 year of age. Reports from the University Teaching Hospital in Lusaka have shown that the proportion of maternal deaths due to malaria have risen from 13% in 1989 to 20% in 1998 (National Malaria Control Centre, 2001). The current maternal mortality rate in Zambia is high at 649 per 1000 live births (Central Board of Health, 1999). In a recent study, the perinatal mortality rate (still births plus deaths in the first week of life) was found to be 52.3 per 1000 deliveries, while neonatal mortality rates (live births who die within the first 7 days of life) was found to be 31.1 per 1000 live births (Chintu, 1976).

At Loloma mission hospital in rural area of North –Western province of Zambia, complicated malaria is the most common reason for admissions in children under five years of age. During the 1997 to 1998 rainy season (1st November to 31st May) 5882 cases of malaria (an average of 840 per month) were diagnosed in children under five, during the same period, 19(16%) of 116 in-hospital deaths (all ages) were due to malaria. (Mc Clean *et al,* 2002).

Malaria associated anaemia is a serious cause of morbidity and mortality in this region (Lackritz et al, 1992). Severe malarial anaemia accounted for a significant proportion of malarial admissions. Transfusions are reserved for children demonstrating signs of decompensated anaemia with respiratory distress, and with haemoglobin of less than 40g/*l*. Two hundred and fifty-six transfusion (68% of all transfusion) where given for treatment of severe malarial anaemia (Mc Clean *et al,* 2002).

1.3 Current Status of Malaria Control in Zambia

Between 1999 and 2000, the Roll Back Malaria Movement partnership was initiated (Chimumbwa, 2001;Masaninga, 2001;Masaninga, 2002). This public-private partnership initiative stimulated the development of the National Malaria Control Strategy covering the period 2000-2005 whose overall goal was *to reduce malaria morbidity and mortality in Zambia by 50%* (National Malaria Control Centre, 2001). The Strategy emphasized both malaria treatment and prevention. The prevention component focused on the need for developing a targeted vector control strategy that prioritized ITN distribution.

Zambia used the RBM Strategic plan (2000-2005) to build and develop strategies, systems and structures for National Malaria Control. In this regard, the Ministry of Health with the national Roll Back Malaria (RBM) partnership has developed a new National Malaria Strategic Plan (NMSP 2006-2011) whose vision is *"A malaria free Zambia"*. The major innovation in the new policy is that in addition to, a new and highly effective drug policy with the deployment of, *Artemether Lumefantrine* (Coartem®) (National Malaria Control Centre, 2004). The prevention component is based on the Integrated Vector Management (IVM) strategy of evidence-based and cost-effective package of interventions. A strong Monitoring and Evaluation has been built into together with strong collaboration of various public and private agencies that impinge on vector breeding, such as agriculture and urban development such as local authorities and engagement of communities (National Malaria Control Centre, 2005; World Health Organization, 2004).

The main interventions under the IVM strategy are Indoor Residual Spraying (IRS) in eligible urban and Insecticide Treated Nets (ITNs) implemented in rural areas. Larviciding and simple environmental management (canalization, draining and land filling) are implemented in collaboration with the local authorities and communities as supplementary vector control interventions in urban areas during

the dry season when the breeding sites for *Anopheles* vectors are discreet and accessible.

1.4. Statement of the problem:

The most important vectors of malaria in Zambia are *Anopheles gambiae sensu stricto*, *Anopheles arabiensis* and the riverine *Anopheles funestus* (Bransby – Williams, 1979). However, very little is known about the stratification of these vectors in terms of actual distribution and breeding characteristics in peri-urban Lusaka. Bransby –Williams (1979) determined the relative densities of *Anopheles gambiae* and *Anopheles arabiensis* and found that *Anopheles arabiensis* was the most predominant species caught in houses in Zambia and was probably the only one transmitting peri-urban and urban malaria. Since the distribution and abundance of both species are strongly influenced by climatological factors especially annual precipitation (Lindsay *et al* 1998), there should be a marked change in their distribution, breeding characteristics and habitat preferences in peri-urban Lusaka considering not only the duration of time that has elapsed since the study was conducted but also the extensive environmental changes occurring in these areas that have invariably created new breeding sites. The stratification of malaria could not have remained the same.

1.5. Study Justification:

The importance of this study lies in the fact that the assessment of malaria transmission, bionomics and breeding characteristics of the *Anopheles* species present in peri-urban Lusaka will help overcome the difficulties encountered in the selection and implementation of malaria vector control strategies. The yielded information will not only lead to new understanding of the epidemiology of malaria, and of the ecology of vectors and parasites but also assist in the implementation of evidence based malaria vector control interventions tailored towards the distinct bionomics, ecological and vectoral characteristics of the present species.

In the last 30 years, only a few publications have dealt with entomological and transmission dynamics in either rural or urban areas in Zambia (Bransby–Williams, 1979; Shelly, 1973). There is, therefore, a need for vector studies in peri-urban Lusaka to obtain base line data in order to appraise present and future situations with a view to bridge the the existing knowledge gap in the decision support systems. Transmission in peri-urban is likely to have changed owing to the creation of new breeding sites on the periphery of the cities by extensive building activities, uncontrolled population growth, nature of housing and proximity to the malaria endemic rural areas. In Lusaka, the sprawling peri-urban areas are a potential source of malaria in the densely populated urban area.

The extensive environmental changes; including agricultural practices in peri-urban Lusaka that parallel those of rural areas perpetually create ideal breeding sites for Anopheline mosquito vectors. Also, public health facilities and malaria control activities in most peri-urban location in Lusaka are either non-existent or have been vandalized and lie in a state of dilapidation and effective malaria control efforts are difficulty due to unplanned construction. This scenario has led to the proliferation of mosquito breeding sites, and snap surveys indicate that individuals and whole communities have inadequate knowledge, attitude and practice as regards malaria (Chimumbwa, 1998). The assessment of malaria transmission risk occurring in peri-urban Lusaka is imperative before any attempt to execute malaria control strategies is made.

Information on Malaria existence in Lusaka is scanty. *In vivo* sensitivity test have been conducted with *Plasmodium falciparum* patients in Lusaka, Zambia (Blom, 1995), but whether these infections were acquired in Lusaka itself or in rural areas is not clear. However, Ngandu *et al,* (1989) reported the presence of urban or sub-urban Malaria transmission in Lusaka, Zambia. Data from these areas are important, and further confirmation is necessary before approaches to urban malaria control can be considered (Mc Wilsons *et al*, 1999).

CHAPTER 2.0: LITERATURE REVIEW

2.1 Mosquitoes of Medical Significance

Mosquito vectors of medical significance belong to several genera such as *Aedes, Culex, Culiseta, Mansonia, Psorophora, Anopheles and Haemagoguos. Culex* are the vectors of filariasis and Japanese encephalitis, *Aedes* of dengue, dengue hemorrhagic fever, and yellow fever. However, the malaria parasites can only be transmitted by the females in the genera *Anopheles* (Manson-Bahr and Bell, 1991). Currently 422 species of *Anopheles* mosquitoes have been identified through out the world (Bruce-chwatt, 1985), many of which are species complexes. However, only about 80 are capable of transmitting malaria, 70 species are vectors of malaria under natural conditions and approximately 45 are of major significance (Nicholas, J. White, 2000). *Anopheles* mosquitoes are most frequently found in tropical regions, but are also found in temperate climates and in the arctic during summer. As a rule, they are not found at elevations above 2000 to 2500 meters. The exceptionally high malaria transmission rates in sub-Saharan Africa are in large part due to the constant presence of the efficient and competent mosquito vectors with high vectoral capacity. Most of these Species belong to the *Anopheles* (Cellia) *gambiae* Giles complex and *Anopheles (Cellia) funestus* Giles group (Rogers et al, 2002).

2.2 Malaria vectors: *Anopheles gambiae* complex and *Anopheles funestus* group

The *Anopheles gambiae* and *An. funestus* species complexes are biologically diverse groups of mosquitoes that contain the most recognized and widespread malaria vectors in sub Saharan Africa. Each of the two complexes is a group of morphologically indistinguishable yet genetically and behaviourally distinct mosquito species that vary dramatically in their importance malaria vectors (Coluzzi et al., 1979): *An. gambiae* s.s. and *An. arabiensis* in the *An. gambiae* complex, and *An. funestus* s.s. in the *An. funestus* complex (Gillies and DeMeillon

1968, Gillies and Coetzee 1987). The *An. gambiae* complex is comprised of seven species, and the *An. funestus* complex of nine species (Gillies and DeMeillon 1968). The *Anopheles gambiae* Giles complex comprises fresh-water breeders; *Anopheles arabiensis* Patton, *Anopheles gambiae sensu stricto,* Giles and *Anopheles quadriannulatus* Theobald. Salt-water breeders *Anopheles melas* Theobald *and Anopheles merus* Dönitz as well as the mineral- water breeder *Anopheles bwambae* White (Scott *et al*, 1993). Whereas, the *Anopheles funestus* group is composed of nine members: *Anopheles funestus, Anopheles vaneedeni* Gillies and Coetzee, *Anopheles leesoni* Evans, *Anopheles rivulorum* Leeson, *Anopheles parensis* Gillies, *Anopheles fuscivenosus* Leeson, *Anopheles aruni* Sobti, *Anopheles brucei* Service, and *Anopheles confuses* Evans and Leeson (Koekemoer *et al*, 2002).

2.3 Malaria Vector Bionomics

In sub-Saharan Africa, the most important vectors of malaria are members of the *Anopheles gambiae* complex. The complex comprises seven sibling species that differ in their efficiency to transmit malaria (White, 1974; Hunt *et al*, 1998). The most prevalent and key malaria vectors in sub-Saharan Africa are *Anopheles gambiae s.s*, *Anopheles arabiensis* and *Anopheles funestus.* These species differ in both their vectorial capacity and populations largely due to variation in their propensity towards anthropophagy, dispersal and temporal activity throughout the season (Norris 2002). The distribution of these malaria vectors shows similarities with the pattern of annual precipitation across Africa (Rogers et al, 2002).

Of the three malaria vectors, *Anopheles gambiae* s. l. and *Anopheles funestus* are highly endophagic and anthropophagic. *Anopheles arabiensis* is largely zoophagic and endophilic (Gillies *et al*, 1987). *Anopheles gambiae s.s* is anthropophagic in that it possesses a remarkable tendency to feed preferentially on humans. Besides, it is a long- lived species, breeds through out the year and bites man frequently. The temperatures in the tropics are ideal and favour parasitic

proliferation and hence, *Anopheles gambiae* contributes significantly to the stability of malaria transmission in sub-Saharan Africa. *Anopheles funetus* is anthropophagic and endophilic and is a highly efficient vector of malaria (Gillies *et al*, 1968). The distribution of *Anopheles funestus* is widespread through out subtropical Africa, and can be found in sympatry with other members of the complex (Gillies *et al*, 1987). *Anopheles gambiae* breeds in temporary water bodies without vegetation and in open sunlight, where as *Anopheles funestus* breeds in permanent water bodies with vegetation, including ponds and swamps.

Anopheles gambiae and *Anopheles arabiensis* are both the most broadly distributed and the most efficient vectors of malaria (White, 1974; Coetzee *et al*, 2000). The range and relative abundance of *Anopheles gambiae* and A*nopheles arabiensis* appear to be strongly influenced by climatological factors, especially annual precipitation (Lindsay *et al*, 1998). In general terms, *Anopheles arabiensis* tends to predominate in arid savannas, where as *Anopheles gambiae* is the dominant species in humid forest (White 1974; Lindsay *et al*, 1998; Coetzee *et al*, 2000). Moreover, where the two species occur in sympatry, large changes in species composition often occur with A*nopheles arabiensis* predominating during the dry season and A*nopheles gambiae* becoming more abundant during the rainy season (Di Deco *et al*, 1981). However, *Anopheles gambiae* may sometimes be more abundant than *Anopheles arabiensis* during the dry season or vice-versa (Service, 1970; White and Rosen, 1973).

2.4 *Anopheles gambiae* Chromosomol and Molecular forms

Anopheles gambiae is the most efficient Afro-tropical malaria vector. In West Africa, *A. gambiae* has been divided into five chromosomal forms designated with a non-Linean nomenclature: bamako, mopti, savanna, forest and bissau (Colluzzi *et al.*, 1985; Toure *et al.*, 1994,1998). The first evidence for genetic heterogeneity within this mosquito species came from cytological observations of the banding pattern of polytene chromosomes that revealed a complex system of

polymorphic paracentric inversions leading to different chromosomal arrangements (Wondji *et al.*, 2002) and frequencies of alternative arrangements, especially involving inversions on chromosome 2, (Coluzzi *et al.*, 1985; Touré *et al.*, 1994, 1998).

These chromosomal forms have been characterized based on the presence or absence of certain diagnostic inversions combinations (e.g. inversion 2Rb in Savanna, 2Rj in Bamako, 2Rbc/u in Mopti, 2Rd in Bissau), different relative frequencies of polymorphic arrangements (e.g. the standard arrangement 2R+/2L+ is almost fixed in Forest, but rare in the other forms), geographical distribution and ecological data (e.g. Mopti is adapted to dryer environments where it breeds all year long in irrigated fields, while Forest is exclusively found in more humid forested areas). Their distribution is, therefore, associated with particular climatic zones.

To provide more insights into their taxonomic status, recent efforts have focused on the pattern of variation observed with molecular markers. This revealed the existence of two genetic variants referred to as the molecular M and S forms (Favia *et al.*, 1994; Della Torre *et al.*, 2001; Charles *et al.*,2001; Wondji *et al.*, 2002). The characterization of the forms is based on either internal transcribed spacer (ITS) or intergenic spacer (IGS) variants (Masendu *et al.*, 2004). Both forms are anthropophagic and effective vectors of human malaria parasites (Awolola *et al.*, 2005).

2.5 Vector Resistance to Insecticides

Insecticide resistance is defined by the WHO as "the development of an ability in a strain of some organism to tolerate doses of a toxicant that would prove lethal to a majority of individuals in a normal population of the same species" (Zlotkin, 1999). There are four classes of insecticides that play a significant role in agriculture and public health, *viz.*, organophosphates (OP), organochlorides (OC),

carbamates and pyrethroids. DDT (dichlorodiphenyltrichloroethane) was first introduced for mosquito control in 1946. In 1947 the first cases of DDT resistance occurred in *Aedes* (Brown, 1986). Since then over a hundred species of mosquito have become resistant to one or more insecticide (WHO, 1992). Insecticides used for malaria control have included benzine hexachloride (BHC), organophosphorus, carbamate, and pyrethroid insecticides. Other insecticide groups, such as the benzylphenyl ureas and *Bacillus thuringiensis* (Bti), have had limited use against mosquitoes.

Benzine hexachloride/dieldrin resistance is still widespread, despite the lack of use of these insecticides for many years. OP resistance, in the form of either broadspectrum resistance or malathion-specific resistance, occurs in many vectors (Hemingway, 1982, 1983; Hemingway and Georghiou, 1983; Herath *et al.*, 1987). OP resistance is widespread in all the major *Culex* vectors (Hemingway and Karunaratne, 1998) and pyrethroid resistance occurs in *C. quinquefasciatus* (Chandre *et al.*, 1998). Pyrethroid resistance is widespread in *Ae. aegypti* (Hemingway *et al.*, 1989) and cases of OP and carbamate resistance also occur in this species (Mourya *et al.*, 1993). The development of pyrethroid resistance in *An. gambiae* is particularly important given the recent emphasis on the use of pyrethroid impregnated bednets for malaria control (Vontas *et al.*, 2001).

2.6 Insecticide Resistance Management

The management of insecticide resistance, or more precisely, the management of vector susceptibility is crucial. There are two possible options in the event of insecticide resistance: one is to use an insecticide until resistance becomes a limiting factor or, preferably rotating or alternating insecticides as a resistance management strategy before resistance reaches measurable levels (Curtis *et al.*, 1993). The use of an insecticide until resistance becomes a limiting factor is rapidly eroding the number of suitable insecticides for insect control. Rotations, mosaics, and mixtures have all been proposed as resistance management tools

(Tabashnik, 1989). However, these models have rarely been tested under field conditions due to the practical difficulties in estimating changes in resistance gene frequencies in large samples of insects (Hemingway *et al.*, 1997). With the advent of different biochemical and molecular techniques for resistance gene frequency estimation, field trials of resistance management strategies have become more feasible. A large-scale trial of the use of rotations or mosaics of insecticides compared to single use of DDT or a pyrethroid is currently under way in Mexico (Penilla *et al.*, 1998).

2.7 The Malaria Parasites

The microorganisms causing malaria are commonly referred to as malaria parasites. These protozoa of the genus *Plasmodium* are pigment–producing amoeboid intracellular parasites, with one habitat in red blood cells and another in hepatocytes (Manson-Bahr and Bell, 1991; Markel and Voges, 1999). Four principal species infect man and cause the four specific types of malaria; *Plasmodium falciparum* that causes Falciparum malaria, *Plasmodium malariae*, causing Quartan malaria, *Plasmodium vivax,* causing Vivax malaria and *Plasmodium ovale* that causes Ovale malaria (Bruce-chwatt, 1985). Accurate knowledge of the geographical and longitudinal distribution of the four *Plasmodium* species infecting man is of crucial significance, since they differ markedly with respect to their morphological features, biology and clinical manifestations (Brumpt, 1949; Garnham, 1966; White, 1996).

Falciparum malaria and Ovale malaria are primarily diseases of the tropics (Markel and Voges, 1999). *Plasmodium falciparum* is cosmopolitan in distribution but confined to the tropics and subtropical areas. It has a 48 hours developmental cycle with a prepatent period of 8 to 15 days and invariably parasitizes both young and mature red cells (of all ages) in man but envades young cells to a greater extent (Manson-Bahr and Bell, 1991). As high as 10% red blood cells may be infectious rate leading to high parasitaemia and therefore death

ensues. *Plasmodium ovale* has been known since 1922. It is widely distributed in tropical Africa especially in West Africa were it has apparently supplanted *Plasmodium vivax* almost entirely (Markel and Voges, 1999). *Plasmodium ovale* has a 48 hourly schizogony and 17 days prepatent period.

Quartan malaria is seen in the subtropics and temperate zones as well, while Vivax malaria is usually the most common in all endemic areas. *Plasmodium malariae* occurs in Tropical Africa, Asia, used to be in Europe and United States of America (Manson-Bahr and Bell, 1991). It has a 72 hourly schizogony and paroxysms occur every 4^{th} day with a prepatent period of 28 hours. *Plasmodium vivax* is the most predominant malarial parasite in most parts of the World. It is found in endemic areas between latitudes 16°N and 20°S and is the only species whose range extends into temperate regions (Markel and Voges, 1999). It has a prepatent period of 15 days with a 48 hourly cycle. This species has a predilection for young and immature red blood cells with infection rates of about 1%.

Although the four aetiological agents of human malaria differ clinically and in global distribution, all parasitize red blood cells, destroy them and release many factors. The presence of only 10 parasites per cubic centimeter of blood cause clinical malaria. However, the transmission of all the species of malaria parasites depend on the presence both of suitable species of *Anopheles* mosquitoes and of infected (gametocyte-bearing) humans.

2.8 Malaria Transmission in Peri- urban Areas

Population expansion and it's social, economic and health impact has been epitomized by Sub-Saharan Africa, in which cities have grown faster than those in any other region (United Nations Population Fund, 1996). For instance, Zambia is the most urbanized country in the region, with 42% of its population living in urban and peri-urban areas. In the past, malaria was regarded as a typical rural disease, and it was a notifiable disease in major towns, as such, its occurrence in

urban and peri-urban areas was attributed to the influx of infected visitors from endemic rural areas. This migration resulted into the rapid growth of urban and peri-urban population that has in turn led to a rise in the incidence of the disease (Mc Wilsons *et al*, 1999). Lusaka was, for instance, initially free from malaria until the late 1970s and early 1980s (Shinondo, 2004 unpublished; Barat *et al*, 1998).

The rapid increase in the rate of urbanization and the inherent population increase have raised great concern over its direct and significant impact on Malaria epidemiology. For example, it has been proved that, although formal urban development typically reduces mosquito densities, the informal development occurring in Sub-Saharan Africa often simply changes the vector species composition, as has been well documented in Dar-es-Salaam (Tanzania), Edea (Cameroun) and Benin City (Nigeria) (Feachem and Jamison, 1991; Wagbatsoma and Ogbeide, 1995). This is further augmented by the emergency, in areas which where once classic rural environment, of densely populated communities on the periphery of established cities where most new growth takes place. This scenario can be ideally exemplified by Lusaka, which has virtually been engulfed by the shanty compounds that have mushroomed all over the city perimeters and have thus encroached on the capital city. In the process, some well-known mosquito breeding sites have disappeared and new ones have appeared.

In areas of endemicity, encroaching transmission has been reported in areas previously free of transmission. This is likely to be a response to warming climatic conditions or else to periods of prolonged summer rains, which occur as a result of extreme climatic episodes, which are becoming more frequent. Examples of this can be seen with the advent of malaria epidemics in the highlands of Madagascar (Albonico *et al*, 1999), Kenya, where transmission may encroach on Nairobi and the Kenya highlands; and Uganda where similar encroachment is happening (Lindblade *et al,* 1999) as well as in Lusaka, where malaria transmission occurs although the city was previously free of transmission (Barat *et al,* 1998).

In peri-urban areas the activities of the people parallel those in the rural settings and their agricultural ventures such as small garden plots, irrigation facilities, excavation associated with estate development continue to provide ideal breeding sites for the *Anophelines.* For example, *Anopheles gambiae*, the primary malaria vector in Africa, has been known to exploit even transient water sources such as hoof prints.

It was originally thought that urban areas do not support significant levels of malaria transmission. The concentration of human population in small areas is normally accompanied by pollution and the destruction of clean water sources required by the anopheline mosquito vectors of malaria. This process usually proceeds through predictable sequence of changes. As the population grows, clean water breeding sites are overwhelmed with sewage and other pollutants, and anopheles can no longer develop. In many cases, however, as one area becomes polluted, a new one with malaria vector breeding develops a short distance away (Mc Wilson*set al*, 1999). This has proven true of the *Anopheles gambiae* complex in Africa, and is the position taken by many malaria investigators with exclusive experience in tropical Africa (Trape, 1987).

However, in Ghana *Anopheles gambiae* has adapted to breeding in household water containers (Chinery, 1984). Unpublished observations that describe the adaptability of *Anopheles gambiae* to changing environments cause considerable concern. This mosquito has been observed to develop in relatively polluted sites, even those containing household wastes. (Mc Wilsons *et al*, 1999).

As can be seen, there is frequent but indirect evidence of urban transmission of malaria in large cities in Sub-Saharan Africa. Most of these investigations had a primary focus other than establishing urban malaria transmission. Therefore specific local investigation would be needed to confirm that malaria is truly endemic in these cities. These investigations will have to establish the source of infection in urban malaria and also examine travel histories (Ng'andu *et al,* 1989).

This information would also help establish priorities for further studies. For example, in one study, malaria was diagnosed in under one year olds with no travel history in Lusaka (Shinondo, 2004 unpublished data).

The presence of malaria in urban environments presents a number of challenges whether locally transmitted or imported from peri-urban or rural areas. In many situations, the rate of acute disease may be higher in urban populations than in peri-urban and rural areas where the levels of transmission are high in susceptible children but low in adults due to relatively high levels of immunity (Trappe and Zoulani, 1987). To illustrate, Watts et al (1990) called the attention to the vulnerability to malaria of populations with low resistance in Lusaka, Zambia. They further reported that there was low malaria transmission in Lusaka with parasites rates of 2.4 % at the beginning of the rainy season (November) and 10.3% at the end of the end of the rainy season (April). Spleen rates were estimated at 3% in the same period. In another study it was observed that in some cases transmission in urban and peri-urban areas of Sub-Saharan Africa is intense but seasonal and for shorter time periods than in rural areas (Mc Wilsons *et al,* 1999).

2.9 Re-appraisal of malaria parasites in Zambia

In a recent survey, malarial parasites rates in Zambia were estimated to range from 2 to 25% (National Malaria Control Centre, 1995). However, Greenwood and Mutabingwa (2002) observed that the gravity of the risks of malaria differs from area to area across Africa indicating that there are marked disparities in the prevalence of malaria in different countries of sub-Saharan Africa. In studies conducted on the Copperbelt Province of Zambia, on urban transmission of malaria, it was observed that patterns of transmission were different in the dry and rainy seasons and that this should be taken into consideration when formulating control strategies (Sukwa, *et al*, 1998).

There are still gaps in our knowledge of malaria transmission due to the fact that the human, parasites and vector relationships are poorly defined. Therefore, there is need to continually update our knowledge regarding the parasite and the vector. For example, in a survey conducted between 1969 and 1972 in Ndola rural, it was confirmed that 26.4% of the people in the area harboured malaria parasites in their blood (Utzinger *et al*, 2001). *Plasmodium falciparum* was the predominant species detected, accounting for 86.8% of the cases, while *Plasmodium malariae* was found in 13.2% of the cases (Wenlock, 1978). Recent empirical approaches, assessing the distribution limits of *Plasmodium falciparum* transmission in Sub-Saharan Africa, confirmed that Ndola rural district is characterized by stable endemic malaria conditions (Snow *et al,* 1999). Another recent study conducted in 1999 by the National Malaria Control Centre indicated that in Zambia 95% of microscopically diagnosed malaria cases are caused by *Plasmodium falciparum* followed by *Plasmodium malariae* (3%) and *Plasmodium ovale* (2%). *Plasmodium vivax* is rarely detected in Zambia (National Malaria Control Centre, 2001).

2.10 Re-appraisal of Malaria Vectors in Zambia

The first evidence of the existence of *Anopheles* mosquitoes in Zambia comes from identifications made when malaria control activities commenced on the Roan Antelope Copper Mine in Luanshya in 1929 (Watson, 1953; Utzinger *et al*, 2001). De Meillon visited the Copper mines in 1937 and conducted a number of entomological evaluations. He determined the flight range of the *Anopheles* species, and recovered *An. gambiae* Giles s.l. 4.2 kilometers from the point of release, while *An. funestus* Giles was recovered 4.5 km from the release site (Coetzee, 1945). Paterson (1963) shed more light on the Zambian vectors when he drew a distribution map showing the dispersion of fresh and salt-water breeding members of the *An. gambiae* Giles s.l. group on the African continent.

Entomological baseline surveys revealed that *Anopheles funestus* and *Anopheles gambiae* were the predominant malaria vectors (Bransby–Williams, 1979), though, recent studies have implicated *Anopheles arabiensis* as another important vector (National Malaria Control Centre, 2001). While *Anopheles funestus* accounted for approximately 80% of adult catches, *Anopheles gambiae* was the most abundant species encountered in larval catches (Utzinger *et al*, 2001) in Luanshya. Detailed ecological studies on the larvae habitat preferences showed that *Anopheles gambiae* was found in open and unshaded natural or man made pools close to the Luanshya river and it's tributaries, as well as open water tanks and in native wells loosely over grown with grass. Reduction and estimation of such habitats was relatively straightforward. In contrast *Anopheles funestus* larvae preferred the shaded banks of the Luanshya river and it's tributaries, and were also found in flooded areas and swamps which were normally formed and sustained for an extensive period after the rainy season (Utzinger *et al*, 2001).

Adams (1940) studied the flight ranges of *Anopheles gambiae* and *Anopheles funestus* in N'Kana, a village in the Copperbelt province bordering the Democratic Republic of the Congo, however these results come decades prior to the delineation of the *Anopheles gambiae* and *Anopheles funestus* species complexes. Pielou (1947) confirmed the presence of *Anopheles gambiae* (s.l.) and *Anopheles funestus* (s.l.) in various types of breeding sites in Northern Rhodesia (Zambia), but makes no mention of the exact locations where the collections were performed.

The most important vectors in Zambia are two fresh water members of the *Anopheles gambiae* complex (*Anopheles gambiae s.s* and *Anopheles arabiensis)* and *Anopheles funestus,* also a fresh water breeder (Bransby–Williams, 1979). However, little is known about the actual distribution of these mosquito species in Zambia. Knowledge about the availability of malaria vector species in Zambia is based on a limited number of empirical observations and on the general

knowledge of mosquito species distribution in the Ethiopian Zoogeographical Region, which includes Zambia (Davidson and White, 1972).

In Zambia, especially in the peri-urban and urban areas, there is lack of knowledge on malaria vectors as indicated by the low number of publications on research carried out in Zambia. However, the majority of these publications are case studies and only a few dwell wholly on entomologic and transmission dynamics in either urban or rural areas. Bransby-Williams (1979) in a study carried out in 1972 showed that house caught mosquitoes in rural areas at Chirundu, Southern province of Zambia had a sporozoite rate of 0.18%. Vector speciation was 100% *Anopheles arabiensis*. Peterson (1963) recorded the co-existence of three *Anopheles gambiae* complex sibling species *Anopheles gambiae*, *Anopheles arabiensis* and *Anopheles quadriannulatus*. Ten years later in the same area in Chirundu it was found that *Anopheles gambiae* had disappeared and was replaced by *Anopheles arabiensis* (Shelly, 1973). Baseline data for Zambia is not readily available or accessible because of the paucity of publications on malaria vectors.

The period between 1973 and 2003 comprised a lull in entomological publications involving Zambian mosquitoes until Lehmann et al. (2003) examined the population structure of *Anopheles gambiae* in southern Africa using microsatellites, and Weeto et al. (2004) validated the presence of *Anopheles funestus* s.s. and *Anopheles leesoni* Evans in 11 countries including Zambia with a novel *Anopheles funestus* species-specific PCR diagnostic.

Mosquito speciation studies are significant in that, although *Anopheles gambiae* complex sibling species are morphologically indistinguishable, there are numerous important vector behavioural differences. *Anopheles gambiae s.s* is characteristically endophagic, anthropophilic and endophilic which renders it a good vector. Residual insecticides and pyrethrum impregnated bed nets can easily control this sub species. On the other hand, although *Anopheles arabiensis* which

also is an effective vector exhibits exophagy, anthropophily or zoophily, and therefore it is a much more difficulty species to control by residual insecticides and impregnated bed nets. In West Africa, it is now known that there is a chromosomal inversion polymorphic basis facultative behaviour of *Anopheles arabiensis*. Chromosomal inversions 2Ra and 2Rb are associated with behavioural traits influencing feeding and resting patterns. Females homozygous for the 2Rc chromosomal inversion are more exophilic than those heterozygous for the 2Rc trait. The latter are exophilic and zoophilic (Coluzzi *et aI*, 1977). Thus residual insecticide causes behaviouralistic avoidance and resistance in *Anopheles arabiensis*.

In this regard, it is epidemiologically significant to determine the relative densities of *Anopheles gambiae* s.s and *Anopheles arabiensis*, but it seems that *Anopheles arabiensis* is the predominate species caught in houses in Zambia (Bransby–Williams, 1979), and it may be the only one transmitting peri-urban and urban malaria. There has been no similar assessment in the last twenty years. There is need to revisit the malaria vector studies to cover peri-urban Lusaka, especially in view that there has been many environmental changes. Some well known breeding sites have disappeared and new ones have appeared. Mc Wilsons et al (1999) indicated that as peri-urban areas become better established; they tend to become less malarious due to the disappearance of non-polluted surface water as these areas are occupied for longer periods. However, due to the development of new breeding sites in the nearest vicinity as a result of estate development and their proximity to the hyper-endemic rural areas transmission is maintained (Feachem and Jamison, 1991; Wagbatsoma and Ogbeide, 1995).

An understanding of local vector's spatial and temporal bionomics and the environment in which they occur is a critical prerequisite in planning and implementation of effective malaria control programmes, vis sa viz, Vector control interventions.

1.6 General Objective

To assess malaria transmission factors and attributes in peri-urban Lusaka.

1.7 Specific objectives

1. To identify the breeding habitats for Anopheline mosquitoes in peri-urban Lusaka.
2. To establish larval habitat preferences by the anopheline mosquitoes in peri-urban Lusaka.
3. To identify the *Anopheles* species present in peri-urban Lusaka.
4. To determine parasite rates in the resident population.
5. To provide evidence of local transmission of malaria.
6. To determine levels of Knowledge, Attitudes and Practices of people on malaria in the study site.
7. To recommend appropriate malaria control strategy in the study site.

CHAPTER 3: MATERIALS AND METHODS

3.1 Study Site

This study was conducted in four locations of peri-urban Lusaka North, namely Kabanana, Chazanga and Chipata, which is geographically located about 10Kms on the northern part of Lusaka city (15°20.701S, 028°18.484E). The Great North Road on the western side and rail-line on the eastern boundary border this area. The site for the collection of mosquitoes included the area along the stream that runs from east to west and ending up in Chazanga dam.

Entomological surveys were also conducted in Kalikiliki (15°24.681S, 028°22.382E), a peri-urban area in the eastern part of Lusaka as shown in figure I. Only those locations on the peripherals of urban areas and exhibiting ideal peri-urban environmental conditions were selected for the study. By definition, a peri-urban area is one on the peripheral of urban settlement and in a process of urbanization. An urban area is a settlement with population concentrations, residential, commercial and industrial characteristics. A rural area relates to the countryside and includes agriculture, forested areas and landscapes.

3.2 Pilot Study

A pilot study was conducted in the selected study area. Two health centres (Chipata and Chazanga) located within the vicinity of these areas were visited to ascertain the parasite rates. This parasitological survey revealed 300 febrile cases were diagnosed as malaria per month basis at both centres. An entomological survey was simultaneously conducted at different randomly selected houses and an average of one female Anopheline mosquito vector in every ten mosquitoes caught was established. These findings were not included in this study.

3.3. Study design

This study was purely empirical in nature and therefore involved the following; a specified malaria incidence study site in peri-urban Lusaka, appropriate Scientific methods were employed and an ideal sample size was used.A written concent form was also used. Also 60 randomly collected female *Anopheles* mosquitoes were studied. The collected data was analyzed by employing Epi -Infor version 3.2.2. The end of the study was determined by both the lapse of the study period and the attainment of the targeted sample size.

Figure 1: Map of greater Lusaka showing the Peri-urban study site locations and Breeding sites

Sample Size

The sample of residents was calculated as follows

$$N=\frac{z^2 \times pq}{e^2}$$

where N = required sample size

Z = confidence limit = 1.96 @ 95%.

P = known or estimated proportion =30% or 30±5%

Q = 100 – P = 70%

E = worst acceptable error = 5%

$$N = \frac{1.96 \times 30 \times 70}{5^2}$$

$$= \frac{3.84 \times 30 \times 70}{25}$$

$$= \frac{3.84 \times 30 \times 70}{25}$$

$$= \frac{8064}{25}$$

$$= \mathbf{323}$$

Therefore,

Sample size = N

$$= 1 - \frac{(N)}{(\text{Population})}$$

Population of Lusaka = 2,000.000.

N = 323

Therefore $= 1 - \frac{323}{2 \times 10^6}$

$= 1 - 0.0001615$

$= 0.9998385$

Therefore, 323/0.9998385 = **323**

Mosquito vector sample size:

$$N = \frac{Z^2 \times pq}{e^2}$$

Where; N=required sample size

P = 10

E = 5%

Z= 1.96

Q = 100-10 = 90

Therefore $N = \frac{1.96^2 \times 10 \times 90}{5^2}$

$= \frac{3.84 \times 900}{5^2}$

$= \frac{3456}{25}$

= **138**

3.4. Entomological Surveys

Entomological Surveys were conducted in order to determine Anopheline breeding sites, to identify larval habitat preferences and to determine the presence of Anopheline mosquitoes in homes and mosquito-human contacts. Data were collected through entomological surveys of larval, pupal and adult mosquito collections.

3.4.1. Larval and pupal collections

The ecological preferences by larvae of the malaria vectors were determined by identifying potential breeding sites and collecting the larvae from the water bodies using the dipping method. Anopheline larval densities were determined by counting larvae in standard 250 mls of water scooped from their breeding habitats

which were also mapped by the hand held Global Positioning System unit (MAGELLAN GPS 315) for future location.

Breeding sites were determined by taking larval samples in areas identified as potential breeding sites. Potential breeding sites included surface water, swampy areas, abandoned wells, water collections in vegetable gardens and building foundations.

Larvae and pupae were sampled using a WHO-standard 250 ml capacity mosquito dipper (WHO 1992). They were removed from the dipper with a wide mouthed pipette (an ordinary medicine dropper with the narrow end cut-off) and placed in collection bottles. Sampling was conducted in a deliberately non-random fashion to maximize sensitivity of collections using standard procedures (Bogh et al. 2003). The sampling sites were surveyed once per week. Anopheline larvae and pupae are normally found at the surface of a breeding place, unless disturbed by water turbulence, a shadow, or movement of the vegetation or object to which they may be clinging and when disturbed they dive towards the bottom and may remain hidden for several minutes. It was, therefore, important to attempt to get surface water into the dipper without previously disturbing the larvae and pupae. When the breeding area was open the dipper was skimmed with its brim just beneath the surface but for small paddles a long pipette fitted with a suction bulb was used.

Collected larvae and pupa were transported in appropriately labeled jars to the Insectary at the National Malaria Control Center in collection bottles. At the insectary, the larvae were reared to adults at 28 degrees centigrade and maintained on 1 part yeast and 2 parts dog biscuit. The imagos were maintained on 10% sucrose solution at 27degrees centigrade and relative humidity of 70-80% according to the Dry and Wet bulb Thermometer readings in the Insectary. The specimens were preserved individually on desiccated silica gel in Eppindorf tubes and stored in airtight desiccators for DNA extraction.

3.4.2. Adult mosquito collections

Adult mosquitoes were collected by the Pyrethrum spray catch method. A Deltamethrine based knock down pyrethroid (Doom) was used with large white Calico sheets in both formal and informal houses. The collections were conducted between 04:00hrs and 06:00hrs after the rains from May to October in randomly selected households on different days. A total of 12 households located near the stream and shallow wells were sampled. Most houses were surrounded by cultivations of sweet potato and vegetables. The mosquitoes collected in this manner were transported to the insectary at National Malaria Control Centre for morphological identification.

3.4.3 Morphological Identification

Anopheles mosquitoes were morphologically distinguished from all caught mosquitoes and examined for the presence of blood. Adult mosquitoes including those reared from larvae was identified by using the morphological keys from the Supplement to the Anophelinae of Africa South of the Sahara, publication No.55 (Gillies and Coetzee, 1987). This was supplemented by publication N0.54 on Anophelinae of Africa South of the Sahara (Gillies and De Meillon, 1968).

3.4.4 Molecular Identification

The modified version of the Scott *et al* (1993) Polymerase Chain Reaction (PCR) analysis was used to differentiate the species within the *Anopheles gambiae* complex. DNA from specimens identified as *Anopheles gambiae s.s* were subjected to PCR assays for definitive identification of the molecular M and S forms (Flavia *et al*, 1994).

3.4.5 Marriott DNA extraction procedure

Mosquito specimens were rehydrated in Buffer (2X SSC+ 1% SDS) for 20 min and then rinsed in 100ul 1X TE for 10 min at room temperature. The dehydrated mosquitoes were homogenized with microfuge pellet pestles in 100 µl Bender buffer (0.1 M NaCl, 0.2 M sucrose, 0.1 M Tris-HCl, 0.05 M EDTA Ph 9.1, 0.5%SDS in DEPC water) The used microfuge pellet pestles were then placed in 1M Sodium hydroxide (NaOH) to make the loading buffer. The homogenized samples were incubated at 65 degrees Celsius for 1 hour, and 15 µl cold 8M Potassium acetate was added, then mixed gently and incubated on ice for 45 minutes. The samples were spun in a micro centrifuge at 14000rpm for 10 minutes, and then the supernatant was transferred to a new 1.5Ml microfuge tube. To precipitate DNA, 250 µl 100% ethanol (2.5X volume) was added to each supernatant. The samples were mixed well by inverting the tubes and incubated at room temperatures for 5 minutes and then centrifuged for 15 minutes at 14000rpm. The supernatant was carefully discarded, leaving the pellet behind in the tube and the pellets were respinned for 2 minutes and allowed to dry completely before resuspending in 20ul 0.1X SSC + 80ul dH20 and stored overnight at 4 degrees Celsius before use or stored at –20 degrees Celsius. To prevent DNA contamination in PCR-based analysis, pestles were soaked in 1M NaOH after use and then washed in soapy water, rinsed off in distilled water, and finally autoclaved before reuse.

3.4.6Anopheles Species Identification

The sub-species within the *An. gambiae s.l.* were differentiated by the modified version of the Scott *et al.,* (1993) protocol. A total of 25 µl reaction mix was used for the reaction with 1-1.5 µl template DNA. This contained; 15.25 µl of sterile deionised water, 2.5µl of 10X PCR buffer (15mM $MgCl_2$, 2.0 µl dNTP mix, 1 µl of 12.ng/µl of UN, 1µl of 6.25ng/µl of GA, 1µl of 12.5ng/µl of ME primers, 1µl of

25ng/μl of QD, 18.75ng/μl of AR and 0.125 μl of 5 units/μl of Taq DNA polymerase).

A positive markers for *An.gambiae s.s.* and *An arabiensis* were run on each agarose gel to ensure that the scoring was uniform across gels. A negative control with no template DNA was run with each set of the 25μl reactions mix but with an excess of 1μl deionised water. The primer sequences used to amplify the 28S coding region of the rDNA intergenic spacer and their amplification lengths of the nucleotides base pairs are as shown in the table below (Scott *et al.*, 1993).

Primer name	**Primer sequence (5"to3")**	**T (*C)**	**Base pairs**
UN	GTC TGC CCC TTC CTC GAT GT	58.3	
GA	CTG GTT TGG TCG GCA CGT TT	59.3	390
ME	TGA CCA ACC CAC TCC CTT GA	57.2	464/466
AR	AAG TGT CCT TCT CCA TCC TA	47.4	315
QD	CAG ACC AAG ATG GTT AGT AT	42.7	153

Table I: *Anopheles gambiae complex* ribosomal DNA (rDNA) intergenic spacer species-diagnostic primers and their respective melting temperatures and nucleotide base pair lengths. (GA-A. gambae, ME- A. merus, AR- A.arabiensis and QD- A.quadriannulatus) (Scott *et al.*, 1993).

Amplification begun by initial denaturing at 94 ° C for 2 minutes, followed by 30 cycles of denaturing at 94 ° C for 30 seconds, annealing of primers at 50 ° C for 30 seconds and polymerisation at 72 ° C for 30 seconds, and lastly by a cycle of extension for 7 minutes at 72 ° C.

PCR products were visualised on an agorose gel. 6μl of the post PCR product was mixed with 4μl of loading dye blue/orange dye and a volume of 10μl was run on the 2.5% agorase gel stained with ethidium bromide (10mg/ml). The first lane was

2µl of a 100 base pair DNA ladder. The gels were run at 120 Volts for 30 - 45 minutes and the bands visualized by illumination with short wave ultraviolet light.

3.5 Parasitological Surveys at Health Centres

Parasitological assessments were conducted to determine the prevalence of *Plasmodium* parasitaemia. Blood from from subjects who presented to the health center with febrile symptoms. The blood sample was screened for parasite species, parasite densities and gametocytaemia by microscopy using Giemsa thin and thick blood smears. Parasitological Surveys were conducted at Chipata and Chazanga health centres.

Inclusion criteria: Subjects presenting to the health center with febrile symptoms and consenting to participate in the study were included.
Exclusion criteria: Infants below six months were excluded from the survey.

The survey was conducted at the end of the rainy season, May to October. One hundred microscopic oil immersion fields were systematically examined and parasite densities were estimated. *Plasmodium species* were differentiated in thin blood smears.

3.5.1. Giemsa stained blood smears

3.5.1.1. Procedure for thin blood smears

Air dried thin film of finger prick blood was fixed by covering it with two drops of absolute methyl alcohol and the slide allowed to dry in the horizontal position. The fixed smear was stained in 1:10 Giemsa/Buffer solution (pH 7.2) for 15 minutes and rinsed in the gentle stream of tap water. The smear was then air-dried and studied for malaria parasites under oil immersion.

3.5.1.2. Procedure for thick blood smears

A thick blood smear was made from a finger prick and completely air-dried. The prepared smear was completely dehemoglobinised in distilled water, stained in 1:10 Giemsa (pH 7.2) for 15 minutes, and rinsed in a gentle stream of tap water. The smear was then air- dried and studied for malaria parasites under oil immersion.

3.5.1.3. Determination of parasite densities

This was achieved by reporting the parasite density in thick smears using plus signs with an accompanying interpretation of the grading scheme.

1-10 parasites per 100/O.I fields ---------- +

11-100 parasites per 100/O.I fields ---------- ++

1-10 parasites per every O.I fields ---------- +++

>10 parasites per every O.I fields ---------- ++++

At least 100 oil immersion (O.I) fields were examined for trophozoites and gametocytes before reporting, for example *Plasmodium falciparum* trophozoites +++, gametocytes +.

3.6 Determination of Knowledge, Attitude and Practices

A questionnaire was the tool used to determine KAP levels on malaria. History of travel and possible importation of malaria from rural or urban areas, family demographic data were obtained from the questionnaire (Appendix A).

3.7 Ethical consideration

Ethical approval for the research was sought and granted by the Ethics committee of the University of Zambia, School of Medicine (Appendix B). Written concent forms were provided to human subjects for inclusion in entomological and blood sample collections (Appendix C).

3.8 Data Analysis

The collected data was analyzed using the Epi- Info version 3.2.2. and manual calculations.

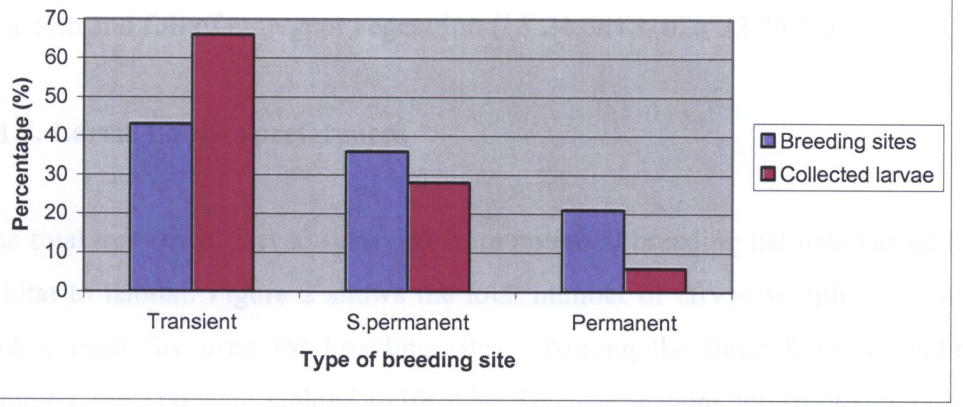
CHAPTER 4: RESULTS

4.1. Entomological Survey

4.1.1. Anopheles habitat identification

In this study, larvae were collected from the fourteen water bodies using the dipping method. The habitats ranged from Transient, through Semi-permanent, to Permanent water bodies. Transient fresh water collections are exemplified by open pools in the fields or stagnant streambeds, pools in cart tracks and building foundation furrows. Permanent or semi-permanent fresh water refer to open streams with vegetation, stream beds running over gravel, flowing water in canals and ditches, and streams in the forest, or plantations (Bruce-Chwart, 1985). The abundance of the breeding sites in peri-urban Lusaka was as shown in Figure 2 below.

Figure 2: Abundance of breeding sites and larvae collected



Transient water bodies constituted 6(43%) of the 14 breeding sites sampled. These habitats included: stagnant, sun-lit muddy water teeming with algae. The were located between newly established vegetable gardens, near banana plants, and in tall grass covered areas (15°20.701S, 028°18.484E); slightly muddy, sun-lit stagnant stream bed; clear un-shaded fresh water overgrown with grass; muddy stagnant water collected between sweet potato mounds with emergent vegetation (15°20.754S, 028°18.154E); stagnant fresh water bed, sun-lit and teeming with algae (15°24.689S, 028°22.345E); and stagnant sun-lit fresh water collection in an abandoned building foundation trough(15°20.685S, 028°18.515E).

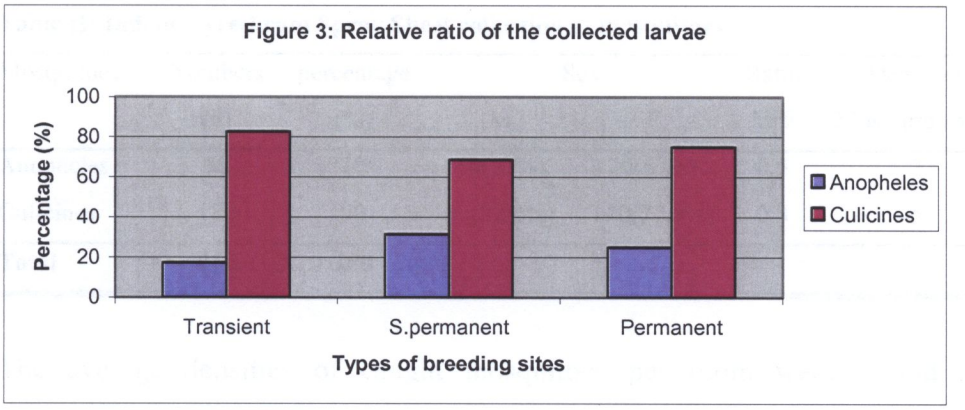
Semi-permanent water bodies constituted 5(36%) breeding habitats (Figure 2). These included, small collection of sun-lit water overgrown with grass (15° 24.679S, 028°22.374E); water standing in an abandoned open well overgrown with reeds and teeming with algae (15°24.696S, 028°22.285E); sun-lit water at the edge of a sugarcane garden (15°24.706S, 028°22.221E); abandoned well that was overgrown with reeds and surrounded with burnt grass; and water at the edge of the stream with reeds and grass (15°20.676S, 028°18.568E).

Permanent water bodies contributed 3(21%) to the sampled breeding habitats (Figure 2). This category included, running water at the edge of a permanent open stream with Banana and Mango tree stamps, teeming algae gave the water a characteristic greenish appearance (15°20.699S, 028°18.472E); fresh slow moving water of a permanent open stream, heavily overgrown with grass and teeming with algae (15°20.703S, 028°18.436E); a permanent water collection at the edge of a dam and full of emergent vegetation (15°24.681S, 028°22.382E).

4.1.2. Larval habitat preferences

The total number of larvae surveyed from potential breeding habitats varied from habitat to habitat. Figure 2 shows the total number of larvae sample from water bodies most favoured for breeding sites. Among the three kinds of habitats sampled, the Transient yielded 1210(66%) Semi-permanent contributed 510(28%) and Permanent provided the rest 120 (6%) of the total larvae collected.

In this study, the prevalence of both Anopheline and Culicine mosquito larvae was determined according to the breeding sites sampled as illustrated in Figure 3 below. The Anopheline and Culicine ratios as well as average larval densities per 250mls of water scooped were also calculated.



Anopheline larvae accounted for 21.7% (400) and the Culicine larvae 78.3% (1440) out of the total of 1840 larvae collected (Figure 3). Semi- permanent breeding habitates were most prefferd by anopheles larvae followed by permanent and transient habitats. The total Anopheline-Culicine ratio was 0.28 (400/1440) in Chazanga, Kabanana, Chipata and Kalikiliki. Anopheline to Culicine ratios where highest in the Semi-permanent habitats was 0.46 (160/350) followed by Permanent habitats 0.33 or (30/90) and the Transient habitats 0.21 (210/1000) the lowest. The contribution of the Anopheline larvae towards the breeding source of mosquitoes in different habitats of peri-urban Lusaka was determined ($x^2 = 3.275$, $P = 0.05$).

4.1.3. Pyrethrum Spray sheet Collection

Results of the knock down spray sheet catches conducted in twelve houses showed that anopheline mosquitoes constituted 10% of the total catch and the culicine accounted for 90% as shown in table II below.

Table II: Indoor Pyrethrum Spray Sheet collection of mosquitoes

Mosquitoes	Numbers	percentage	Sex		Ratio	Densities
	(#)	(%)	M	F	M/F	Mosquito/room
Anopheles	30	10	10(33%)	20(67%)	0.5	2
Culicines	180	90	40(22.2%)	140(77.8%)	0.3	5
Total	**210**	**100**				

The average densities of caught mosquitoes per room were 2 and 5 for Anopheline and culicine mosquitoes respectively. Among the Anopheline mosquitoes, 10 (33%) were male and 20 (67%) were females. The male to female ratio was 0.5 and the average engorged Anopheline mosquito collected was 2 per room.

4.1.4 Identification of *Anopheles* species in peri-urban Lusaka

Morphological identification revealed that all the caught *Anopheles* were members of the *Anopheles gambiae* complex (Gillies and Coetzee, 1987;Gillies and De Meillon, 1968). Molecular differentiation of the *Anopheles gambiae s.l* species by the modified version of the Scott *et al.*,(1993) Polymerase Chain Reaction (PCR) protocol, revealed the presence of both *Anopheles gambiae s.s* and *Anopheles arabiensis*. Thirty specimens were subjected to PCR, however, eleven could not be identified due to the degeneration of their DNA. Identification was achieved by the Polymerase Chain Reaction using rDNA molecular markers for *An. arabiensis* and *An. gambiae* (Figure 4 and Figure 5). Of the nineteen well preserved specimens, Eighteen (95%) *Anopheles* specimens collected from Chazanga, Kabanana and Kalikiliki were amplified for *Anopheles gambiae s.s* at 390 bp and only one (5%) specimen collected from Chazanga was amplified for Anopheles *arabiensis 315 bp* (lane 12 figure 4).

Table III: Summary of molecular identification of Anopheles mosquitoes

Site	Anopheles gambiae	Anopheles arabiensis
Kalikiliki	11(58%)	0
Chazanga	7(37%)	1(5%)
Kabanana	0	0
Chipata	0	0
Total	**18(95%)**	**1(5%)**

The specimens used for identification to species level were collected either as adult by Pyrethrum Spray sheet collection method or as larvae that were reared to adulthood in the insectary. In Kalikiliki, eleven mosquitoes were identified as *An. gambiae* constituting 58% of the total mosquitoes identified as *Anopheles gambiae* where as in Chazanga seven (37%) were identified as *An. gambae* and only one (5%) was identified as *An. arabiensis* as shown in Table III above.

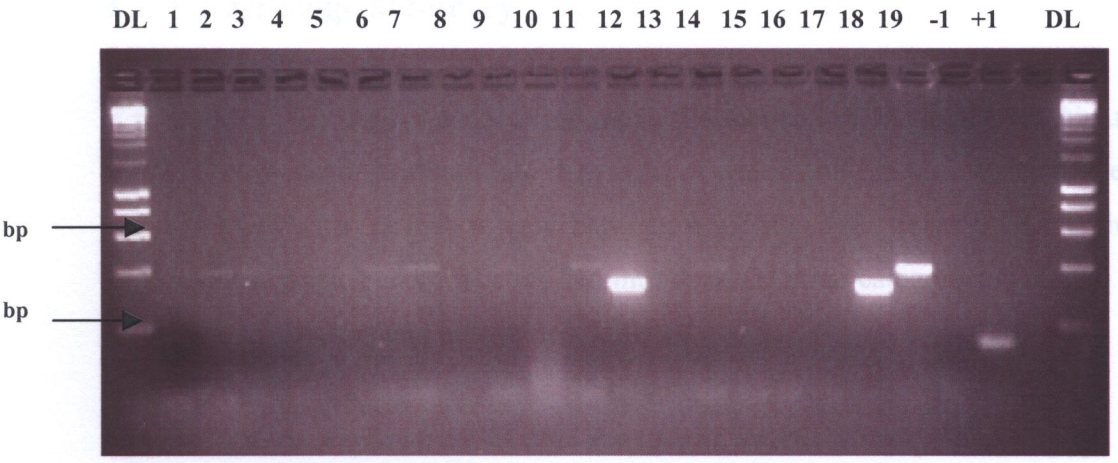


Fig 4: DNA bands produced by ribosomal DNA- Polymerase chain reaction (PCR) amplification from the different species in the Anopheles gambiae complex from Chazanga. DL = 1-kp DNA ladder size standards, +1 = positive control (*A.arabiensis* ,) , -1= negative control. The sample DNA in each of the lanes was as follows: 1,2,3,6,7,11 and 14 were amplified for *A. gambiae.ss* (390bp). 12 was amplified for *A,arabiensis* (315bp). The complete 25ul reaction volume was electrophoresed through an ethidium bromide-containing 2.5% agarose gel. The amplified fragments were visualised by illumination with short wave ultraviolet light.

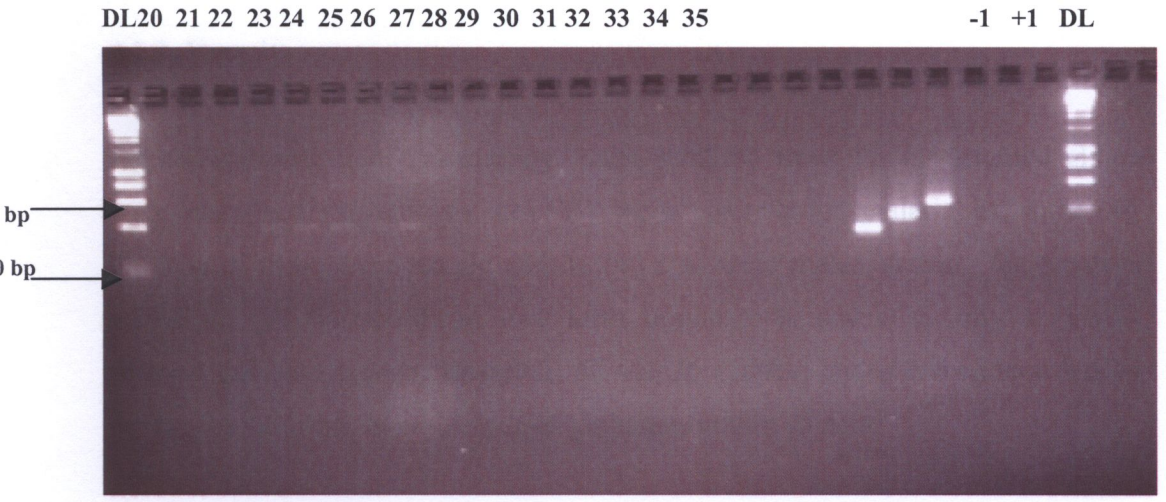


Fig 5: DNA bands produced by ribosomal DNA- Polymerase chain reaction (PCR) amplification from the different species in the Anopheles gambiae complex from Kalikiliki. DL = 1-kbp DNA ladder size standards, +1 = positive control (*A.arabiensis* ,) , -1= negative control. The sample DNA in each of the lanes was as follows: 23,24,25,26,27,30,31,32,33,34 and 35 were amplified for *A. gambiae.ss* (390bp). The complete 25ul reaction volume was electrophoresed through an ethidium bromide-containing 2.5% agarose gel. The amplified fragments were visualized by illumination with short wave ultraviolet light.

4.2. Parasitological Survey

4.2.1. Study Population

A total of 297 febrile patients seeking medical attention at Chazanga and Chipata Health Centres were recruited into the study, of which 168 (56.6%) were females and 129 (43.4%) were males, aged between 6 months and over 60 years. The population of participants was stratified into three (3) main categories by age: 0 – 4, 5 – 15 and 15 years and above. See Table IV

Table IV: Malaria prevalence at Chazanga and Chipata Health Centres

Main Age Groups	Numbers Tested			Malaria positives	
	(F)	(M)	Total	(f)	(%)
0-4 Yrs	38	52	**90**	27	30
5-15 Yrs	21	14	**35**	9	25.7
>15 Yrs	109	63	**172**	40	23.3
Total	168	129	**297**	**76**	**100**

4.2.2. Parasite rates

By conventional light microscopy, three hundred thick Giemsa-stained slides were examined. Seventy-six (25.6%) were positive for malaria parasites, 45 (59.2%) of these were female and 31 (40.8%) were male. Among the positive slides, 75 (98.7%) exhibited ring form trophozoites where as only 1 (1.3%) showed gametocytaemia. However, all the 76 (100%) slides were identified as having only *Plasmodium falciparum* parasites on thin smears.

Prevalence of Malaria according to sex was determined and Table IV shows the data separated into groups with respect to sex. To test whether the prevalence of malaria was independent of sex, a 2 x 2 contingency table was constructed in which data were classified according to sex and malaria. X^2 was calculated, using Yate's correction factor. No significant difference in the prevalence of malaria

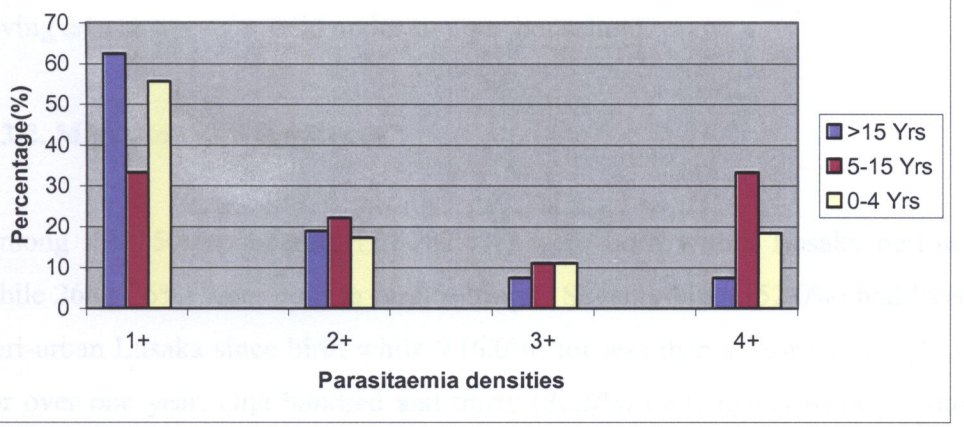
was found between sexes (X^2 = 0.977, P= 0.05). Data for both sexes were therefore combined in the analysis.

The prevalence of malaria with regards to age was also determined. (Table IV). A 2x2 contingency table was constructed for each pair of associations and the X^2 test, with Yate's correction, was applied. There was a significant difference in the prevalence of malaria by age group (P= 0.05). The 0 –4 years group had the highest infection rate of 30.0%, followed by 5to15 years and over 15 years age groups with 25.7% and 23.3% respectively.

4.2.3. Parasite densities

Thick and thin blood films were interpreted as negative only after examination with an oil immersion lens at X 1000 magnification for at least 100 oil immersion fields by a competent microscopist. In all positive slides, Parasitaemia was quantified using the plus signs (Bruce-Chwatt, 1985). Figures 6 below shows the parasite densities in thick smears according to age group.

Figure 6: Parasite Densities According to Age



There was a significant difference of parasite densities according to age (P=0.05). The 5-15 years age group had the highest parasitaemia density of 4+ (18.5%) and the above 15years age group had the lowest parasitaemia density of 1+ (62.5%).

Children aged between 6 months and 4 years supported low grade parasitaemia (55%) and gametocytaemia was not observed in this study.

4.3 Determination of Knowledge, Attitude and Practices

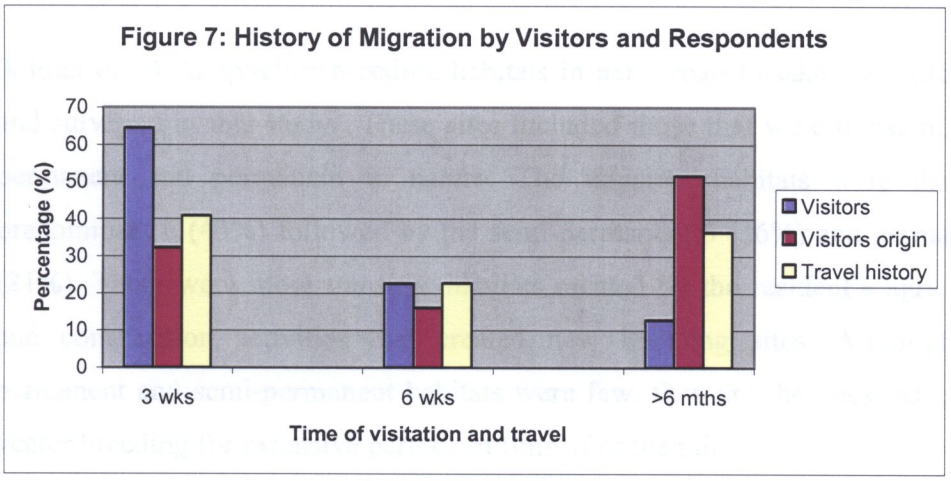
One hundred and fifty participants in this study responded to the questionnaire. The respondents furnished information regarding their family demography, residence and knowledge of the causative agent, vector, prevention and control of malaria. The questionnaire gauged the attitudes and practices as regards to malaria.

4.3.1. Family demographic data

Family demographic data in Chazanga, Kabanana and Chipata yielded an average of seven residents per household. Eighty-four (56.3%) of the residents in the entire sampled population were adults and 66 (43.7%) accounted for children under15 years of age. Among these children, 23 (34.2%) were under five years giving an average of 1 child under five per household.

4.3.2. Migration and Residence

Among the 150respondents, 124 (82.4%) were born within Lusaka peri-urban while 26 (17.6%) were born in rural settings. Seventy-eight (52.0%) had lived in peri-urban Lusaka since birth while 9 (6.0%) for less than a year and 63 (42.0%) for over one year. One hundred and thirty (86.8%) participants were peri-urban permanent residents whereas 20 (13.2%) constituted those that had migrated to peri-urban Lusaka as a result of visiting or family relocation. Fifty-one (34.1%) of the respondents hosted visitors from out side Lusaka in the previous three weeks, up to six months (Figure 7). Hundred and seven (71%) respondents had a history of travel outside Lusaka.



Further analysis showed that there was no significant contribution of migration towards malaria incidence/prevalence in peri-urban Lusaka (X^2= 1.704, P=0.05).

4.3.3. Knowledge, Attitude and Practice

The levels of knowledge about malaria among the hundred and fifty respondents were varied but 73 (48.4%) exhibited a good knowledge of malaria. In terms of the knowledge of the causative agents and the vector, more than half of the respondents showed knowledge of malaria transmission and causes 94 (62.6%) and signs and symptoms119 (79.1%) . Ninety-two (61.5%) showed awareness of what to do when they suspected malaria. As regards prevention of malaria69 (46.2%) of the respondents were knowledgeable about ITNs, IRS, Environmental management and Larviciding. This result shows that there was a poor KAP in peri-urban Lusaka. The association between knowledge levels and transmission of malaria was determined (X^2 = 26.463, P = 0.05) and it was found that there was positive association between knowledge and malaria incidence in peri-urban Lusaka in that the people who are knowledgeable had less malaria.

CHAPTER 5: DISCUSSION

A total of 14 Anopheline breeding habitats in peri-urban Lusaka were identified and surveyed in this study. These sites included those that were transient, semi-permanent and permanent in nature. The transient habitats were the most predominant 6 (43%) followed by the semi-permanent 5 (36%) and permanent 3 (21%). There were more transient habitats created by the resident's agricultural and construction activities that created new breeding sites. Although both permanent and semi-permanent habitats were few, they are the ones that sustain vector breeding for extensive periods of time after the rains.

A total of 1840 mosquito larvae were collected from the three kinds of the habitats: there were more larvae in transient habitats (66%) than the semi-permanent (28%) and the permanent habitats (6%).

It is known that urban areas do not support significant levels of malaria transmission due to pollution and the destruction of clean water sources required by the Anopheline mosquito vectors (Mc Wilsons et al 1999). This study revealed that *Anopheles* can breed in clean, clear water of open and unshaded natural pools close to the stream, abandoned wells overgrown with grass, abandoned building foundations, water collected between sweet potato mounds, and in newly established vegetable gardens. Furthermore, some Anopheline mosquito larvae were found in the muddy water and this could be attributed to biological adaptability of the members of the *Anopheles gambiae* complex (Mc Wilson et al 1999). This adaptability of *Anopheles gambiae* to changing environments has allowed this species to develop in relatively polluted water including sewage (Mc Wilsons et al 1999) and the spread of these species across the country could undermine current vector control strategies. .

The numbers of *Anopheles* larvae collected from the three different habitats varied: The Semi- permanent habitats supported more Anopheles larvae (31.4%)

followed by the permanent habitats (25%) and transient habitats (17.4%).The low prevalence of anopheline larvae in transient water can be ascribed to their high levels of pollution and therefore were not ideal for Anopheline breeding as evidenced by the high percentage (82.6%) of the Culicines collected from these habitats.

Permanent water bodies which include perennial streams are important in the perennial malaria transmission in peri-urban areas as evidenced by the comparatively high rates of Anopheline larvae observed in both semi permanent (31.4%) and in permanent habitats (25%).

The adult Anopheline mosquitoes accounted for only 10% of the mosquitoes collected by the knock down Pyrethrum Spray sheet Collection method.. The catch of two female Anopheles per room should be considered to be high and explains the high infection rates of 25.6% among the febrile subjects at the health centre. Notably, it is not the number of mosquitoes present that is critical in the transmission cycle, but rather the longevity, which contributes to the efficient transmission of malaria (Shiff, 2002).

Morphological identification revealed that all the caught *Anopheles* were members of the *Anopheles gambiae s.l.* However, molecular differentiation of the *Anopheles gambiae s.l* species by the modified version of the Scott et al, (1993) Polymerase Chain Reaction (PCR) protocol revealed the presence of both *Anopheles gambiae s.s* and *Anopheles arabiensis*. Data on the distribution and speciation of *Anopheles* in Zambia is fragmentary, but studies conducted in rural settings of Zambia by Shelly in 1973 and Bransby-Williams in 1979 indicated vector speciation to be 100% *Anopheles arabiensis.* This study has shown the co-existence of *Anopheles gambiae s.s and Anopheles arabiensis*, in peri-urban Lusaka. In the absence of any other data, the predominance of *Anopheles gambiae s.s* in contrast with the findings by Shelly (1973) and Bransby-Williams (1979), implies that *Anopheles gambiae s.s* is the major vector in peri-urban Lusaka. The

finding of *Anopheles arabiensis* , a species that is more exophilic and abundant in arid –Savannas (Onyabe and Conn, 2001), shows that it is still an important malaria vector in peri- urban Lusaka. The predominance of the *Anopheles s.s* vector makes it more amenable to control by IRS and ITNs interventions. This finding of co-exisistence of *Anopheles species,* therefore, demonstrates the need for an integrated vector management strategy for malaria control in this peri-urban setting.

Seventy-six (25.3%) of the participants in this study were positive for malaria parasites by the end of the rainy season (May 2003). This finding is in agreement with those by National Malaria Control Centre (1995) of 2-25% parasite rates across the country and 26.4% in Ndola rural survey between 1969 and 1972 (Utzinger et. al 2001). This study confirms that malaria is endemic and stable in peri-urban Lusaka. It was also observed that 100% of the parasitaemia were *Plasmodium falciparum* mono infections with 98.7% trophozoites and only 1.3 % showing gametocytaemia. Country studies have shown 86.8% *Plasmodium falcipurum* and 13.2% *Plasmodium malariae* infections rates in Ndola rural (Wenlock, 1978) and the National Malaria Control Centre country-wide infection rate is 95% *Plasmodium falciparum*,(1999).

Prevalence of malaria according to sex was determined and no significant difference was found between sexes. Similar observations have been reported Whittle et al (1969) who showed that sex and malaria prevalence are not related.

The National Malaria Control Centre (2001) reported that the highest prevalence of malaria occurs in the 0-4 years age group. This concurs with the findings in this study that malaria is associated with age and a significant difference was found in the prevalence of malaria by age group (P=0.05). Prevalence and age are thus related with infections being highest (30.0%) in the early age group (0-4 years) and lowest (23.3%) in the 15 year and above group as a result of acquired immunity. The high parasite rates in the 0-4 years group is a good indicator of a

recent transmission of malaria (Bruce- Chwatt 1985). For an urban area, if 10% or more of the children under 5 with fever have malaria confirmed by microscopy, malaria is considered to be endemic in the area (Mc Wilsons *et al*, 1999). Therefore, it must be concluded that malaria is endemic in peri-urban Lusaka.

Malaria parasites were quantified using the plus sign to determine the parasites densities according to age group. There was a significant difference of parasite densities in the different age groups. The 0 – 4 years group had the highest infection rates with low-grade parasitaemia. The highest parasite densities occurred in the 5 to 15 years age group and the lowest in the 15 years and above age group. These findings are in agreement with the observation that the prevalence rate of malaria in children under five years is dependant on the intensity of the transmission and declines with age as immunity develops (Whittle et al, 1969).

Family demographic data showed that there was an average of 7 residents per household in peri-urban Lusaka. The minimum and maximum number of residents per household was 3 and 25 respectively with an average of one child under five years per household. The population constituted 56.3% adults and 43.7% children (those under 15 years of age). Among these children, 34.2% were under 5 years of age.

Congestion in households is therefore one of the factors contributing to the increasing burden of malaria in this setting. According to Greenwood and Mutabingwa (2002) the population of many malaria endemic countries has doubled in the past two decades, thus greatly increasing the absolute numbers of those at risk. Consequently, the immunity levels against the malaria parasite are quite high especially in adults (Trape and Zoulani, 1987) that could explain the low-grade parasitaemia observed in older subjects in this study.

It has been established that human migration contributes markedly to malaria transmission,and as many as 7000 imported cases of malaria are recorded in Europe each year (Muentener et al, 1999). In this study, there was a lot of exchange of visits and 71% of respondents had a history of travel outside Lusaka and 34.1% of the respondents had hosted visitors from outside Lusaka In the 1970s, malaria was an imported and notifiable disease in Lusaka but it appears that the disease has become endemic in Lusaka as a result of increased rural urban traffic. This study has confirmed that there is local transmission in Lusaka as 80% subjects with malaria confirmed by microscopy had history of travel. It was established in this study that there is no significant contribution of in migration towards malaria transmission in peri-urban Lusaka (P 0.05)

Although the residents had high KAP, as regards to the signs and symptoms, causative agent and the vector, health seeking behaviour, the infection rates were high in the study area. This can be ascribed to the low KAP as concerns prevention.

CHAPTER 6: CONCLUSION

There are three kinds of Anopheline mosquito breeding habitats in peri-urban Lusaka, namely Transient, Semi-permanent and Permanent. The semi- permanent were the most prevalent and most preffered breeding sites for *Anopheles* species.

Anopheles gambiae s.l constituted 10% of the indoor pyrethrum collected mosquitoes. Molecular speciation of both house caught and laboratory showed that Eighteen (95%) *Anopheles* specimens collected from Chazanga, Kabanana and Kalikiliki amplified for *Anopheles gambiae s.s* at 390 bp and only one (5%) specimen collected from Chazanga amplified for *Anopheles arabiensis* 315 bp.

Parasite rates in peri-urban Lusaka were at 25.6%. This parasitaemia was completely due to *Plasmodium falciparum* mono infections with 98.7% trophozoites and 1.3% gametocytaemia rates. The 0-4 years group had the highest infection rate (31.8%) and the 5-15 years age group supported the highest parasitaemia density. Those above the 15 years age group had the lowest parasitaemia density.

There are appreciably high levels of knowledge on malaria as regards the disease among peri-urban Lusaka residents but low knowledge of control and prevention explaining the high infection rates. The congestion in this peri-urban area probably contributed to the high transmission in peri-urban Lusaka.

Local transmission of malaria in peri-urban Lusaka was strongly inferred in that 31.8% of febrile children under the age of five had malaria confirmed by microscopy, it was also established that migration does not contribute significantly towards transmission. Furthermore, the presence of children under five years (78%) who lived in peri-urban Lusaka since birth and the presence of gametocyte bearers and the vector in the community perpetuate the transmission cycle. Local transmission is also strongly supported by the proximity of ideal

breeding habitats and the presence of efficient *Anopheles gambiae* complex species (*Anopheles gambiae and Anopheles arabiensis)* in the houses. Following the determination of this local transmission of malaria, appropriate control strategies can be instituted in peri-urban Lusaka based on this transmission paradigm.

Recommendations

The presence of the *Anopheles gambiae* complex species and their discreet and accessible breeding sites in peri-urban Lusaka creates, a critical need for an integrated vector management (IVM) approach to control the shown local transmission. Environmental management, Larviciding and biological control as supplementary interventions to Insecticide Treated Bed nets and Indoor Residual Spraying approaches would effectively reduce malaria transmission.

In order to implement evidence based vector control, there is need to conduct further entomological studies to establish other parameters (Insecticide resistance, molecular forms, Entomological Inoculation Rates) that are essential prerequisites of an informed implementation of effective vector control interventions.

Behavioural Change for Communication (BCC) should also be strengthened in peri-urban Lusaka

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Appendix A

QUESTIONNAIRE

TITLE: TRANSMISSION ATTRIBUTES OF PERI-URBAN MALARIA IN LUSAKA, ZAMBIA

Clinic Name:.....Area:.....

Date:.....Interviewers Code:.....

Result of Malaria test (+ve/-ve):.....

SECTION 1: FAMILY DEMOGRAPHIC DATA

Respondents Name:.....Sex (M/F):.....

Home address:.....Age (yrs):.....

1. What is the total number of residents in the home?
   (a) Adults (.....)
   (b) Children- under 15 yrs of age (.....)

2. Total number of children under five years in the home?
   .....

3. How long have you lived with the child?
   (a) Since birth
   (b) Less than one year
   (c) Other (specify).....

SECTION 2: MIGRATION

4. Where was the child born?
   (a) Urban
   (b) Rural
   (c) Other (specify).....

5. Why is the child in Lusaka?
   (a) Lives here
   (b) Visiting
   (c) Family moved
   (d) Other (specify).....

6. Have you ever received a visitor from outside Lusaka who were diagnosed with malaria during their stay with you?
   Yes/No.....

7. If yes, how long ago
   (a) 3 weeks
   (b) 1-6 months ago
   (c) Over 6 months
   (d) Other (specify).....

8. Where did he or she come from?
   (a) Within Lusaka urban
   (b) Within Lusaka rural
   (c) Other (specify).....

9. During the last three weeks have you (and your child) spent a night out of town or made a trip outside town
   Yes/No.....

10. If so, where did you go
   (a) Within Lusaka urban
   (b) Within Lusaka rural
   (c) Other (specify).....

11. How long have you been living here in Lusaka?
   (a) Since birth
   (b) Less than one year
   (c) Over one year
   (d) Other (specify).....

SECTION 3: GENERAL KNOWLEDGE ABOUT MALARIA

12. What is Malaria?
   (Write exactly as respondent states).
   .....
   .....
   .....
13. What causes Malaria?
   .....
   .....
14. What are the signs and symptoms of Malaria?
   .....
   .....
15. What do you do when you suspect Malaria?
   .....
   .....
16. How can you prevent Malaria?
   .....
   .....

Appendix B

THE UNIVERSITY OF ZAMBIA

RESEARCH ETHICS COMMITTEE

Telephone: 256067
Telegrams: UNZA, LUSAKA
Telex: UNZALU ZA 44370
Fax: + 260-1-250753
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Dean's Office
P.O. Box 50110
Lusaka, Zambia

Assurance No. FWA00000338
IRB00001131 of IOR G0000774

Ref: 005-05-03
15 July, 2003

Mr Emmanuel Chanda
Department of Pathology & Microbiology
School of Medicine
P.O. Box 50110
LUSAKA

Dear Chanda,

RE: SUBMITTED RESEARCH PROPOSAL

The following research proposal was presented to the Research Ethics Committee Meeting on 28 May, 2003, where changes were recommended. We would like to acknowledge receipt of the corrected version. The research proposal has now been approved. Congratulations!

Title of proposal: 'The transmission attributes of per-urban malaria in Lusaka'

Conditions:

- This approval is based strictly on your submitted proposal. Should there be need for you to modify or change the study design or methodology, you will need to seek clearance from the Research Ethics Committee.
- If you have need for further clarification please consult the Research Ethics Committee. Please note that it is mandatory that you submit a detailed progress report of your study to this committee every six months and a final copy of your report at the end of the study.

Yours sincerely

Prof. J. T. Karashani, M.B., Ch.B, Ph.D
CHAIRMAN
RESEARCH ETHICS COMMITTEE

Date of approval: 15 July, 2003 Date of Expiry: 14 July, 2004

Appendix C

CONSENT TO PARTICIPATE IN RESEARCH

1. **TITLE: THE TRANSMISSION ATTRIBUTES OF PERI-URBAN MALARIA IN LUSAKA, ZAMBIA**

2. **PURPOSE:**

The main objective of this study is to assess the intensity of malaria transmission in peri-urban Lusaka. It is also intended to determine the kind of mosquito species predominant in the area, and to determine infection rates in the population. The data from this study will be a basis for implementing malaria control.

3. **METHOD**

If you accept to participate in this study, you will undergo any of the following procedures.

a. You will be asked to submit blood, collected from the thumb pricked with a lancet, for malaria parasite examination.
b. You will be required to allow house mosquito spotters into your home to collect adult mosquitoes using knock down insecticides at early in the morning between 6 and 8 hours.
c. You will also be asked a few questions pertaining to your family demography, travel and your knowledge about malaria.

You may choose to participate or not to and you can withdraw if you so wish.

4. **RISKS AND DISCOMFORTS**

There are few risks involved for example,
- Short-lived kinds of discomfort such as the smell of the knock –down insecticides but this clear rather fast.
- Also the pricking of the thumb to collect the blood causes a slight pain.
- The collection procedure involves gaining access to peoples homes, but this instills fear of property loss.
- The mosquito spotters are likely to be subjected to adverse environmental conditions around ideal breeding sites such as snake, and mosquito bites.

5. **BENEFITS AND ALTERNATIVES**

The information collected from the study will benefit the community and individual participants in that,

1. The data obtained will be used for tailoring appropriate malaria vector control strategies in the community.
2. The participants will have free malaria parasite diagnosis.
3. If diagnosis for malaria parasites is positive, treatment will be recommended for the participant at health centers.

Translation of this consent form will be made in appropriate languages.

CONSENT: PARTICIPANT OR CARE GIVER OF MINOR PARTICIPANT

I have been asked to participate in the above research and give my consent by signing this form.

I understand that:

1. My consent to participate is voluntary and I may withdraw at any time if I wish to do so.
2. I have understood the information that I have read/has been read to me in my vernacular language and I understand the procedure fully.

Participants signature or thumb .....

Witness signature or thumb .....

Investigators signature .....

Date ..... Place .....

N.B: In case you have any questions or there is something you need to clarify PLEASE CONTACT

Emmanuel Chanda
University of Zambia
Pathology and Microbiology
School of Medicine
P.O. Box 50110
Lusaka.

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Appendix D

P.O. Box 50127
Lusaka
Tel: 235554
Fax: 236429

MINISTRY OF HEALTH
LUSAKA DISTRICT HEALTH MANAGEMENT BOARD

24th July, 2003

The Program Coordinator
Department of Medical Parasitology
UNZA – School of medicine
P.o. Box 50110
LUSAKA

Dear Doctor,

RE: RESEARCH UNDERTAKING – MR EMMANUEL CHANDA

Be informed that permission has been granted for the above named student to carry out his dissertation research on "The transmission attributes of peri urban malaria in Lusaka Zambia".

By copy of this letter the In-Charges of Chipata and Chazanga.

Health Centres are informed forthwith.

Yours faithfully,

DR. M. KABASO
CLINICAL CARE EXPERT
FOR DISTRICT DIRECTOR OF HEALTH

c.c. In-Charges – Chipata and Chazanga