CHAPTER 1: INTRODUCTION

1.1 Background.

Adult mosquitoes frequent a wide variety of places for various purposes, and since they are frail insects and subject to desiccation, they are generally found where the air is relatively static and the humidity is high (WHO, 1975; Goma, 1966). Such preferred places include human dwellings. Mosquitoes entering and resting inside human habitations are collectively referred to as endophilic species. They have been well studied, mostly for their role as vectors of human diseases (Fonseca *et al*., 2004; White, 1989; WHO, 1982).

Endophilic mosquitoes include vectors of Malaria, Filariasis, Yellow fever and Dengue. Viruses of Yellow fever and Dengue are primarily transmitted from human to human by the mosquito species *Aedes aegyti* (Vanlandingham *et al*., 2005; Davies and Martin, 2003), while mosquito species of the genus *Culex* are known to transmit filarial worms, as well as viruses that cause encephalitis (Fonseca *et al*., 2004; WHO, 1982). Among the four diseases transmitted by endophilic mosquitoes, malaria is the most devastating. It is estimated that 40% of the world's population is at risk of malaria and with more than 500 million people becoming severely ill with the disease annually (WHO, 2007). Sub-Saharan Africa bears much of this burden (WHO, 2006). In Zambia, more than 3 million clinical cases and 8,000 deaths occur annually due to malaria (Chanda *et al*., 2004). Transmission of all the four diseases mentioned above is achieved by female mosquitoes during episodes of blood sucking from an infected human host to another (both infected and non-infected), during the insects' gonotrophic cycle (Lucas and Gilles, 1990).

In sub-Saharan Africa, endophilic mosquitoes known to efficiently transmit human Malaria belong to the *Anopheles gambiae* complex or *A. gambiae* sensu lato (s.l.) (Coetzee, 2004;

Coetzee *et al*., 1993; Gillies and Coetzee, 1987; Gillies and de Meillon, 1968). This complex comprises seven sibling species namely; *Anopheles gambiae* sensu stricto (s.s.), *Anopheles arabiensis, Anopheles melas, Anopheles merus, Anopheles bwambae, Anopheles quadriannulatus* species A and *Anopheles quadriannulatus* species B (Coetzee *et al.,* 1993; Gillies and Coetzee, 1987; Gillies and de Meillon, 1968). *Anopheles gambiae*, s.s. and *A. arabiensis* are the major malaria vectors, the most widespread species being *A. arabiensis,* which is found throughout the Afro-tropical region except in the equatorial forest-belt (Morlais *et al*., 2005; Coetzee *et al.,* 1993; Gillies and Coetzee, 1987). Although these species can be separated morphologically from related mosquito species, they are indistinguishable within the complex (Gillies and Coetzee, 1987). Identification of these species can easily be done using molecular genetic techniques (Hoy, 2003; Scott *et al*., 1993; Kent and Norris, 2005; Charlwood and Edoh, 1996; Van Rensburg *et al*., 1996)**.**

In Zambia, Gillies and de Meillon (1968) reported the occurrence of *A. gambia*e s.s. in Kitwe, Copperbelt Province. Hervy and co-workers (Hervy *et al*., 1998) documented 27 *Anopheles* species in Zambia. *Anopheles arabiensis* is among them. However, the distribution and abundance of these species in many regions of the country is still largely unknown (MoH, 2000).

Currently in Zambia, malaria vector control through indoor insecticide spraying, commonly known as Indoor Residual House Spraying (IRHS) has become a cornerstone in the fight against the malaria vector. The programme is being implemented in 36 districts of the country (NMCC, 2007 unpublished). However, the impact of IRHS on the abundance and distribution of endophilic malaria vectors has not fully been assessed. Such an assessment, would involve collection, identification and classification, as well as comparison of mosquito diversities in insecticide sprayed and unsprayed houses, in the districts implementing the IRHS programme.

This study aimed at assessing impacts of IRHS on the diversity, abundance and distribution of endophilic human malaria vectors in Chongwe district, Zambia. The term diversity here implies the kind of mosquito species; whereas abundance relates to the relative numbers of mosquitoes that can be found per human dwelling. The term distribution refers to the extent of occurrence of mosquitoes in terms of geography.

1.2 Statement of the Problem.

Presently, very little is known about the diversity, abundance and distribution of malaria vector species of mosquitoes in Zambia. Further, there is inadequate information on which of the mosquito species in the *A. gambiae* complex are the major vectors of malaria in different parts of the country. The little available data on malaria vector mosquito species are based on unpublished data on mosquito distribution in the Sub-Saharan Africa (MoH, 2000).

In addition, despite IRHS being implemented in the 36 districts of the country to date, under the Roll Back malaria programme, very little has been done to assess the effect the programme has had on endophilic *Anopheles* mosquito, abundance and distribution to justify scaling up this mosquito control strategy by adopting it in other districts of the country.

1.3 Study Justification.

Adequate local knowledge of species' diversity, abundance and distribution is vital not only for the success of IRHS programmes, but also for other malaria intervention programmes such as the promotion of the use of Insecticide Treated Nets (ITNs). Use of ITNs in Zambia is one of the key

malaria control interventions (MoH, 2000). This study will contribute to knowledge on *Anopheles* mosquito diversity and distribution and will assess the effectiveness of the IRHS programme implemented by the government of Zambia through the Ministry of Health in the Chongwe district.

Mosquito control through spraying of residual insecticides inside human dwellings requires adequate knowledge of mosquito species being targeted. This is because of the enormous diversity in feeding and resting behaviour that different mosquito species display. In areas where *A. arabiensis* is abundant, for example, it has been reported that control may require more than just spraying the inside of houses. The behavior of this species is such that in sprayed areas, it resorts to feeding on cattle and other animals outside of the human dwellings in avoidance of the insecticide. Molineaux and Gramiccia (1980) in a study in Nigeria reported that in areas sprayed with residual insecticide, a significant proportion of both populations of *A. arabiensis* and *A. gambiae*, s.s. were resting outside. It is thus important to know the mosquito species occurring in target areas so that the control programme may be tailored to the target mosquito species.

Further, genetically based malaria control methods using sterile *A. gambiae* mosquitoes have been developed (Morlais *et al*., 2005). Adoption of such genetically based malaria control methods in many African countries, including Zambia, can be a challenge without adequate molecular data on mosquitoes in the country. Hence this study included molecular methods of mosquito identification.

Therefore, this study aimed at contributing information on diversity, abundance and distribution of malaria vector species of Chongwe district in Zambia, that could be replicated in other districts in which IRHS is being implemented in the country.

1.4 Objectives.

1.4.1 General Objective.

The general objective of this study was to assess the effects of Indoor Residual House Spraying on the diversity, abundance and distribution of human malaria vectors in Chishiko and Chiota Villages of Chongwe district, Lusaka Province, Zambia.

1.4.2 Specific Objectives.

The specific objectives were to:

a) Identify and classify endophilic mosquito species of Chongwe district, Lusaka Province, Zambia using morphological methods.
b) Identify which of the different species within the *Anopheles gambiae* complex is dominant in Chongwe district using molecular techniques.
c) Assess effects of IRHS on endophilic malaria transmitting mosquito species, abundance and distribution in the study area.

1.5 Hypotheses.

Three hypotheses were tested in this study namely:

a) Endophilic mosquito species of Chongwe district do not include malaria vector species.
b) The major vector(s) of human malaria in the Chongwe district does not belong to the *Anopheles gambiae* sibling species complex.
c) The IRHS programme in Chongwe district has not changed the diversity, abundance and distribution of endophilic human malaria transmitting mosquito species.

CHAPTER 2: LITERATURE REVIEW

2.1 Mosquitoes and Human Malaria Disease.

Mosquitoes are two-winged insects belonging to the order Diptera, family Culicidae (Service, 1990; Gillies and de Meillon, 1968). They are characterized by having long conspicuous needle-like mouthparts, which in the female are used for sucking plant juices, like nectar and vertebrate blood. Female mosquitoes feed on plant juices but also need vertebrate blood as a source of proteins for their egg production. Male mosquitoes do not feed on vertebrate blood; they survive only on nectar and other plant juices. Mosquitoes are widely distributed all over the world. The number of species exceeds 3000 and this is separated into two large tribes namely, Anophelini and Culicini, the former being the smaller of the two and includes vectors of human malaria (Jupp, 1996; Service, 1993).

Human malaria, particularly, in the sub-Saharan Africa is transmitted by mosquitoes of two species complexes; the *Anopheles gambiae*, sensu lato (s.l.) or the *Anopheles gambiae* complex and the *Anopheles funestus* complex.

The *Anopheles gambiae,* sensu lato (s.l.) is composed of seven biological species, these being; *Anopheles gambiae*, sensu stricto (s.s.), *Anopheles arabiensis*, *Anopheles melas*, *Anopheles merus*, *Anopheles bwambae*, *Anopheles quadriannulatus* species A and *Anopheles quadriannulatus* species B (Coetzee *et al.,* 1993; Gillies and Coetzee, 1987; Gillies and de Meillon, 1968). *Anopheles gambiae*, sensu stricto (s.s.) and *A. arabiensis* are widely distributed and are the primary vectors of human malaria in tropical African countries, including Zambia (Morlais *et al*., 2005).

The *Anopheles funestus* complex consists of nine members: *Anopheles funestus*, sensu stricto (s.s.), *Anopheles parensis*, *Anopheles aruni*, *Anopheles vaneedeni*, *Anopheles confusus*, *Anopheles leesoni*, *Anopheles rivulorum*, *Anopheles brucei* and *Anopheles fuscivenosus* (Gillies and de Meillon, 1968; Knight and Stone, 1977; Ward, 1984, 1992; Gillies and Coetzee, 1987). Among the nine species, *Anopheles funestus* s.s. has been implicated in the transmission of malaria and is dominant both in numbers and distribution in the sub-Saharan Africa region.

Although mosquito species of the two named complexes can be distinguished morphologically from other similar mosquito species, they are difficult to separate within the complexes. Distinguishing these species within the respective complexes require the application of the molecular technique called Polymerase Chain Reaction (PCR). This technique involves extraction and subsequent amplification of species-specific nucleotides on ribosomal DNA from the mosquitoes of the complex.

2.2 Mosquito Classification.

Mosquitoes, like all other insects are principally classified on the basis of morphological differences or similarities that occur among them. Morphological characters used in classifying adult mosquitoes include those of the head, thorax, legs, wings and the abdomen (Edwards, 1941; Coetzee *et al.,* 1993; Gillies and Coetzee, 1987; Gillies and de Meillon, 1968; Huang, 2004). However, there are certain cases where morphological data fail to separate mosquito species within a group, as is the case with the *Anopheles gambiae* complex which is a group comprising seven sibling mosquito species. In such cases, molecular techniques are used.

The head of an adult mosquito has a pair of large compound eyes with a pair of antennae joined to the head between the eyes. A pair of palps forms below the antennae and in female culicine

mosquitoes they remain very short and are approximately one fifth of those found on male anopheline mosquitoes. The term “anopheline mosquitoes” here describes all mosquitoes of the tribe Anophelini and conversely the term “culicine mosquitoes” is used to describe mosquitoes of the tribe Culicini. In anopheline mosquitoes, the palps are composed of five parts and are covered with scales, which may be of different colours. This variation in colour is often used in species classification. In culicine mosquitoes, the palps are covered with scales generally brown or black in colour. A proboscis protrudes from the ventral part of the head and extends forward and in the case of culicine mosquitoes is approximately five times greater in length than the palps. White banding patterns are present on the proboscis of some culicines and are used in the classification of the species.

The thorax of an adult mosquito supports a pair of wings; a pair of halteres on the upper surface and three pairs of legs on the lower or ventral surface. Like in many other winged insects, the wings of a mosquito have a network of veins on them. These veins are important in the classification of mosquitoes and each is assigned a number and or a name. The vein along the front edge of the wing is called the costa and the short vein is called the subcosta. There are six other veins numbered 1 up to 6. These veins are covered with scales, usually brown, black, white or cream in colour. The back edge of the wing has fine scales which in anopheline mosquitoes, appear as dark and pale spot or bands. The pattern of these scales on the wing is a very useful feature in the classification of mosquitoes. Like anophelines, some culicines have wing patterns that form from dark and light contrasting scales, which with the combination of other characteristics such as the length of the wing veins are used for species classification (Edwards, 1941; Coetzee *et al.,* 1993; Gillies and Coetzee, 1987; Gillies and de Meillon, 1968; Huang, 2004).

The leg of a mosquito is long and made up of a short coxa joined to the body, followed by a short trochanter, and then a long femur, a long tibia, and long tarsus which are made up of five parts. The five parts are numbered 1 up to 5 with segment 1 being closest to the body. At the end of the leg is a pair of claws. The leg is also covered with scales which may be of different colours. The structure of the leg for culicines is the same as that of the anophelines. Pattern combinations of scales on the femur, tibia, and tarsomeres are important for classification of the mosquito species. Claws are also useful characters especially when dealing with culicine mosquitoes (Edwards, 1941; Coetzee *et al.,* 1993; Gillies and Coetzee, 1987; Gillies and de Meillon, 1968; Huang, 2004).

In both the anophelines and the culicines, the abdomen has eight visible segments. The upper plates of the segments are called tergites, and the lower plates are called sternites. They are joined by a membrane which allows the distension of the stomach when the mosquito takes in fluids. Markings or apical bands on some or all tergites are also used in the classification of the mosquito species (Gillies and Coetzee, 1987; Gillies and de Meillon, 1968).

Based on the morphological characters briefly described, the family Culicidae is divided into three sub-families, the Anophelinae, Culicinae and Toxorhychitinae (Service, 1990). The subfamily Anophilinae has in turn three genera, namely, *Anopheles, Bironella,* and *Chagasia* (Goma, 1966). The subfamily Culicinae is the largest accounting for more than 27 genera (Service, 1990; Goma, 1966). The subfamily Toxorhychitinae is the least among the three sub-families with only one genus. Globally, about 3,324 mosquito species have been named in 37 mosquito genera. In the Afro tropical region alone, more than 640 species and sub-species in 14 genera are known to occur (Service, 1993).

In Southern Africa, there are 40 mosquito species of the genus *Anopheles* that have been described representing the sub-family Anophelinae. The subfamily Culicinae is represented by 13 genera, these being: *Aedomyia*, *Aedes*, *Conquillettidia*, *Culex*, *Culiseta*, *Eretmapodites*, *Ficalbia*, *Hodgesia*, *Malaya*, *Mansonia*, *Mimomyia*, *Orthopodomyia*, and *Uranotaenia.* Among these mosquito genera, *Aedes* and *Culex* are the majority. The genus *Toxorhychites* of the sub-family Toxorhychitinae is represented by two species; *Toxorhychites brevipalpis* and *Toxorhychites lutescens*, the former being commoner (Jupp, 1996).

2.2.1 Classification of the Anopheline Mosquito Fauna of the Sub-Saharan Africa Region.

Mosquitoes of the genus *Anopheles*, particularly the species of the afro-tropical region are of particular interest in the present study, being a group where malaria vectors are found.

The classification of the anopheline mosquito fauna of the sub-Saharan Africa falls into two subgenera; *Anopheles* Christophers and *Cellia* Theobald. It must be noted that the use of the name “Anopheles” which denotes a genus has also been extended to denote a sub-genus. Key morphological features of adult anopheline mosquitoes that distinguish the two groups include; the presence or absence of the pharyngeal armature, the terminalia, and banding patterns of the wing. The pharyngeal armature in females of the subgenus *Anopheles* is absent or not well developed; and in males, the terminalia has 1-3 spines at the base of the coxite, set on distinctly raised tubercles; the wings are usually dark with the costa possessing less than 4 main dark areas. Pale scales of wing-veins when present, are commonly intermingled with dark ones, thus not forming distinct pale areas. In the subgenus *Cellia,* however, the pharyngeal armature is well developed except in the species *A. wilson* and *A. jebudensis*. The terminalia in males of the subgenera *Cellia* has 4-6 spines at the base of the coxite and are usually not set on tubercles. The

wings are usually with distinct pale markings, including a series of spots on the costa (Gillies and de Meillon, 1968).

The two subgenera are further divided into series and sections. The concept of series and sections was introduced to accommodate some of the morphological differences observed among members of the subgenera. For the purpose of the current study, characteristics of the series and sections are only briefly described. Specific details are well elaborated in Gillies and de Meillon (1968).

The subgenus *Anopheles* comprise three series; *Myzorhynchus, Anopheles* and *Christya*. Their distinctive features are summarized as follows; in the series *Myzorhynchus* that comprises 10 species, the fore femora are swollen at the base. In females, the palps are shaggy. The wings have 3 or fewer small pale spots on costa and except in the *Anopheles obscurus*, the seventh abdominal segment (tergite) has ventral scale tuft. The series *Anopheles*, which is a large cosmopolitan group, is represented by but a single species, *Anopheles concolor*. The fore femora is not swollen at the base; the wings are entirely dark and the palps not shaggy except at the base. Under the series *Christya* two species are recognized; *Anopheles implexus* and the *A. okuensis*. The abdomens of members of this series have no scales on the dorsal part but instead have lateral segmental tufts of very long and narrow scales (Gillies and de Meillon, 1968).

The subgenus *Cellia* comprises six series; *Neomyzomyia*, *Myzomyia*, *Pyretophorus*, *Paramyzomyia*, *Neocellia* and *Cellia*. Members of the series are characterized by having a single row of teeth on the pharyngeal armature, which are not differentiated into rods and cones. Three sections are recognized under this group. The Myzomyia series comprises a very large number of species in the African fauna. The *A. funestus* complex falls under this series. The pharyngeal armature in this group consists of 2 rows of teeth, differentiated into rods and cones. The series

Pyretophorus comprise a small group of savanna species adapted to a wide range of habitats. Members of this group are closely associated with man. It is a group where vectors of malaria are found. The pharyngeal armature in these is also differentiated into rods and cones. The *Paramyzomyia* include a small group of species, which are occasionally found in semi-arid conditions. The pharyngeal armature is differentiated into rods and cones; the cones in these have well-developed roots. The series *Neocellia* comprises adults that have brightly marked legs and broad mesonotal scales. Very few species of the African fauna are found in this group. The *Cellia* is a very distinct group of savanna species. The adults have shaggy palps and heavily scaled thorax and abdomen. The abdomen has lateral projecting tufts of scales. Pharynx with rods and cones; roots of cone are well developed (Gillies and Coetzee, 1987; Gillies and de Meillon, 1968).

2.3 Identification of Adult Mosquito Vectors of Human Malaria of the Sub-Saharan Africa Region.

In the sub-Saharan Africa, mosquitoes known to efficiently transmit human malaria belong to two groups; the *Anopheles gambiae* complex and the *Anopheles funestus* group (Coetzee *et al.,* 1993; Gillies and Coetzee, 1987; Gillies and de Meillon, 1968).

The *Anopheles gambiae* complex comprises seven sibling species; *Anopheles gambiae* sensu stricto (s.s.), *Anopheles arabiensis, Anopheles melas, Anopheles merus, Anopheles bwambae, Anopheles quadriannulatus* species A and *Anopheles quadriannulatus* species B (Coetzee *et al.,* 1993; Gillies and Coetzee, 1987; Gillies and de Meillon, 1968). The distribution of the species varies across the sub-Saharan Africa (Appendices 7-10).

Identification of adult mosquitoes usually involves examination of the females as their activities are directly linked with disease transmission. Female adult mosquitoes of the *Anopheles gambiae*

complex group are identified by the following major characteristics: the legs are speckled; the palps have 3 pale bands; the pale markings on the wing are usually yellowish or cream; the third main dark area of vein 1 has a pale interruption (marking); the abdomen is light brown and is mainly clothed with hairs; and the 8th tergite is usually covered with scales, which may extend onto the 7th tergite (Gillies and Coetzee, 1987; Gillies and de Meillon, 1968.)

The *Anopheles funestus* group on the other hand consists of nine members namely: *Anopheles funestus* sensu stricto (s.s.), *Anopheles parensis*, *Anopheles aruni*, *Anopheles vaneedeni*, *Anopheles confusus*, *Anopheles leesoni*, *Anopheles rivulorum*, *Anopheles brucei* and *Anopheles fuscivenosus* (Gillies and de Meillon, 1968; Knight and Stone, 1977; Ward, 1984, 1992; Gillies and Coetzee, 1987). Among the nine species, only *Anopheles funestus* s.s. is dominant both in numbers and distribution in the sub-Saharan Africa region. These mosquitoes are distinctly small and dark. The wing size is about 3.3mm long. On the wing, the main dark and light areas of the costa and the first vein are well developed; the spots on other veins are rather reduced (Gillies and Coetzee, 1987; Gillies and de Meillon, 1968).

Although mosquito species of the *A. gambiae* complex can be separated morphologically using mosquito taxonomic keys from other related mosquito species, they are indistinguishable within the complex (Coetzee *et al.,* 1993; Gillies and Coetzee, 1987). Similarly, identification of members of the *A. funestus* within the complex based on morphological differences is rather difficult. Molecular genetic techniques have thus been developed to resolve these difficulties (Hoy, 2003; Scott *et al.,* 1993; Kent and Norris, 2005). The techniques involve amplification of species-specific nucleotides on ribosomal DNA of a mosquito. These nucleotides vary in size depending on the number of base pairs (bp) involved. The nucleotide sizes specific to *Anopheles gambiae* s.s. and *Anopheles arabiensis* are 390 bp and 315bp, respectively (Table 3.2). In the *Anopheles funestus* species complex, five members that can be identified using these techniques

include: *Anopheles funestus* s.s. (505bp), *Anopheles leesoni* (146bp), *Anopheles vaneedeni* (587), and *Anopheles rivolulum* (411). When subjected to gel electrophoresis these DNA fragments appear as bands at different positions on the gel image. Amplified sample DNA fragments corresponding to these are thus interpreted accordingly.

2.4 Geographical Distribution of Mosquitoes.

Mosquitoes are found in all the six major zoogeographical regions of the earth. These being; the Palaearctic region, which includes Europe, Africa north of the Sahara including parts of Asia; the Ethiopian region, Africa South of the Sahara and including Madagascar; the Oriental region, the area around Himalayas which includes India, Pakistan, China south of the boundary of the Palaearctic region including islands of the East Indies; the Australian region including Australia, New Zealand, Tasmania and islands of the South and Southwest Pacific; the Nearctic region, the entire North America and Mexican frontier; Neotropical region, South America and including North America south of the United states and Mexican frontier (Goma, 1966).

2.4.1 Distribution of Human Malaria and its Vector Mosquito Species Globally.

Human malaria is caused by sporozoa of the genus *Plasmodium* comprising four species, namely: *P. falciparum*, *P. vivax*, *P. malariae* and *P. ovale*. It is predominantly a disease of the tropics, found in regions roughly between latitudes 60° N and 40° S. The most affected regions include most parts of Africa, South America, South-East Asia, the Arabian Peninsula and the Western Pacific (Lucas and Gilles, 1990). The order of prevalence of the malaria parasites is *P. falciparum*, *P. vivax*, *P. malariae* and *P. ovale*. *Plasmodium falciparum* is more prevalent in Africa than in any other region of the world; *P. vivax* is commonly found mainly in the United

States, Latin America, and in some parts of Africa; *P. malariae* is widespread throughout sub-Saharan Africa, Southeast Asia, Indonesia, Western pacific, and in many areas of the Amazon basin in South America. *P. ovale* is very limited in its distribution being found mainly West Africa, the Philippines, Eastern Indonesia, and Papua New Guinea (Lucas and Gilles, 1990).

Malaria parasites are transmitted by females of certain species of the *Anopheles* mosquitoes. There are 60 *Anopheles* species that are vectors of malaria, of which about 38 are of major epidemiological importance globally. Global distribution pattern of the vector species involved predictably follow the distribution pattern of malaria itself. In the United States of America, potential vectors of malaria include *Anopheles quadrimaculatus, A. freeborni* and *A. albimanus*. In South America, the major vectors are *Anopheles darling* and *A. marajoara.* As earlier mentioned, *Anopheles gambiae* s.s, *A. arabiensis* and *A. funestus* s.s dominate the Afro-tropical region as major vectors. In the Asian region, *Anopheles stepheninsi*, *A. sundaicus*, *A. culicifacies* are known to transmit malaria. A complete global distribution map of dominant or potentially important malaria vector has been worked (Kiszewksi *et. al*., 2004) (Appendix 6).

In the Afro-tropical region, mosquitoes of the *Anopheles gambiae* s.l. surpass all other species known to transmit malaria in terms of efficiency. In the oriental region, malaria is transmitted by several species including *Anopheles culicifacies*, *A. fluviatilis*, *A. minimus*, *A. philippinensis*, *A. dirus* and *A. stephensi* (Lucas and Gilles, 1990).

2.4.2. Distribution of Human Malaria and its Mosquito Vectors in Zambia.

Malaria generally affects all the nine provinces of Zambia. The prevalence is however higher in rural parts of the country than in urban centers. The low prevalence of malaria in urban areas is

perhaps largely due to the absence of suitable breeding grounds for the malaria transmitting mosquitoes. Malaria transmitting mosquitoes do not breed in polluted water mostly found in urban centers. There are four species of plasmodia (malaria parasites) that cause infections in humans; *Plasmodium falciparum, P. vivax, P. malariae* and *P. ovale*. The most common species in Zambia is *Plasmodium falciparum*, which accounts for about 95% of all malaria cases. *Plasmodium malariae* accounts for 3% while *P. ovale* is responsible for 2 % of malaria cases. *Plasmodium vivax* is rarely encountered in Zambia (MoH, 2000).

The pattern of distribution of mosquitoes responsible for transmission of malaria parasites is yet to be thoroughly investigated in Zambia. However, *Anopheles gambiae* s.s. has been reported to occur in several areas of the Copper belt, Northwestern, and Luapula Provinces of Zambia (TDRC, 2011, Survey Report). *Anopheles arabiensis* appears to be concentrated in certain areas of the Southern Province of Zambia such as Macha (Kent, 2006). The Macha area has similar climatic conditions and geographical features as the Chongwe area. The two mosquito species, which are thought to be responsible for transmitting malaria in most parts of the country, may exist together within a given locality. Service (1970) reported occurrence of *A. gambiae* and *A. arabiensis* together in the same locality in a study in Nigeria. White and Rosen (1973) had similar findings in the same country. In Zambia, this has yet to be established.

2.5 Life Cycles and Feeding Behaviour of Mosquito Vectors of Human Malaria.

Like all other mosquito species, *Anopheles* mosquitoes require two completely different environments to complete their life cycles. The immature stages which comprise eggs, larvae and pupae require an aquatic environment and the adult mosquito an aerial and terrestrial environment (Goma, 1966; Gillies and de Meillon, 1968).

Adult female Anopheline mosquitoes lay eggs singly over the surface of clean stagnant water. These eggs, which possess air floats, remain buoyant for about two to three days before hatching can take place. Once hatched, the larva, which is about 1.5millimeters, spends its time feeding on various floating microscopic organisms such as bacteria, algae, yeasts, fungal spores and protozoa. During growth, the larva casts its skin four times before developing into another form called a pupa. A pupa is a non-feeding stage, of several days duration, providing for the morphological and physiological changes required for transformation of the larva to the adult (Goma, 1966; Gillies and de Meillon, 1968; WHO, 1982).

Once an adult female mosquito has emerged from the pupa, it remains for some hours in the vicinity of the breeding site for mating with the emerging males. Mating usually occur during 24-48 hours after emergence of adult female mosquitoes. Generally mating occurs once in the life of the female mosquito. The female adult mosquito then begins a journey in search of a nearest host. After feeding, the mosquito then rests for about two to three days usually in hidden places to allow for digestion of blood as well as for ovary development. Once developed the adult mosquitoes searches for a breeding site and the cycle continues (Goma, 1966; Gillies and de Meillon, 1968).

The longevity of adult mosquitoes varies according to species but normally the life span of the male mosquitoes of a given species is much shorter than that of the females. The longevity of mosquitoes generally is shortened in prolonged high temperature coupled with a low humidity. Under conditions of high temperature and low humidity, an adult mosquito loses a lot of water as a result of rapid evaporation (Goma, 1966). Generally, adult mosquitoes survive longer at a temperature of 27°C and a humidity of 70%. In hot climates, the average longevity ranges between 1-2 weeks (Service, 1993). Gillies (1988) in coastal Kenya compared the longevities of *Anopheles gambiae* s.s. and *Anopheles arabiensis*. The average longevity for *A. gambiae* s.s. was

1.5 weeks and 1.3 weeks for *A. arabiensis*. Under approximately natural conditions, the longevity of *Anopheles atroparvus* is six months in winter and about six weeks in summer (Hill, 1937). Similarly, *Anopheles freeborni* can live for six months in winter but can only survive for four weeks in summer (Horsfall, 1955).

Both male and female adult mosquitoes take nourishment in liquid form. The most readily available sources of food are plant nectars, honeydews and fruit juices, which are taken using the sucking mouthparts. Although both sexes can exist on these food types, female mosquitoes require a blood meal, which is primarily vital for development of eggs. Thus only female mosquitoes feed on blood. However, there are a few exceptions to this; certain mosquito species like those of the genera T*oxorhychites* and *Malaya* can still develop the eggs without the need for a blood meal. The source of a blood meal ranges from warm-blooded to cold blood animal hosts. The choice of hosts varies greatly with the species of mosquito and the opportunity available to it. Some mosquitoes exclusively feed on a particular animal host and would only switch to another in the absence of the preferred host.

Mosquitoes have also been classified on the basis of various preferences in terms of places of feeding, type of hosts on which they feed as well on physiological conditions of water in which they breed. Mosquitoes that predominantly feed on hosts outdoors are referred to as exophagic mosquitoes. Conversely, those which feed on hosts indoors are called endophagic mosquitoes, such as *Anopheles arabiensis*. Mosquitoes that have a preference for feeding on human hosts are termed as anthropophagic mosquitoes, majority of which have been incriminated in the transmission of human diseases. Zoophagic mosquitoes have a preference for feeding on animal hosts while those without a fixed preference are considered as indiscriminate biters. *Anopheles gambiae* s.s. and *Anopheles arabiensis* are highly anthropophagic but, when alternative mammalian hosts are available, *A. arabiensis* exhibits greater affinity to feed on animals

(Duchemin *et al.*, 2001; Pates *et al.*, 2001). Both species are endophilic in behaviour. The biting activity of *Anopheles gambiae* and *Anopheles arabiensis* generally starts at around 21-22 hours and reaches peak in the period between midnight and 04 hours (Gillies and de Meillon, 1968).

Mosquitoes which breed in salty water bodies are referred to as salty-water species. In the genus *Anopheles* such species include *A. melas* and *A. merus* (Gillies and Coetzee, 1987). Fresh-water species prefer water bodies devoid of salt, *A. gambiae s.s.* and *A. arabiensis* are such examples (Gillies and Coetzee, 1987).

2.6 Control of Human Malaria and Its Mosquito Vectors.

2.6.1 Global Malaria Control Programme.

The World Health Organization through the Global Malaria Control Programme recommends three primary interventions for reduction or elimination of malaria. These being: Chemotherapy, distribution of insecticide-treated nets (ITNs) and Indoor Residual Insecticide House Spraying (IRHS) in all communities at risk of malaria (WHO, 2006).

Three major types of drugs used in the treatment of various stages of malaria are of quinine, sulfadoxine-pyrimethamine and artemisinin combination therapy (ACT).

In the case of Zambia, the malaria treatment policy can be looked at from two aspects: treatment of uncomplicated and complicated malaria cases. An uncomplicated malaria case is defined by a combination of clinical symptoms that include fever, headache, aching joints, back pain, nausea

and vomiting, and general body discomfort. Whereas a complicated malaria case, usually life threatening includes symptoms such as, excessive vomiting, inability to drink or breast feed, extreme weakness, convulsions, drowsiness, loss of consciousness and abnormal breathing. Other symptoms include haemoglobinuria, hypoglycaemia, splenomegaly and hepatomegaly (NMCC, 1999 unpublished).

For uncomplicated malaria cases, artemether-lumefantrine (Co-artem®) is administered as the first line drug for adults and children weighing above 5kgs (NMCC, 1999 unpublished). Artemether-lumefantrine contains two active substances, which act together to kill the malaria parasites- *Plasmodium falciparum*. Artemisinins are the most rapidly effective anti-malarial drugs known. Clinical improvement in malaria patients usually shows within 1-3 days of starting treatment (NMCC, 1999 unpublished). Artemisinins are derived from the leaves of *Artemesia annua*, a plant used in traditional Chinese medicine. The potency of artemisinins as an antimaralial drug was discovered and developed by the Chinese researchers in the 1970s. Since then, a number of derivatives have been developed some of which include: dihydroartemisinin and arteether (NMCC, 1999 unpublished).

In Children weighing below 5kgs, sulfadoxine-pyrimethamine (SP) is recommended. This drug belongs to the antifolate group of antimalarials. Sulfadoxine works synergistically with pyrimethamine against the parasite-specific enzymes, dihydropteroate synthase and dihydrofolate reductase. The two drugs inhibit parasite synthesis of folate, which is essential for DNA replication and therefore cell growth (NMCC, 1999 unpublished).

In cases of treatment failure in all age groups to the first line drugs, quinine is administered as a second line drug. Quinine is an alkaloid extracted from the bark of the cinchona tree. It is a blood

schizonticide effective against *Plasmodium falciparum* infections; including resistant strains (NMCC, 1999 unpublished).

In pregnant women, quinine is used as the first line drug for treatment of uncomplicated malaria in the first trimester of pregnancy. In the second and third trimesters, sulfadoxine-pyrimethamine or artemether-lumefantrine is administered. For intermittent preventive treatment (IPT) during the second and third trimester of pregnancy, sulfadoxine-pyrimethamine is recommended (NMCC, 2010 unpublished).

Insecticide treated nets (ITNs) are widely used in many countries where malaria is endemic. Insecticide treated nets work in two ways. Firstly, they act as physical barriers against any mosquito seeking to feed on hosts sleeping under it. Secondly, the Pyrethroid insecticides on the net have repellent and insecticidal effects to the host seeking mosquitoes. Mosquitoes that come in contact with the insecticide on the net eventually die due to the effects of the insecticide. This way, malaria vector populations are reduced overtime. However, significant results are only obtained when usage is at a large scale. A recent study has shown that usage of ITNs by 60% of all adult and children in a community could reduce cases of malaria by as much as 50% (WHO, 2006).

Indoor Residual Insecticide House Spraying (IRHS) is the application of long-acting chemical insecticides on the walls and roofs of houses and other domestic shelters in a given area, in order to kill the adult vector mosquitoes that land and rest on these surfaces. This is usually applied by means of hand compression sprayers. The primary effect of IRHS is to reduce the life span and density of the vector mosquitoes. Consistent application of IRHS over time in large areas has been shown to alter the vector distribution and subsequently the epidemiological pattern of malaria in countries like Botswana, Namibia, South Africa, Swaziland and Zimbabwe. In these

countries, the density of *A. funestus* and *A. gambiae* which are the major vectors of malaria were reduced to negligible levels through IRHS (WHO, 2006).

However, in areas where *A. arabiensis* is dominant, IRHS even at high spraying coverage levels is usually not an effective method in reducing the density of the malaria vector. The behavior of *A. arabiensis* is such that it tends to avoid entering sprayed houses in preference to seeking alternative animal hosts outside the houses (Gillies and Coetzee, 1987). For instance, in Nigeria, Molineaux and Gramiccia (1980) reported a significant proportion of populations of *A. arabiensis* resting outside in areas that had been sprayed with residual insecticides. In cases such as this, environmental management becomes a second option. Environmental management for mosquito control would involve destroying all possible water pools likely to act as breeding places for the mosquitoes (WHO, 2006).

Another promising method of malaria control involves reducing malaria vector populations using the sterile-insect technique (SIT) (Benedict and Robinson, 2003; Helinski *et al*., 2008). It is a technique which has yet to be adopted in many countries in Africa. But in some countries like India, the technique has been used to control *Culex* mosquitoes and *Anopheles albimanus* in El Salvador, though on a limited scale (Helinski *et al*., 2008). Field tests are yet to be conducted on sterile *A. gambiae* s.s. mosquitoes, a major vector of malaria in the Afro-tropical region (Morlais *et al*., 2005).

The sterile-insect technique operates on the principle that when a female mosquito mates with a male counterpart whose sperm has been rendered unviable, the female will have no progeny. Thus overtime, the mosquito population declines. Sterilization is commonly done by exposure of male mosquitoes to high doses of radiation, which damages chromosomes, thus rendering the sperm unviable.

But in order for SIT to be effective the sterile males have to be released in large numbers to outcompete the local male mosquitoes in mating with females. This ratio aspect is very crucial. Ideally the ratio of the number of released sterile males to that of the local male population is 10:1 (Morlais *et al*., 2005). This requirement for large numbers of sterile male mosquitoes renders this method unsuitable for many African countries where the densities of the target mosquitoes are high (Morlais *et al*., 2005). Infrastructure and human resource for this sort of malaria intervention in least developed countries would be a challenge. However, SIT is a tool that can be more effective compared to the use of chemicals (insecticides) where development of resistance in the malaria vectors to the insecticides seem to be a major challenge.

2.6.2 Mosquito Vector Control in Zambia.

Mosquito control may be undertaken either to prevent mosquito-borne diseases or to protect humans and their livestock from the vicious attacks by the insects. This entails both destroying mosquito-breeding places as well as using other intervention methods such as spraying of chemicals inside human habitations to kill the mosquitoes (www.malariasite.com). One of the interventions presently being applied in Zambia is the Indoor Residual Insecticide House Spraying (IRHS).

Indoor Residual House Spraying involves spraying insecticides, which have a persistent lethal effect on mosquitoes, on all indoor surfaces where mosquitoes are likely to rest. The persistent or residual effect varies with the kind of insecticide used, its formulation, the dosage applied, the type of surfaces sprayed, and the climatic conditions at the time of spraying. The duration of the residual effect usually varies from a few weeks to over a year (WHO, 1982). The attack is

mainly directed at disease vectors that frequent human habitations, bite man and rest indoors. These vectors, while resting on sprayed surfaces, come into contact with the insecticide and later die, therefore, unable to transmit the disease (WHO, 1982).

Zambia is one of the countries known to have had recorded success in IRHS programmes in the late 1950s (MoH, 2000). DDT at the time was the most effective insecticide that was used. However, because of its negative effects on non-target organisms and the environment, the World Health Organization imposed a global ban on its use in the 1970s. Consequently public IRHS interventions began to decline in the country. Malaria cases, on the other hand, began to increase, particularly in the urban areas and recent studies have estimated that the incidence rates of the disease more than trebled over the past three decades. Whereas, there were 121.5 cases per 1000 in 1976, this rose to 398.8 per 1000 in 1998 (NMCC, 1999 unpublished). In 2006, WHO lifted the ban on the use of DDT on condition that the insecticide would only be used in IRHS campaigns. Previously DDT was extensively used in both health and agriculture programmes to control disease vectors and insect pests, respectively. Zambia is one of the countries that have once again reverted to the use of DDT to control malaria vectors. Presently, IRHS is being implemented in 36 districts across the country including Chongwe district in Lusaka Province where the program was began in 2005 using pyrethroids. Use of DDT for IRHS programmes in Chongwe district began in 2006/2007 malaria transmission season (NMCC, 2010 unpublished).

2.6.2.1 Indoor Residual Insecticide House Spraying (IRHS) in Zambia.

Indoor Residual House Spraying (IRHS) using DDT (2g/m²) to combat malaria was first adopted in 1950 by the Rhodesia-Nyasaland Federation under the Federal Malaria Eradication Organization. The Rhodesia-Nyasaland Federation was made up of three member countries;

Northern Rhodesia, Southern Rhodesia and Nyasaland. It was the role of the Federal Malaria Eradication Organization to develop and ensure implementation of malaria programmes in these member countries. However, following the break up of the federation in 1963, each country had to develop its own programmes (MoH, 2000).

To ensure continuity of the IRHS programme, the Northern Rhodesian government, now called Zambia, involved three independent authorities: the Municipal councils, the Ministry of Health and the Mining companies. These were to administer the IRHS programme in various areas across the country. The Municipal councils were responsible for urban areas; the Ministry of Health was responsible for the rural areas while the Mining companies were assigned to mine compounds (MoH, 2000).

Through this method, malaria in the urban communities was kept to a minimum for at least three decades (1940-1970). While the disease had almost been eliminated in major towns along the line of rail, malaria in the rural areas was increasingly becoming a serious problem. The situation got worse following the drop in copper prices on the international market on which the economy of Zambia depended to sustain malaria programmes. Increased responsibilities associated with struggles for freedom in the Southern Africa affected the economy of Zambia as well. Funding to the IRHS thus reduced significantly. The situation got even worse following the ban of the use of DDT world wide in IRHS programmes by the WHO in the 1970s. The IRHS programme continued to decline until it ceased completely around 1990 (MoH, 2000).

2.6.2.2 Current Status of IRHS in Zambia.

The Abuja Declaration in 2000 marked the revival of the IRHS programme in Zambia. Under this declaration, African countries were mandated to develop mechanisms that would ensure

attainment of interim Progress Indicators towards the Roll Back malaria (RBM) goals (Manyeme *et al*., 2005). Being a signatory to this declaration, Zambia developed mechanisms that focused at improving policy frameworks, piloting and scaling up of effective malaria interventions, mobilizing resources, capacity building as well as increasing partnerships.

As a follow-up to these mechanisms, 10 sentinel districts were identified under the RBM programme across the country. These were to serve as operational districts under which the impact of the RBM interventions would be monitored. In 2001, the first surveys were embarked on. Vector control, which embraced ITNs and IRHS, was among other components included in the survey. In line with the findings of the survey, recommendations were made to scale up use of ITNs and IRHS to particularly target rural communities across the country. The programme started with two districts and later increased to 15 then to 36 in 2007 (Appendices 11a and 11b) and presently the programme operates in 36 districts of the country (NMCC, 2007 unpublished).

2.6.2.3 Impact of the IRHS Intervention on Mosquito Vectors of Human Malaria in Zambia.

There has not been thorough surveillance of vector population since the inception of the programme in most parts of the districts due to inadequate trained human resource (qualified entomologists), as the country lack appropriate entomological equipment (NMCC, 2007).

CHAPTER 3: MATERIALS AND METHODS

3.1 Study Design.

This study was a case-control type of study; Chishiko Village being the case where houses had been sprayed with a residual mosquito insecticide (DDT,) during the 2008-2009 malaria transmission period through the IRHS programme and Chiota village a control where houses had not been sprayed with the residual mosquito insecticide.

3.2 The Study Area.

This study was conducted in a rural area of Chongwe district in Chiota and Chishiko villages, 45 km east of the capital city Lusaka, within Lusaka Province (Longitude, between 28º and 30ºE., and Latitude, between 15º and 16ºS) (Figs.1 and 2).

Chongwe district, which is an area of about 10,500 km^2 lies at an average altitude of about 1180 m above Sea Level. About 92% of the district is plateau with the rest being the valley. Two major permanent rivers namely, Chongwe and Chalimbana run through the district. Chongwe river flows through the Chongwe Town Centre. Chalimbana river, which is a tributary of the Chongwe river flows on the southern part of the town centre. Other river bodies are mostly temporally pools which form during the rainy season. The normal rainy season starts from early November, lasting up to March the following year with rainfall of more than 800 mm annually. Temperatures range between 18 and 33 degrees during the hot/wet season and between 14 and 25 degrees celcius in the cool dry season. A savanna type of vegetation characterizes this region, with *Brachystegia-Julbernadia* or miombo tree species dominating. The described topographic,

climatic, and vegetation type render most parts of the Chongwe district prone to high densities of mosquitoes especially during the rainy season.

The district has a total population of 205, 272 people (CSO, 2000). A large proportion of this population (92%) consists of rural dwellers. Chiota Village has a population of 2, 488 people with 333 homesteads, while Chishiko Village is slightly larger, with 2, 688 people and 448 homesteads (Chalimbana district Development Plan 2001-2005). The two study villages are approximately 25 km apart (Fig. 2).

3.2.1 Livelihoods of the People in the Study Area.

The economic activities in the district are largely agricultural. Over 75% of household incomes in the district come from agricultural related activities. Three major types of farming systems exist, these being commercial farming, emergent farming and small scale farming. Maize, cotton, sunflower and groundnuts are the major crops cultivated in the district. In terms of livestock, several small-scale farmers own cattle, goats and chickens, which are mostly traded to the urban community of Chongwe district. The urban community comprises government workers and business individuals (Chalimbana district Development Plan 2001-2005).

3.2.2 Criteria for Selecting the Study Area.

The selection criteria used for the study area were, abundance of mosquitoes known to occur in the area during the rainy season and the fact that Chongwe is one of the districts in which the IRHS programme was being implemented in the country. Among the ten sentinel IRHS districts in Zambia under the Roll Back Malaria programme, Chongwe experiences the highest mosquito densities and malaria incidences during the rainy season (MoH, 2000 Unpublished). The high

annual mosquito densities observed are due to increased number of water pools that develop during the rainy season. These water pools serve as suitable mosquito breeding places, particularly at the beginning and towards the end of the season.

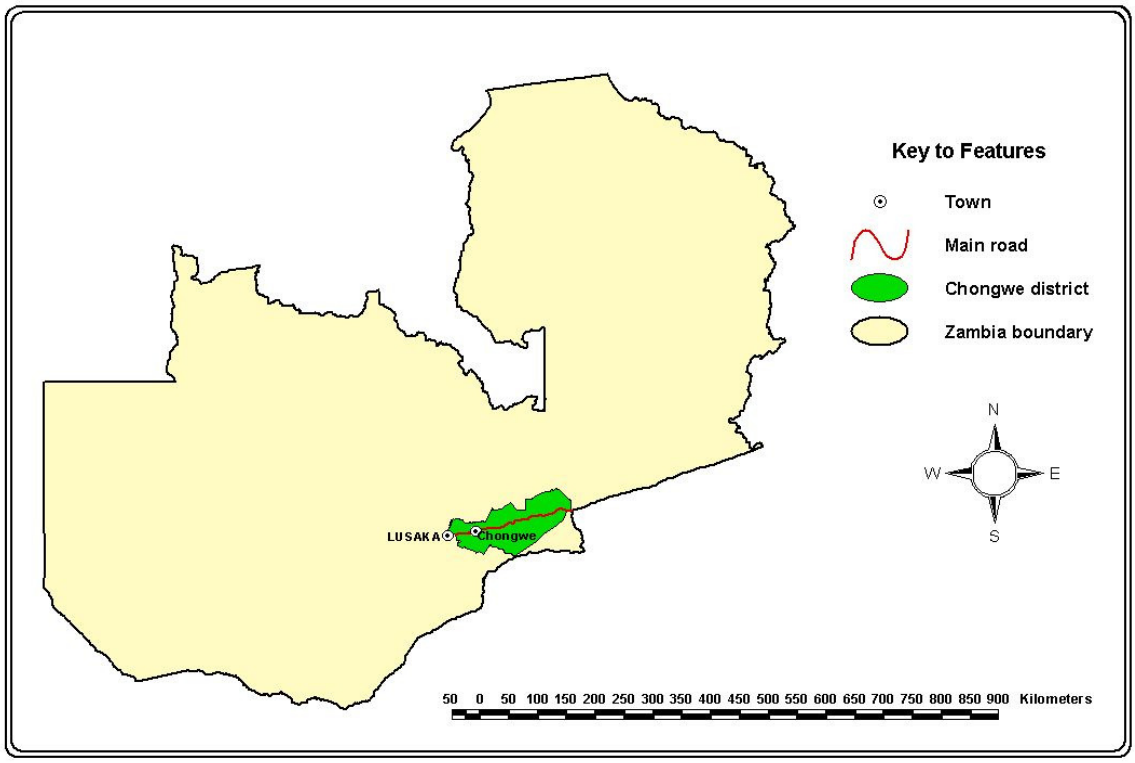

Figure 3.1 Location of Chongwe district in Zambia (Source: Surveyor-General, Lusaka, 1986).

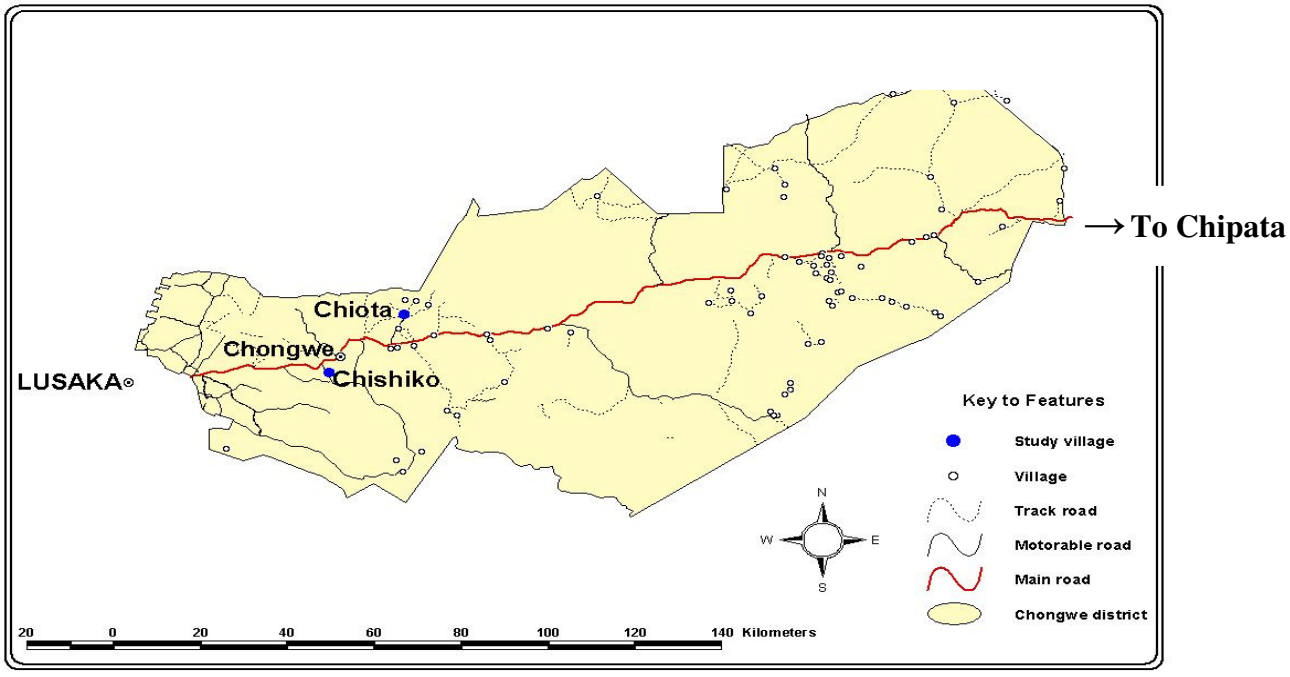

Figure 3.2 Location of the Study Area (Chiota and Chishiko Villages) in Chongwe district, Zambia (Source: Surveyor-General, Lusaka, 1986).

3.2.3 House Designs of the Study Area.

In both study villages, majority of the houses were made of mud and had porous walls, with grass-thatched roofs (Fig. 3). The designs of the houses did not take into account the protection of the occupants against mosquitoes; they had open eaves and unscreened windows and doors. Because of the need to access adequate water for domestic use and for the livestock, a large number of families lived close to streams, rivers and still water bodies which are temporally. Mosquitoes that bred in these waters had therefore easy access to the human and animals for blood meals.

3.3 Mosquito Sampling and Collection.

3.3.1 Sample Size.

Determination of the appropriate sample size for population-based surveys like this one, which employ simple random samples, three factors were taken into consideration: (i) the estimated prevalence of the variable of interest, in this case, the proportion of houses of the Chongwe district in which mosquito can be found (the prevalence estimate for this study was obtained from entomological surveys that had previously been done by the National Malaria Control Centre in Chongwe district in which about 94% of houses were found to have mosquitoes (ii) the desired level of confidence and (iii) the acceptable margin of error.

The following formula was employed (Southwood, 1966; www.ifad.org/)

Formula: $n=Z^2pq/d^2$:

Where n = required sample size

Z = confidence level at 95% (standard value of 1.96)

p = 94% (estimated proportion of houses with mosquitoes in Chongwe district)

q = 5% (100-p)

d = margin of error at 5% (standard value of 0.05)

Calculation:

$n = (1.96)^2 \times 94 \times 5 / (5)^2$

= 72 houses

3.3.2 Mosquito Sampling.

Houses sampled for mosquitoes in the study area were selected using random numbers. This was achieved by firstly assigning numbers to all the houses in the study area. Then the GraphPad random number generator was used to select the required houses to be included in the study. This method was used for both sprayed and unsprayed houses in the study areas. A total of 72 houses were sampled, 36 from either village. The 36 houses used in Chishiko Village were among those that had previously been sprayed with DDT (at a rate of $2g/m^2$) in the month of November 2008 under the national IRHS programme. Due to inadequate resources (Funds), sampling of mosquitoes was done only once in each of the selected houses during the study period. Ideally, strict impact assessments of IRHS are done over a period of at least not less than 3 years. Sampling of mosquitoes in the identified sentinel houses is replicated monthly or every after 3 months, depending on the study design (WHO, 1975).

The selected houses and mosquito breeding sites within approximately 500 meters of each house were then marked using a hand-held Global Positioning System (GPS) receiver. Data were

collected on the number of occupants and on whether the occupants had used mosquito nets the previous night, using a questionnaire (Appendix 2).

On the days of mosquito collections, the participants were requested to prepare their houses by clearing the floor surfaces on which sheets of cloth were to be spread where the mosquitoes would be falling on after being knocked down by the space sprayed insecticide (WHO, 1975).

3.3.3 Mosquito Collection.

For each sampled house two fields assistants were required for mosquito sampling. One assistant collected mosquitoes from inside the house while the other assistant guarded against the escape of mosquitoes from the house through house eaves from outside. The insecticide, Target® was then sprayed along the eaves by both the mosquito collector on the inside and the mosquito guard on the outside, starting from opposite ends of the house. After spraying, doors and windows were closed for ten minutes. Thereafter, the sheets on the floor were removed, lifting them by the four corners to the outside daylight for examination (WHO, 1975). The knocked down mosquitoes were then picked using pairs of forceps and each mosquito stored individually in a dry vial containing Silica gel.

All the collections were done in the morning between 04:30 hours and 8:30 hours (WHO, 1975). Mosquito sampling started on the 31st of December, 2008 and ended on the 25th of January, 2009. The sampling was made once for each of the selected houses during the stated period.

Mapping of mosquito distribution involved collection of way-points of places from where the mosquitoes were sampled. A Geographical Positioning System (GPS) was used for this purpose.

The collected way-points were then fed into the computer software; Arcview GIS version 3.2 to generate the distribution maps (Figures 4.4 and 4.5).

3.3.4 Ethical Considerations.

Before commencing the mosquito sampling, ethical clearance was obtained from the University of Zambia, School of Medicine (Appendix 1), and from the World Health Organization (WHO) Research Ethics Committees in Geneva. Further authorization from the headmen and residents of the two villages was obtained with the assistance of the village secretaries by way of consent forms.

Figure 3.3 Design of Houses in the Study Area.

3.4 Mosquito Species Identification.

3.4.1 Morphological Identification Method.

Morphological identification of mosquitoes was done using a compound microscope and taxonomic identification keys (Gillies and Coetzee, 1987; Service, 1990; Edwards, 1941). All the mosquitoes that were generally identified as *Anopheles gambiae* s.l. were further identified to species level using molecular techniques.

3.4.2 Molecular Identification.

3.4.2.1 Chelex Method of Mosquito DNA Extraction and Polymerase Chain Reaction (PCR) Assay.

DNA was extracted using the Chelex method from each whole female *Anopheles gambiae* s.l. mosquito and the Polymerase Chain Reaction (PCR) was performed according to the protocol described by Scott and co-workers (Scott *et al*., 1993). A total of 11 whole mosquito specimens from the study area were used, in molecular identification of the mosquitoes from the study area. In addition to the 11 mosquito samples from the study area, two (2) positive mosquito samples of *A. arabiensis* from Zimbabwe and four (4) from Macha were included in the molecular assay making a total of 17 samples. The additional mosquitoes served as standards for comparison with the study samples just in case the commercial *A. arabiensis* standard failed to work.

A whole mosquito sample was individually introduced into each 1.5ml microfuge tube, containing 400ul of 1 x PBS/ 1% saponin solution. It was then crushed mechanically using a bent pipette tip until no body part was recognizable. The crushed specimen was then left to stand in

the solution at room temperature for 20 minutes after which it was spun at 14, 000 revolutions per minute (rpm) for 2 minutes at 26°C using an Eppendorf Centrifuge (5417R). The supernatant was then aspirated and discarded, retaining the debris (pellet) in the tubes. Thereafter, 400ul 1 x PBS was added and the tube spun again at 14000 rpm for another 2 minutes at 26°C. As in the previous step, the supernatant was aspirated and discarded. Then 25ul of 20% w/v of Chelex and 75ul sterile water (ddH_2O) were added to the tube. The sample tube was then closed and a fine hole pierced in its lid using a hot sterile hypodermic needle. The needle was flamed between piercing different sample tubes to avoid sample cross contamination. The tube contents were then boiled at approximately 100°C for 10 minutes in a water bath. The fine hole in the lid of the tube was for the purpose of releasing vapour during the boiling step to prevent the lid from popping open. The tubes were then spun at 14, 000 rpm for 1 minute in the centrifuge at 26°C after which the supernatant was aspirated into sterile vials for storage at -70°C pending the PCR assay. The remaining pellets in the tubes were this time discarded.

3.4.2.2 Polymerase Chain Reaction (PCR) and PCR Master Mix.

Before conducting DNA amplification for the 17 samples (11 from Chongwe; 2 additional ones from Zimbabwe and 4 from Macha), a Polymerase Chain Reaction (PCR) master mix for 25 instead of 17 PCR reactions was prepared. The extra volume was to cater for any loss resulting from transfers from stock bottles. This was done by pippetting 268.75µl of double distilled water (ddH_2O) into a 2ml microfuge tube, followed by 62.5 µl 10x reaction buffer, then 50 µl dNTPs, 12.5 µl *Anopheles gambiae* s.s. primer, 75 µl *Anopheles arabiensis* primer, 37.5 µl BSA and 18.75 µl Black Taq DNA polymerase (Table 3.1).

Table 3.1 Reagents and Quantities required for a 25-reaction PCR Master Mix.

Reagent	Volume for one PCR reaction	Volume for 25 PCR reactions
ddH2O	10.75 µl	268.75 µl
10X reaction buffer	2.5 µl	62.5 µl
DNTPs	2 µl	50 µl
A. gambiae s.s. primer	0.5 µl	12.5 µl
A. arabiensis primer	0.5 µl	75 µl
BSA	1.5 µl	37.5 µl
Black Taq	0.75 µl	18.75 µl

The primers used in this protocol amplify species-specific nucleotide sequences on intergenic spacers (non-coding regions) of the ribosomal DNA (Table 3.2).

Table 3.2 Mosquito DNA Primers used in the PCR Assay.

MOSQUITO SPECIES	PRIMER SEQUENCE	PCR PRODUCT SIZE
Anopheles gambiae s.s.	5'-GA CTG GTT TGG TCG GCA CGT TT-3'	390bp
Anopheles arabiensis	5'-AAG TGT CCT TCT CCA TCC TA-3'	315bp

3.4.2.3 Sample DNA Amplification.

One and half microlitres of the mosquito DNA extract were introduced into each microfuge tube. Then 25 µl of the master mix was added. The tubes were then placed in the PCR instrument for

the amplification of the sample DNA. Amplification was conducted through 30 cycles. The initial step involved denaturation of the DNA for 2 minutes at 94°C, followed by primer annealing for 30 seconds at 50°C , then extension for 30seconds at 72°C. The successive cycles were done at denaturation for 2 minutes at 94°C whereas the final extension step was done for 7 minutes at 72°C (Scot *et al*., 1993). The resulting PCR products where temporarily (20 minutes) stored at -70°C as the electrophoresis gel was being prepared.

3.4.2.4 Gel Preparation.

The agarose gel was prepared by mixing 50ml of 0.5 x TBE and 2.50g agarose in a beaker and heat applied in a microwave until the mixture boiled. Then 11.25 µl of Ethidium bromide were added and mixed while gel was still molten. The mixture was then poured into a mould and allowed to solidify at room temperature for approximately 15 minutes.

3.4.2.5 Loading of PCR Products.

Six microlitres of each of the PCR products were mixed with 4 µl of loading dye on a Parafilm using a pipette and then loaded onto the wells on the agarose gel. Wells on the flanks (i.e. well number 1 and 22) were loaded with 1.5 µl of 100 base pair ladder. The rest of the wells in between were loaded in the order of: Negative control (2), *A. gambiae* standard (3), *A. arabiensis* standard (4), samples of *A. arabiensis* from the Macha area (5 and 6), *A. arabiensis* from Zimbabwe (7 and 8), 10 samples from the study area (9 -18), *A. arabiensis* samples from the Macha area (19 and 20), and another sample from the study area (21). Electrophoresis was then performed at 120 Volts and 500mL Amps for 120 minutes in 0.5 x T.B.E. The gel was then captured on a camera for visualization of the DNA bands.

3.5 Determination of Mosquito Diversity, Abundance and Distribution.

3.5.1 Mosquito Diversity.

The alpha (α) diversity index of 0.5 (from William's nomograph for determining α diversity indices) was used to estimate the expected species total (EST) for the study area (Southwood and Henderson, 1978). The following expression was used:

$$EST = \alpha X / 1-X$$

Where, EST= Expected Species Total

α =Alpha Index of Biodiversity

X= Sampling Factor [$X = 1-e^{-N/\alpha}$] where e= 2.7182818 and,

N= Total number of mosquito species collected in the sample.

3.5.2 Mosquito Abundance.

The mean mosquito densities as a measure of abundance were calculated; and analysis of variance (ANOVA) performed in STATISTIX version 2.0 for significance of abundance between Chiota and Chishiko Villages. Percent composition for each of the mosquito species were calculated manually.

3.5.3 Mosquito Distribution.

Distribution of mosquito species in the study areas was determined by comparing the distribution variance (S^2) and arithmetic mean ($\bar{x}$) of mosquito numbers (Southwood, 1978). The

distribution variance was computed manually using the following formula for each of the mosquito species:

$$S^2 = \frac{\Sigma x^2 - [(\Sigma x)^2 / n]}{n-1}$$

Where; Σ denoted summation of all factors to the right

$\bar{x}$ = value of number of mosquitoes per house (mean density)

n = total number of houses

S^2 = distribution variance

3.6 Testing of Specific Research Hypotheses.

3.6.1 Hypothesis 1: Endophilic Mosquito Species of Chongwe district do not include Malaria Vector Species.

This hypothesis was tested through identification of all mosquitoes collected indoors during mosquito sampling from houses in the two villages and screening for the presence of malaria vectors in the collections.

3.6.2 Hypothesis 2: The Major Vector(s) of Human Malaria in the Chongwe district does not belong to the *Anopheles gambiae* Sibling Species Complex.

Examination for the absence of mosquito species from the *Anopheles gambiae* sibling species complex in the mosquito collections made from the houses of the study areas tested this hypothesis.

3.6.3 Hypothesis 3: The IRHS Programme in Chongwe district has not changed the Diversity, Abundance and Distribution of Endophilic Human Malaria Transmitting Mosquito Species.

This hypothesis was tested by comparing the diversity, abundances and patterns of distribution of human malaria vectors in selected DDT sprayed houses in Chishiko village and non sprayed houses in Chiota village.

CHAPTER 4: RESULTS

4.1 Mosquito Sampling and Collection.

Out of a total of 36 houses sampled for mosquitoes in the unsprayed section of the study area, 20 had mosquitoes compared to the sprayed section in which only 12 houses had mosquitoes present. A variation was observed in the species composition of mosquitoes in the houses. In the unsprayed study area, (8)22% of the houses had *Culex quinquefasciatus*, (9)25% had anopheline mosquitoes while (3)8% had both *Culex quinquefasciatus* and the anopheline species. Sixteen (16) (44%) houses had no mosquitoes present (Fig. 4.1). In the sprayed study area, 56% of the houses had *Culex quinquefasciatus* mosquitoes, and no *Anopheles* mosquitoes were collected in this area (Fig. 4.2).

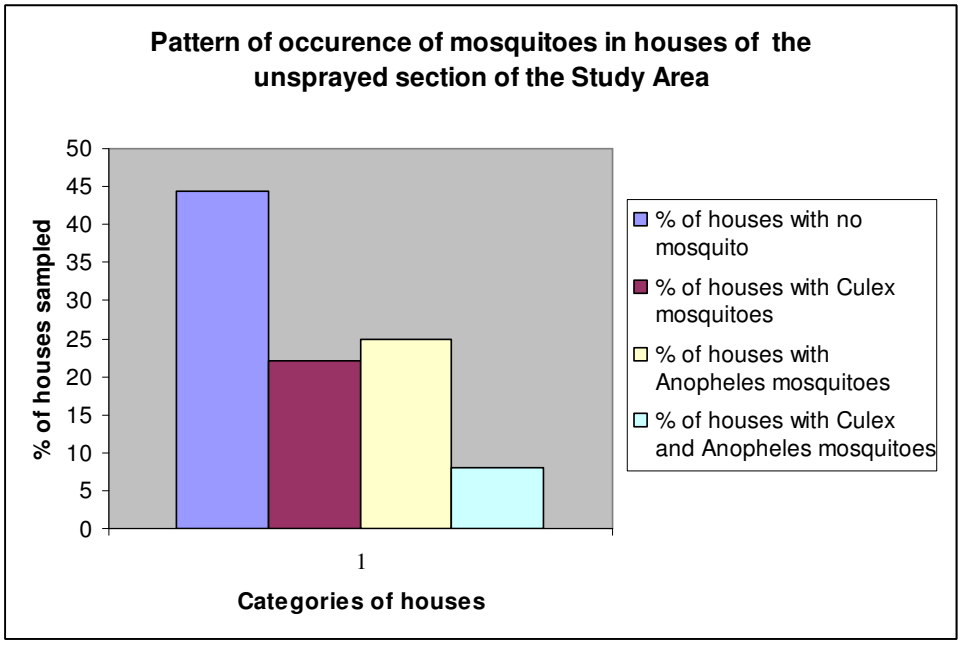

Figure 4.1: Pattern of occurrence of mosquitoes in houses of the unsprayed Study Area.

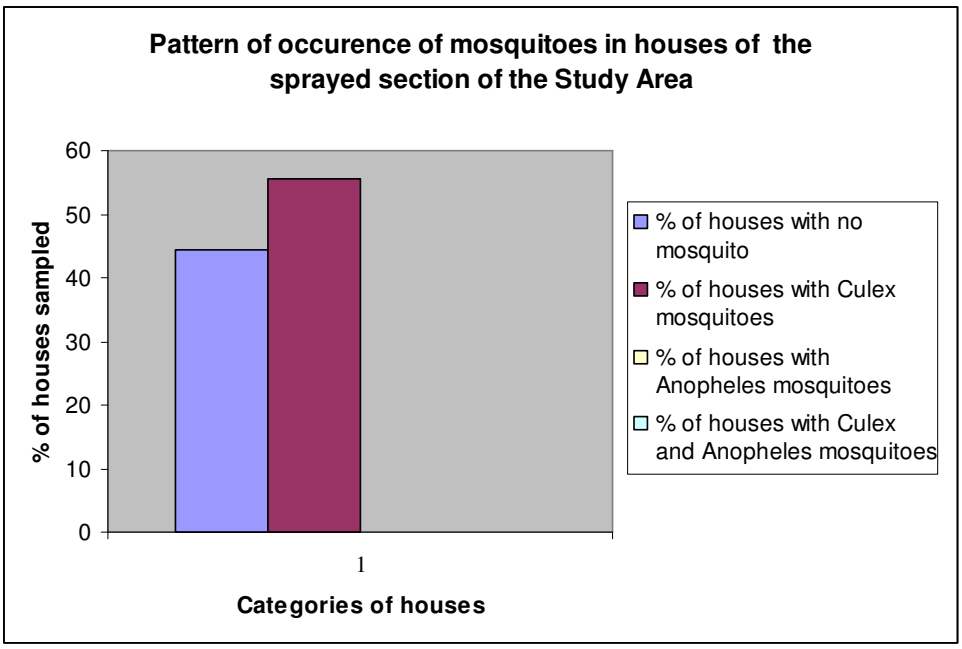

Figure 4.2: Pattern of occurrence of mosquitoes in houses of the sprayed Study Area.

4.2 Mosquito Species Identification.

4.2.1 Morphological Identification.

Three species of endophilic mosquitoes were identified from the study area using taxonomic identification keys. These were *Culex quinquefasciatus* (n=66), *Anopheles squamosus* (n=7) and another species identified only as belonging to the *Anopheles gambiae* s.l. (n=11), which was further identified to sibling species (see section 4.2.2).

4.2.2 Molecular Identification of the *Anopheles gambiae* s.l. Mosquito Specimens using PCR.

All the 11 *Anopheles gambiae* s.l. mosquito specimens subjected to the Polymerase Chain Reaction (PCR) assay were identified as *Anopheles arabiensis* through the molecular identification method.

According to the PCR assay results, the bands of amplified mosquito DNA sample in lanes 9-18 and 21 matched with the DNA bands of positive controls of *Anopheles arabiensis* (315bp) in lanes 4-6; 19 and 20 on the electrophoresis agarose gel (see Figure 4.3).

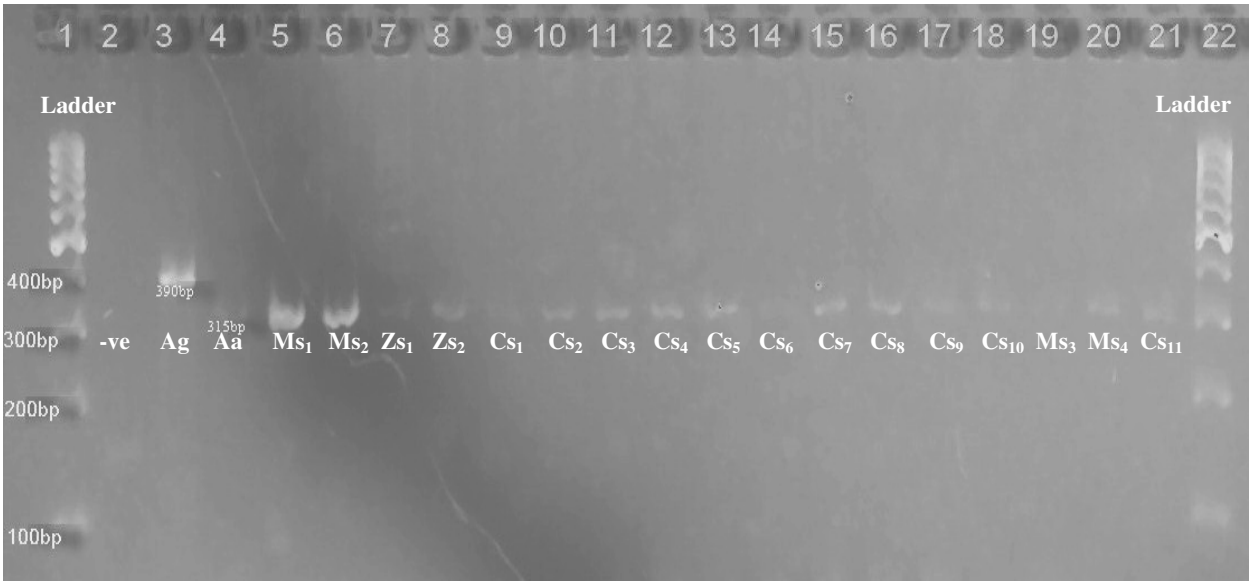

Figure 4.3 Gel Image of the Polymerase Chain Reaction Assay of Mosquito DNA.

Legend

Lanes 1 and 22: 100bp DNA ladder.

-Ve= Negative control (No DNA present).

Ag= Positive control for *Anopheles gambiae* s.s. (390bp).

Aa= Positive control for *Anopheles arabiensis* (315bp).

Ms_1- Ms_4= Macha samples 1-4.

Zs_1 and Zs_2 = Zimbabwe samples 1 and 2.

Cs_1- Cs_{11}= Chongwe samples 1-11.

4.3 Mosquito Diversity, Abundance and Distribution.

4.3.1 Mosquito Diversity.

All sample specimens of endophilic mosquitoes collected from the study area belonged to three mosquito species. These were identified as; *Culex quinquefasciatus* Say, 1823, *Anopheles squamosus* Theobald, 1905 and *Anopheles arabiensis* Patton, 1905. On the basis of this number of endophilic mosquito species identified (three) and the total number of mosquito specimens collected from the study area (n = 84), an estimate of Expected Species Total (EST) of endophilic mosquitoes using an alpha (α) index of diversity value of 0.5 determined for the area, indicated that the study area or Chongwe district had an expected species total of three endophilic mosquitoes.

4.3.2 Mosquito Abundance.

Out of the 84 mosquitoes collected in the study, *Anopheles arabiensis* accounted for 11 representing a proportion of 13.1%; *Anopheles squamosus* accounted for 8.3% representation; and the rest (66) were *Culex quinquefasciatus* which accounted for the largest proportion of 78.6%. All the mosquitoes collected were females (Table 4.1).

Table 4.1 Frequency Data of Mosquito Species Collected in Chiota and Chishiko Villages combined.

Mosquito Species	Male n	Female n
Culex quinquefasciatus Say	0	66
Anopheles arabiensis Patton	0	11
Anopheles squamosus Theobald	0	7
Total	0	84

4.3.2.1 Mosquito Abundance in Chiota Village.

Thirty-four (34) mosquitoes were collected in Chiota Village, out of which 47.0% were *Culex quinquefasciatus*; 32.4% were *Anopheles arabiensis* and 20.6% were *Anopheles squamosus* (Table 4.2).

Table 4.2 Frequency Data of Mosquito Species collected in Chiota Village.

Mosquito Species	Male n	Female n
Culex quinquefasciatus	0	16
Anopheles arabiensis	0	11
Anopheles squamosus	0	7
Total	0	34

4.3.2.2 Mosquito Abundance in Chishiko Village.

There were 50 mosquitoes collected in Chishiko Village all of which were *Culex quinquefasciatus* species. No *Anopheles* mosquitoes were collected in this section of the study area (Table 4.3).

Table 4.3 Frequency Data of Mosquito Species collected in Chishiko Village.

Mosquito Species	Male n	Female n
Culex quinquefasciatus	0	50
Anopheles arabiensis	0	0
Anopheles squamosus	0	0
Total	0	50

4.3.2.3 Comparison of Mosquito Abundance in Chiota and Chishiko Villages.

The difference in the abundance of *Culex* mosquitoes between the two study areas was not significant ($p > 0.05$) (Table 4.4). However, a significant difference was noted in the case of *Anopheles* mosquitoes ($p < 0.05$) (Table 4.5).

Table 4.4 Analysis of Variance for *Culex* Mosquito Abundance in Chiota and Chishiko Villages.

Source of Variation	d.f	SS	M.s	F	p
Between	1	16.06	16.06	2.58	0.1127*
Within	70	435.4	6.22		
Total	71	451.50			

*Significant at p = or <0.05

Table 4.5 Analysis of variance for *Anopheles* mosquito abundance in Chiota and Chishiko Villages.

Source of Variation	d.f	SS	M.s	F	p
Between	1	4.50	4.50	8.08	0.0059
Within	70	39.00	0.56		
Total	71	43.50			

4.3.2.4 Mosquito Mean Density in Chiota and Chishiko Villages.

The mosquito mean density varied between the two villages. In Chishiko Village, 1.39 *Culex* mosquitoes per house were found while in Chiota Village the density was lower (0.44) (Table

4.6). The mean density of the *Anopheles* mosquitoes was 0.5 per house in Chiota while no *Anopheles* mosquitoes were encountered in Chishiko Village (Table 4.7).

Table 4.6 *Culex* Mosquito Mean Density by Village.

Village	Mean density ($\overline{x}$)	Group size	Std. Dev.
Chiota	0.44	36	0.9085
Chishiko	1.39	36	3.4082
Total	0.92	72	2.4941

Table 4.7 *Anopheles* Mosquito Mean Density by Village.

Village	Mean density ($\overline{x}$)	Group size	Std. Dev.
Chiota	0.50	36	1.0556
Chishiko	0.00	36	0.0000
Total	0.25	72	0.7464

4.3.3 Mosquito Distribution.

4.3.3.1 Distribution of Human Malaria Vector Species in Chiota and Chishiko Villages.

The calculated variance (S^2=1.11) to determine distribution of *Anopheles* mosquitoes in Chiota Village was larger than the mean mosquito density (0.50), implying that the mosquitoes were contagiously distributed (Table 4.8). The variance for Chishiko Village could not be determined. The formula for calculation of variance required a mean density of greater that zero.

Table 4.8 Distribution of Human Malaria Vector Species by village.

Study area	Variance (S^2)	Mean density ($\bar{x}$)	Number of houses
Chiota	1.11	0.50	36
Chishiko	-	0.00	36

4.3.3.2 Distribution of Non-malaria Vectors (*Culex* Mosquito Species) in Chiota and Chishiko Villages.

In both study areas *Culex* mosquitoes were contagiously distributed since the values of variance calculated were greater than the mean mosquito densities for the two areas (Table 4.9). See also figures 4.4 and 4.5 for the distribution pattern.

Table 4.9 Distribution of Non-malaria Vector Species (*Culex* Mosquito Species) by village.

Village	Variance (S^2)	Mean density ($\bar{x}$)	Number of houses
Chiota	0.83	0.44	36
Chishiko	406.7	1.39	36

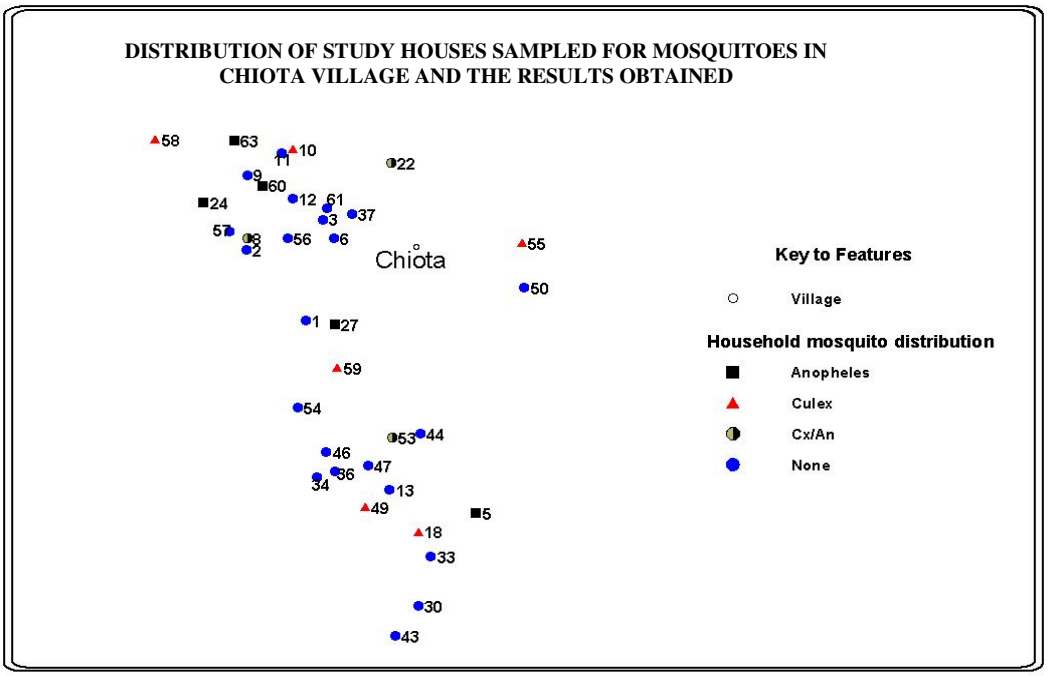

Figure 4.4 Distribution of Study Houses in Chiota Village (see also appendix 3a).

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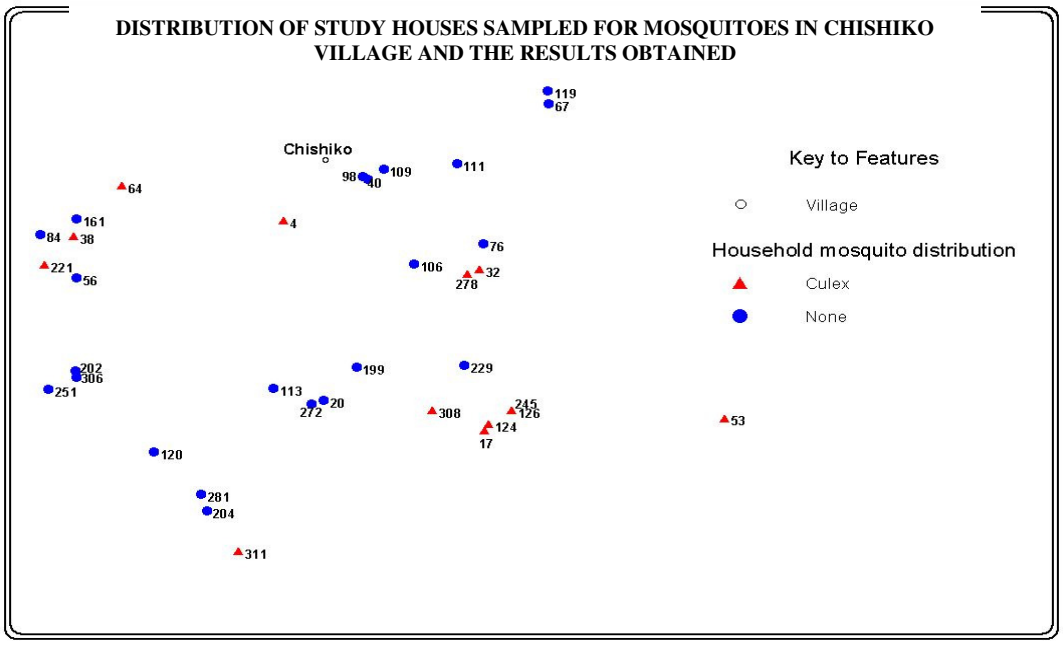

Figure 4.5 Distribution of Study Houses in Chishiko Village (see also appendix 3b).

4.4 Effects of IRHS on the Human Malaria Vector Species Abundance.

The difference in density of *Anopheles arabiensis* and *Anopheles squamosus* between the two sections of the study area was significant ($p < 0.05$); 0.5 mosquitoes per house in Chiota compared to no mosquito Chishiko (Table 4.8). This implied that the IRHS was possibly exerting some positive impact on the abundance of the malaria vectors in the area.

4.5 Effects of IRHS on Human Malaria Vector Species Distribution.

The absence of malaria vectors in the sprayed section of the study area could be attributed to the impact of IRHS on the distribution of the malaria vectors.

CHAPER 5: DISCUSSION AND RECOMMENDATIONS

5.1 Endophilic Mosquito Diversity.

Three species of endophilic mosquitoes were collected from homes in Chongwe district, Zambia, in this study. This number of species collected tallied with the estimate made of the Expected Species Total (EST) for the area using the alpha index of diversity ($\alpha = 0.5$). The three endophilic species identified from the study area were *Culex quinquefasciatus, Anopheles squamosus* and *Anopheles arabiensis*. In comparison to the diversity of endophilic mosquitoes reported for the Macha region of Southern Zambia, the endophilic mosquito diversity determined for this study area was low. In Macha, Kent (2006) reported the presence of 14 different endophilic mosquito species in different houses namely: *Anopheles arabiensis, Anopheles coustani, Anopheles funestus, Anopheles longipalpis, Anopheles rufipes,* and *Anopheles squamosus.* Others were *Culex quinquefasciatus, Culex univittatus, Culex antennatus, Culex poicillipes, Culex tigripes, Uranotaenia balfouri, Mansonia uniformis* and *Aedes achraceus.*

The climate and geographical characteristics of Chongwe are quite similar to those of the Macha region and therefore a similar number of endophilic species could have been expected in the study area in Chongwe. The explanation for the difference in diversity of endophilic mosquitoes observed between the two regions could be either that a yet to be identified factor(s) limits the number of endophilic mosquitoes present in Chongwe district or this could have been as a result of differences in sampling methods in the two regions. Kent used both the CDC (Centers for Disease Control) light traps and the spray-catch method over several days in her endophilic sampling in Macha, and therefore could have had more chances of collecting different mosquito species. The sampling of endophilic mosquitoes in this study was done only once, using only one method and therefore the chances of collecting more of different mosquito species were lower.

The CDC light traps are much more efficient in trapping human host seeking mosquitoes inside of houses compared to other methods (Service, 1977; Lines *et al*., 1991). Some mosquito species such as *Anopheles funestus* readily bite hosts inside of houses but a good proportion of them may not remain indoors after feeding. Such species are usually missed when sampling using the spray-catch method.

5.2 Endophilic Mosquito Abundance.

The relative abundance of endophilic mosquitoes (i.e. number of mosquitoes collected per house) was generally low in this study. Among the three endophilic mosquitoes collected, *Culex quinquefasciatus* was the most abundant species (78.6%) being collected even in houses that had been sprayed with the insecticide (DDT). Its presence in the sprayed houses could imply that it is less susceptible to DDT compared to the anopheline species that were not found in the sprayed houses. But *C. quinquefasciatus* should equally be considered for control because its persistence in the sprayed houses could indirectly affect the deployment of the IRHS programme in the area. Majority of residents are not knowledgeable about the difference between malaria vectoring mosquitoes and mosquitoes that do not transmit malaria; the consequence of this is that they will tend to undermine the relevance of the programme if even after the spraying had been done they can still see mosquitoes in the houses.

The mean density of the anopheline mosquito species collected in the unsprayed houses was unexpectedly low, considering that the study was done during the season when all conditions were generally favourable for breeding. There are several factors that determine the number of endophilic mosquitoes which could explain why very few mosquitoes were collected in the unsprayed study area. First, the distance of mosquito breeding sites to the dwelling area can affect mosquito numbers; the further away the mosquito breeding site is, the less likely to find

mosquitoes in the houses. The integrity of dwelling structures can also considerably affect mosquito abundance. Houses that have too many openings tend to have high densities of mosquito because the mosquitoes easily gain entrance in search of blood meals. Some studies have found that size and design of houses including the amount of furniture present could greatly influence the preference of endophilic mosquito to remain indoors after feeding (Service, 1977). Other factors that can affect mosquito abundance include the number of occupants that had actually slept in the houses the night before the collection; and the utilization of insecticides treated bed nets in the area. An attempt was made to collect data that included these variables but there were a lot of gaps in the data; for example it was not easy to determine whether the bed nets used in the houses had been treated with the insecticide or not. But at least there were some indication that IRHS had suppressed the malaria vector density in the sprayed area.

5.3 Endophilic Mosquito Distribution.

The distribution variance calculated for all the mosquito species in each of the study areas were greater than the mean densities indicating that the mosquitoes were contagious (aggregated) in distribution. The distribution variance for the *Culex* mosquitoes was 0.83 and the mean density was 0.44 for Chiota; and 406.7 and mean density of 1.39 for Chishiko Village. The distribution variance for the anopheline mosquitoes (both *A. arabiensis* and *A. squamosus*) was 1.11 and the mean density was 0.5 for Chiota Village. The variance for Chishiko could not be computed as there were no anopheline mosquitoes encountered in the study area.

The aggregated pattern of distribution for organisms like mosquitoes that are very sensitive to specific ecological conditions for their survival was expected. There are several factors that determine this sort of distribution for mosquitoes in nature. Macro and microclimates are major factors that significantly influence distribution. Conditions of the macroclimate will determine

the distribution of a species over a large area but the conditions of microclimates influence the local distribution of a species within the same macroclimate (WHO, 1975). Variations in terms of temperature, humidity, and rainfall pattern, which is a composite of macro and microclimates, can considerably affect the distribution of mosquitoes. Mosquitoes generally tend to avoid places with extreme temperatures and low humidity.

5.4 Major Anopheline Mosquito Vector.

Reading from the DNA gel image obtained in this study (Fig. 4.1), the bands of DNA of all the *Anopheles gambiae* s.l. specimen corresponded with DNA bands of Positive controls for *A. arabiensis.* This indicated that the major species was *Anopheles arabiensis*.

The absence of the other members of the *A. gambiae* s.s. complex in the study area could be explained by the differences in feeding and breeding habits that the species exhibit. *Anopheles merus* and *Anopheles melas* for example, though endophilic in behaviour prefer to breed in salt water and are therefore mostly found in coastal areas (Appendix 9). In the study area, there were no potential salt-water or brackish water breeding places, which could have sustained these species. *Anopheles quadriannulatus* which is another member of the complex highly prefers to feed on animal hosts such as cattle and is therefore not normally collected in human habitations. On the other hand, *Anopheles bwambae* rarely feeds on human hosts inside of dwellings and strictly breeds around hot springs, normally within a circle of 10km. *Anopheles bwambae* has only been reported around the Buranga Hot Springs in Uganda. Appendix 10 shows the areas where the species has been reported. The only hot spring which probably could have sustained *Anopheles bwambae,* in Chongwe was far from the study area and therefore there was little possibility of the species if present, appearing in the collection in this study. The most likely endophilic member of the *A. gambiae* complex which could have been collected together with *A.*

arabiensis in the study area is *Anopheles gambiae* s.s. *Anopheles gambiae* s.s. and *A. arabiensis* are known to co-exist over much of the Afro-tropical region (Gillies and Coetzee, 1987). Adult females of *A. gambiae* s.s. are equally endophilic. Many studies seem to suggest that there are no major differences between the two species in choice of breeding sites. Service (1970) in Nigeria found both species breeding in the same pools. White and Rosen (1973) also found 42% of the water pools investigated supported both species in Nigeria. In this study, however, co-existence of the two species was not observed. This observation is not unique. Kent (2006) in Macha found only *A. arabiensis*. Gillies and de Meillon (1968) reported only *A. gambiae* occurring in Kitwe, and in North Western Zambia.

Anopheles funestus is another important malaria transmitting mosquito, which might have been present in the study area. It has often been found to co-exist with *A. gambiae* s.s. and *A. arabiensis. Anopheles funestus* belongs to a species group consisting of nine members: *Anopheles funestus* sensu stricto (s.s.), *Anopheles parensis*, *Anopheles aruni*, *Anopheles vaneedeni*, *Anopheles confuses*, *Anopheles leesoni*, *Anopheles rivulorum*, *Anopheles brucei* and *Anopheles fuscivenosus* (Gillies and de Meillon, 1968; Knight and Stone, 1977; Ward, 1984, 1992; Gillies and Coetzee, 1987). Among the nine species, only *Anopheles funestus* s.s. is dominant both in numbers and distribution in the Afro-tropical region. Its distribution in Zambia is not very well documented, although Kent (2006) collected some in houses of the Macha area. The density of adult *Anopheles funestus* s.s. varies according to the pattern of rainfalls. In the Savannas where rains occur once only per year, *Anopheles funestus* s.s. is more abundant at the end of the rainy season and at the beginning of the dry season. This study was done almost halfway into the rainy season. This perhaps was the reason it was not encountered in the study area if it is present there. Another reason is that *Anopheles funestus* s.s. although readily bites human hosts inside of houses, a good proportion prefers to rest outside after feeding. In a study in Ghana, Brady (1974) found a large proportion of *Anopheles funestus* s.s. that had fed on

human hosts resting outdoors in the villages. Brun (1973) had similar findings in Burkina Faso. These observations could suggest therefore that *A. arabiensis* was the predominant endophilic member of the *A. gambiae* complex responsible for transmission of malaria in the Chongwe district. More work is needed to verify these findings.

Although *Anopheles squamosus* was reported in the study area, its role in malaria transmission is generally considered negligible. In Tanzania, out of 1,060 females of this species dissected for malaria vector incrimination, only a single specimen was found positive (Gillies, 1964). Ramsdale (1965) in Zimbabwe also reported a single positive out of 24. No positive specimens were recorded from the following similar studies; Mali, out of 1,021, Holstein in Hammon *et. al*., (1956); Nigeria, out of 83 (Taylor, 1930); Democratic Republic of Congo (DRC), out of 256 (Vincke, 1946); Zimbabwe, out of 68 Messer (in de Meillon 1947); Madagascar, out of 1,324 (Chawet *et. al*., 1964). On account of these findings, *Anopheles squamosus* could not be a major vector in Chongwe district. In addition, *A. squamosus* is largely exophilic and prefers mostly to feed on animal hosts. In Kenya, few positives for human blood from houses and cattle sheds were found (Symes, 1931). Bruce-Chwatt and Gockel (1960) found 6 per cent of specimens collected from various sources positive for man. In Burkina Faso, only 0-8 percent *A. squamosus* was found feeding on man (Hammon *et. al*., 1964)

The presence of *Culex quinquefasciatus* and the *anopheline* mosquitoes in the study area observed in this study is typical. *Culex quinquefasciatus* is a common endophilic mosquito and has been found to co-exist with different other mosquito species in many entomological studies (Lines, *et al*., 1991; Mboera *et al*., 1997). Although *Culex quinquefasciatus* has never been incriminated in the transmission of malaria anywhere in the world, it is known to be a vector of human lymphatic filariasis, a disease common in the tropics. Lymphatic filariasis results from infection with parasite nematodes *Wuchereria bancrofti, Brugia malayi* and *Brugia timori*.

Within the Africa region, as defined by the World Health Organization (WHO), the disease is widespread in 39 of the 46 member countries and with an estimate of 390 million persons at risk (Zagaria and Savioli, 2002). In most of these countries, *Culex quinquefasciatus* has been incriminated in its transmission. In Zambia, the prevalence of filariasis is still being assessed. A recent study, however, indicated that the disease was prevalent in the Western and Eastern Zambia. In Kalabo district, Western Zambia, the prevalence stood at 53.95% while in Luangwa district, Eastern Zambia, close to Chongwe district, the prevalence was reported at 40.47% (Mwase *et al*., 2005).

In Africa generally, vectors of lymphatic filariasis are found in three mosquito species complexes or groups, these being: the *Anopheles gambiae* complex, the *Anopheles funestus* group and the *Culex pipiens* complex. In Eastern and Southern Africa, among the seven sibling species of *A. gambiae* complex, *A, gambiae* s.s., *A. arabiensis* and *A. merus* are known to be the principal vectors of lymphatic filariasis (White, 1989; Zagaria and Savioli, 2002). In the *A. funestus* group, only *A. funestus* has been incriminated in many parts of the Eastern and Southern Africa. The *Culex pipiens* complex includes five species and form, namely. *Culex pipiens, C. quinquefasciatus* Say, *C. quinquefasciatus* form *pallens* Coquillet, *C. globocoxitus* and *C. austricus* Dobrotworsky and Drummonds (Knight and Stone, 1977, 1978; Ward, 1992; Service, 1993). The major vector in the *Culex* complex in Southern Africa is *C. quinquefasciatus* (White, 1989). In Tanzania, for example, White (1971) investigated the relative importance of *C. quinquefasciatus, A. gambiae* and *A. funestus* in the transmission of Filariasis. *Culex quinquefasciatus* was found to be the major vector among the three. In the same region, McMahon *et. al*., (1981) found *C. quinquefasciatus* to be among the other important vectors (*A. gambiae* and *A. funestus*). In a study in one of the villages near Tanga, Bushrod (1981) found that *C. quinquefasciatus* was a vector, the other being *A. gambiae* s.l. Pedersen *et. al*., (1999) in

a comparison study on the status of lymphatic filariasis in three urban and semi urban communities of Pemba Island, found *C. quinquefasciatus* to be the predominant vector.

In Kenya, surveys on lymphatic filariasis vectors in two highly endemic villages along the coast, *C. quinquefasciatus* was found to be the major vector in one of the two villages. In the same country, Mwandawiro *et. al*., (1997) studied the main vectors of lymphatic filariasis in three villages along the Coast, again *C. quinquefasciatus* was found to be among the important vectors of lymphatic filariasis, the other vectors being *A. gambiae* and *A. funestus.* In the Comoros island, a longitudinal survey on lymphatic filariasis vectors showed that *C. quinquefasciatus* was the dominant vector (Brunhes, 1975). Sabatinelli *et. al*., (1994) also in a survey on lymphatic filariasis vectors found *C. quinquefasciatus* to be the dominant in the Comoros Island. On the basis of the findings given, *C. quinquefasciatus* could perhaps be one of the vectors responsible if lymphatic filariasis was present in Chongwe district.

5.5 Impact of IRHS on Malaria Vectors.

Malaria vectors identified for Chongwe district were only in the section of the study area that had not been sprayed with the insecticide. This could suggest that females of the two mosquito species *Anopheles arabiensis* and *Anopheles squamosus* avoided visiting the sprayed houses, while in search of human blood meals or their population had considerably been reduced. If the later be the case, then the IRHS programme in the Chongwe area has a positive impact on the abundance and distribution of human malaria vector species. This is positive in the sense that lower numbers of the malaria vector implies low malaria transmission. But it must be noted that *Anopheles arabiensis* has a tendency of feeding on other animal hosts, such as cattle as an alternative in avoidance of the chemical irritation in insecticide sprayed enclosures. Therefore, more work is required to confirm whether *A. arabiensis* is exhibiting this kind of behaviour in

the study area before a final conclusion can be made on effects of Indoor Residual House Spraying programme on *A. arabiensis* in the sprayed areas of Chongwe district. On the other hand, *A. squamosus* is largely exophilic (tendency to avoid enclosures) and prefers mostly to feed on animal hosts and if there is any impact of IRHS it is difficult to ascertain because logically the chemical sprayed inside of houses hardly affects mosquitoes that rest and feed outside the houses.

As to the reasons why *Culex quinquefasciatus* was also found in the sprayed houses which the malaria vectors seemed to have avoided, these were not clear. A total of 50 *C. quinquefasciatus* specimens were collected from the 36 sprayed houses. In view of this, controlling of *C. quinquefasciatus* in Chongwe would require in addition to IRHS, applying other mosquito control strategies such as use of insecticide treated mosquito bed nets. This could also entail re-evaluating the type and concentration of the insecticide used in the IRHS programme in areas of Zambia where *C. quinquefasciatus* will be determined to occur.

5.6 Conclusions.

The study has shown that endophilic mosquito species in Chongwe district include malaria transmitting mosquitoes, one of which belongs to the *Anopheles gambiae* s.l. complex. Between the two malaria vectors collected in Chongwe District, *Anopheles arabiensis* was possibly the major vector since the role of the other mosquito species in malaria transmission, *Anopheles squamosus,* is generally considered negligible. The absence of the two malaria vectors in the sprayed section of the study area was an indication of some positive impact of IRHS on the abundance and distribution of the two human malaria mosquito vectors in Chongwe district. But it could also mean that the malaria vectors could be feeding and resting outdoors following IRHS.

5.7 Recommendations.

It is recommended that:

(i) Since this study has only provided some evidence of the effectiveness of Indoor Residual House Spraying (IRHS) against the malaria vectors as a malaria control intervention, a similar study should be undertaken longitudinally in other areas of Chongwe district where the IRHS programme is being conducted to make the results more conclusive.

(ii) A follow-up study of the behavior of *Anopheles arabiensis* should be carried out to throw some light on why this insect avoids sprayed houses.

(iii) A study of insecticide resistance of the three species found in Chongwe district would also complement this study.

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APPENDICES.

Appendix 1: Ethical Clearance Letter.

THE UNIVERSITY OF ZAMBIA

BIOMEDICAL RESEARCH ETHICS COMMITTEE

Telephone: 260-1-256067
Telegrams: UNZA, LUSAKA
Telex: UNZALU ZA 44370
Fax: + 260-1-250753
E-mail: unzarec@zamtel.zm

Ridgeway Campus
P.O. Box 50110
Lusaka, Zambia

Assurance No. FWA00000338
IRB00001131 of IORG0000774

9 June, 2008
Ref.: 001-03-08

Mr Namafente Osbert
Department of Biological Sciences
Postgraduate Studies
University of Zambia
LUSAKA

Dear Mr Namafente,

RE: RESEARCH PROPOSAL: **"A STUDY OF DIVERSITY ABUNDANCE AND DISTRIBUTION OF ENDOPHILIC MOSQUITOES AND THE EFFECTS OF INDOOR RESIDUAL INSECTICIDE HOUSE SPRAYING IN CHONGWE DISTRICT, ZAMBIA"**

The above research proposal was presented to the Research Ethics Committee Secretariat meeting held on 19 March, 2008, where changes were recommended. We would like to acknowledge receipt of the corrected version with clarifications. The proposal has now been approved.

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 this office. 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.
- Any serious adverse events must be reported at once to this Committee.

Yours sincerely,

Dr E. Munalula Nkandu, BSc (Hons), MSc, PgD R/Ethics, PhD
CHAIRPERSON

Date of approval: **22 February, 2008** Date of expiry: **21 February, 2009**

Appendix 2: Sample of Data Collection Questionnaire.

Investigator……..Date……

Country……...District…….……Village……

Household number…….

Geographical coordinates of sampling house:

S……..……E…….....Elevation……..…….

Geographical coordinates of nearest potential mosquito breeding site:

S……..……E…….....Elevation……..…….

Sampling technique: Spray-sheet

Mosquito species	**Number of male mosquitoes**	**Number of females mosquitoes**
		-

1. What is the size of your household? Children……...Adults…….
2. How many ITNs do you own? .....
3. How many people slept in the ITNs last night? Children……Adults……

Appendix 3a: Mosquito sampling data for Chishiko Village.

Study No.	GPS co-ordinates		El (m)	*Culex* species	*Anopheles* species
	E	S			
221	15º22.200	028º40.218	1082	1	0
119	15º21.517	028º42.024	1056	0	0
40	15º21.862	028º41.377	1068	0	0
229	15º22.591	028º41.724	1061	0	0
202	15º22.612	028º40.328	1082	0	0
306	15º22.639	028º40.333	1083	0	0
111	15º21.801	028º41.697	1078	0	0
278	15º22.236	028º41.734	1063	1	0
161	15º22.020	028º40.332	1080	0	0
272	15º22.742	028º41.177	1065	0	0
204	15º23.161	028º40.760	1078	0	0
106	15º22.196	028º41.543	1058	0	0
251	15º22.684	028º40.233	1084	0	0
56	15º22.249	028º40.333	1085	0	0
311	15º23.321	028º40.914	1059	1	0
98	15º21.854	028º41.360	1072	0	0
120	15º22.931	028º40.611	1094	0	0
17	15º22.849	028º41.797	1076	6	0
67	15º21.569	028º42.026	1108	0	0
166	15º22.612	028º40.328	1063	0	0
76	15º22.115	028º41.792	1063	0	0
126	15º22.768	028º41.894	1069	1	0
32	15º22.218	028º41.779	1064	4	0
84	15º22.080	028º40.204	1084	0	0
20	15º22.728	028º41.219	1076	0	0
281	15º23.095	028º40.779	1078	0	0
113	15º22.681	028º41.039	1067	0	0
245	15º22.768	028º41.894	1069	16	0
199	15º22.598	028º41.337	1080	0	0
124	15º22.822	028º41.811	1081	4	0
109	15º21.823	028º41.434	1068	0	0
53	15º22.799	028º42.658	1054	1	0
308	15º22.770	028º41.610	1073	12	0
38	15º22.088	028º40.323	1070	1	0
64	15º21.889	028º40.494	1079	1	0
4	15º22.027	028º41.077	1081	1	0

Appendix 3b: Mosquito sampling data for Chiota Village.

Study No.	GPS co-ordinates S	E	El (m)	*Culex* species	*Anopheles* species
10	15º13.094	028º50.289	1101	2	0
50	15º13.526	028º50.985	1107	0	0
3	15º13.314	028º50.380	1094	0	0
8	15º13.372	028º50.151	1111	1	2
2	15º13.409	028º50.150	1112	0	0
46	15º14.040	028º50.390	1075	0	0
63	15º13.069	028º50.113	1109	0	3
30	15º14.519	028º50.667	1080	0	0
56	15º13.373	028º50.273	1105	0	0
6	15º13.373	028º50.413	1104	0	0
11	15º13.107	028º50.256	1101	0	0
36	15º14.101	028º50.415	1070	0	0
1	15º13.629	028º50.328	1107	0	0
43	15º14.614	028º50.597	1067	0	0
12	15º13.249	028º50.289	1104	0	0
58	15º13.065	028º49.877	1124	1	0
57	15º13.351	028º50.098	1106	0	0
44	15º13.983	028º50.672	1088	0	0
18	15º14.289	028º50.667	1074	4	0
13	15º14.157	028º50.580	1076	0	0
53	15º13.995	028º50.588	1094	1	1
47	15º14.082	028º50.515	1077	0	0
60	15º13.208	028º50.198	1112	0	2
9	15º13.175	028º50.153	1093	0	0
5	15º14.230	028º50.840	1070	0	3
33	15º14.366	028º50.704	1077	0	0
49	15º14.212	028º50.507	1098	2	0
27	15º13.642	028º50.415	1095	0	1
59	15º13.778	028º50.423	1105	1	0
54	15º13.901	028º50.305	1091	0	0
24	15º13.261	028º50.022	1084	0	4
34	15º14.119	028º50.362	1114	0	0
37	15º13.297	028º50.468	1104	0	0
61	15º13.279	028º50.392	1092	0	0
22	15º13.138	028º50.584	1099	2	2
55	15º13.388	028º50.977	1128	2	0

Appendix 4a: Insecticide Used in the Study.

Appendix 4b: Spreading of Cotton Sheets on the Floor of a House before Spraying it with an Insecticide to Collect Mosquitoes.

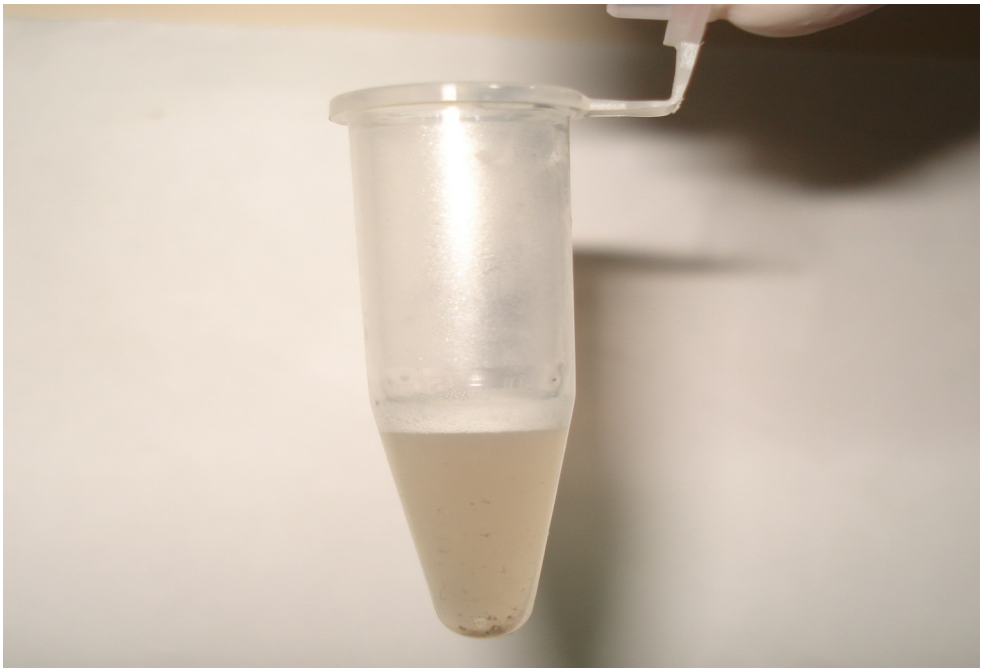
Appendix 4c: Spraying Insecticide Round the Eaves of a House.

Appendix 4d: Collection of Insecticide-knocked-down Mosquitoes from Cotton Sheets.

Appendix 4e: Introducing a Mosquito into a Sample Collection Vial.

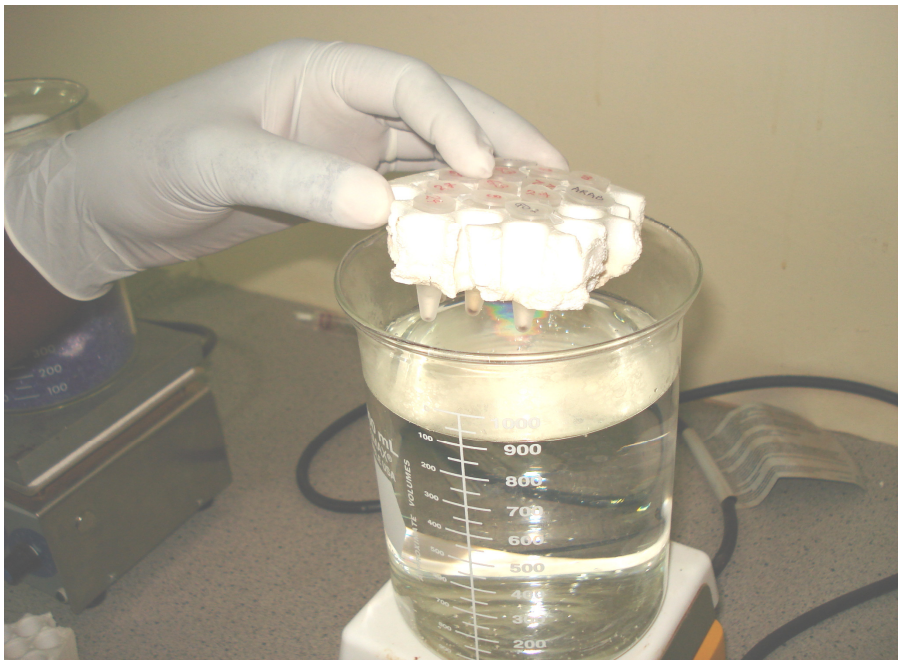
Appendix 4f: Whole Mosquito Sample in a Microfuge Tube.

Appendix 4g: Mosquito crushing in a Microfuge Tube using a Bent-tip Pipette.

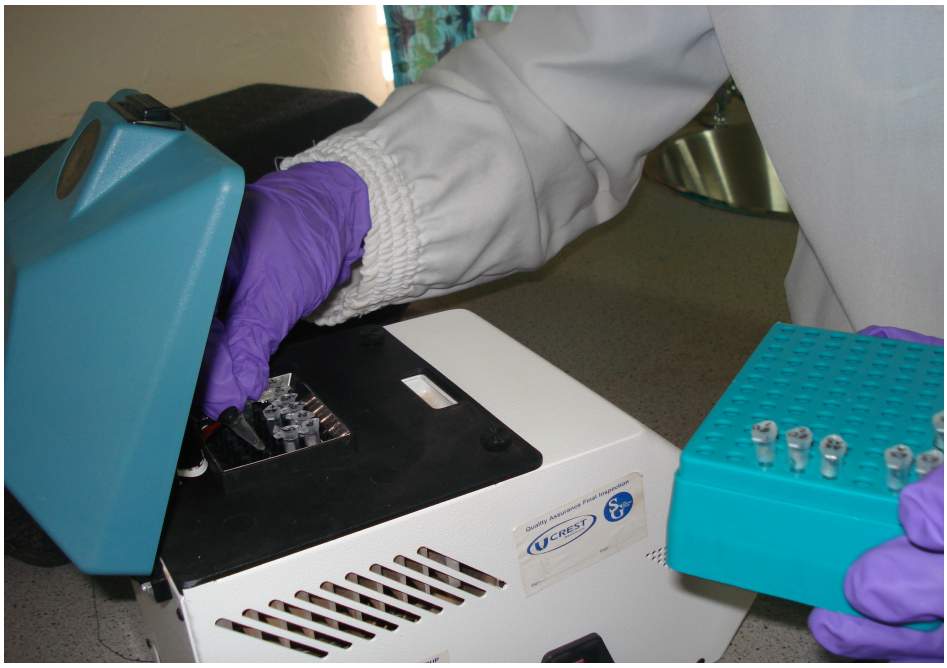


Appendix 4h: Appearance of the Crushed Mosquito in a Microfuge Tube.

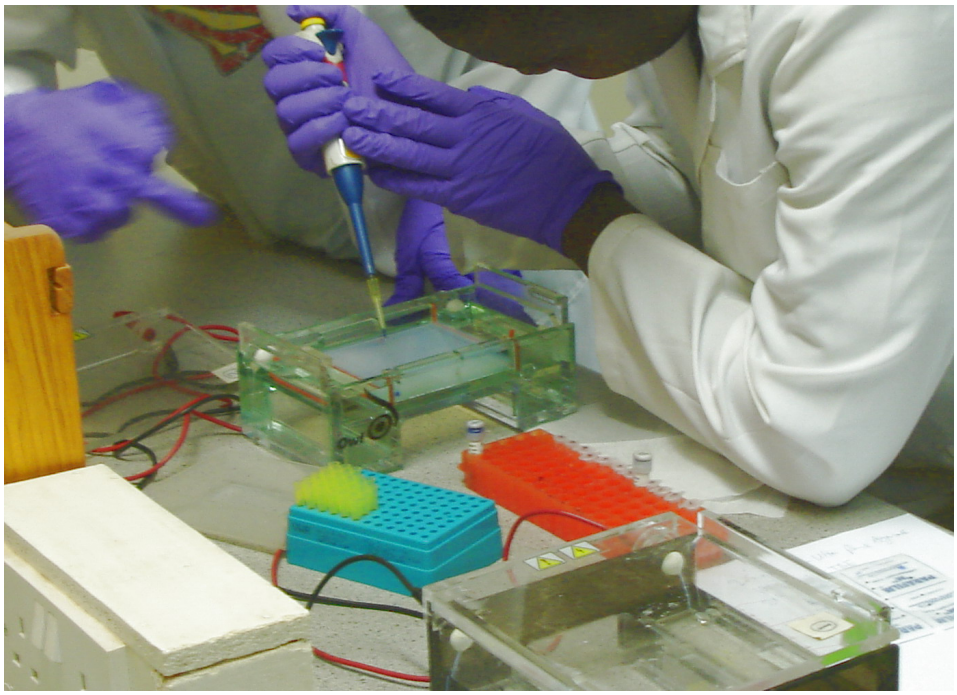
Appendix 4i: Centrifuge used in Mosquito DNA Extraction.



Appendix 4j: Water Bath used during Mosquito DNA Extraction.



Appendix 4k: Loading of Mosquito DNA in the PCR Instrument for Amplification.



Appendix 4l: Loading of the PCR Products and DNA Ladder in wells on an Electrophoresis Gel.

Appendix 5: Data Output in STATISTIX 2.0

ANOVA FOR ANOPHELES MOSQUITO ABUNDANCE BY IRS STATUS

SOURCE	DF	SS	MS	F	P
BETWEEN	1	4.50000	4.50000	8.08	0.0059
WITHIN	70	39.0000	0.55714		
TOTAL	71	43.5000			

COMPONENT OF VARIANCE FOR BETWEEN GROUPS 0.10952
EFFECTIVE CELL SIZE 36.0

IRS STATUS	MEAN	SAMPLE SIZE	GROUP STD DEV
CHIOTA	0.5000	36	1.0556
CHISHIKO	0.0000	36	0.0000
TOTAL	0.2500	72	0.7464

CASES INCLUDED 72 MISSING CASES 0

ANOVA FOR CULEX MOSQUITO ABUNDANCE BY IRS STATUS

SOURCE	DF	SS	MS	F	P
BETWEEN	1	16.0556	16.0556	2.58	0.1127
WITHIN	70	435.444	6.22063		
TOTAL	71	451.500			

	CHI-SQ	DF	P
BARTLETT'S TEST OF EQUAL VARIANCES	48.15	1	0.0000

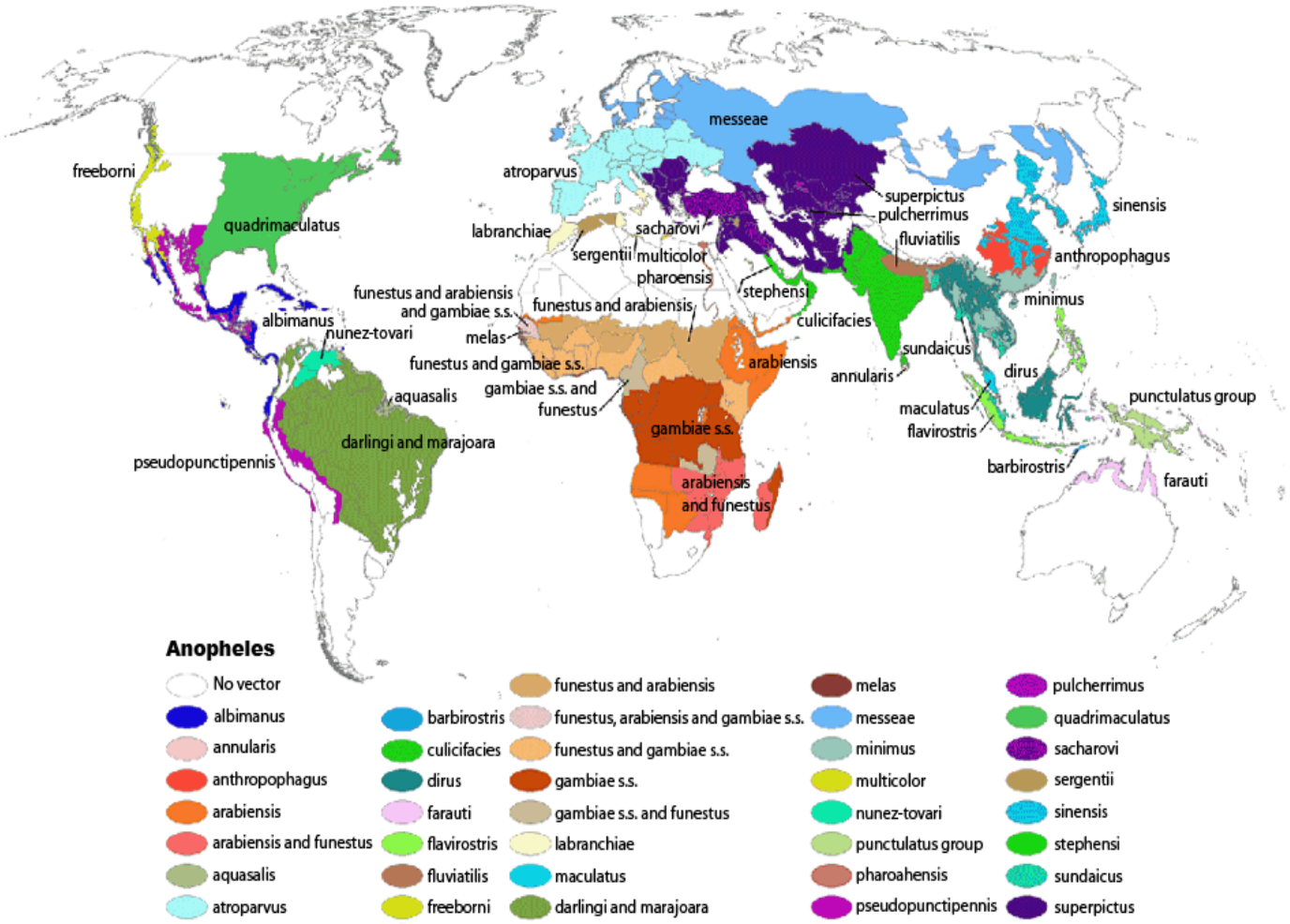
COCHRAN'S Q 0.9337
LARGEST VAR / SMALLEST VAR 14.073

COMPONENT OF VARIANCE FOR BETWEEN GROUPS 0.27319
EFFECTIVE CELL SIZE 36.0

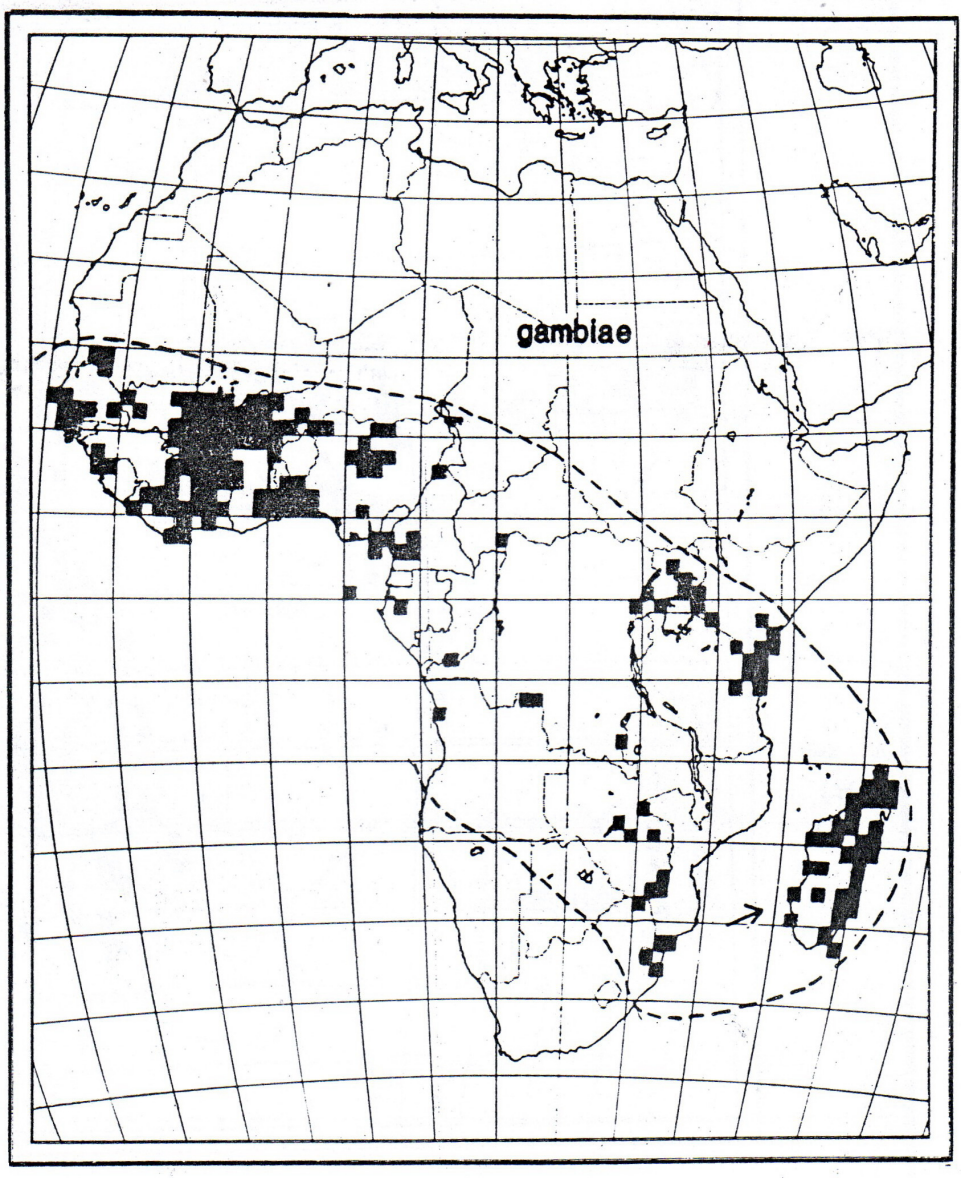
IRS STATUS	MEAN	SAMPLE SIZE	GROUP STD DEV
CHIOTA	0.4444	36	0.9085
CHISHIKO	1.3889	36	3.4082
TOTAL	0.9167	72	2.4941

CASES INCLUDED 72 MISSING CASES 0

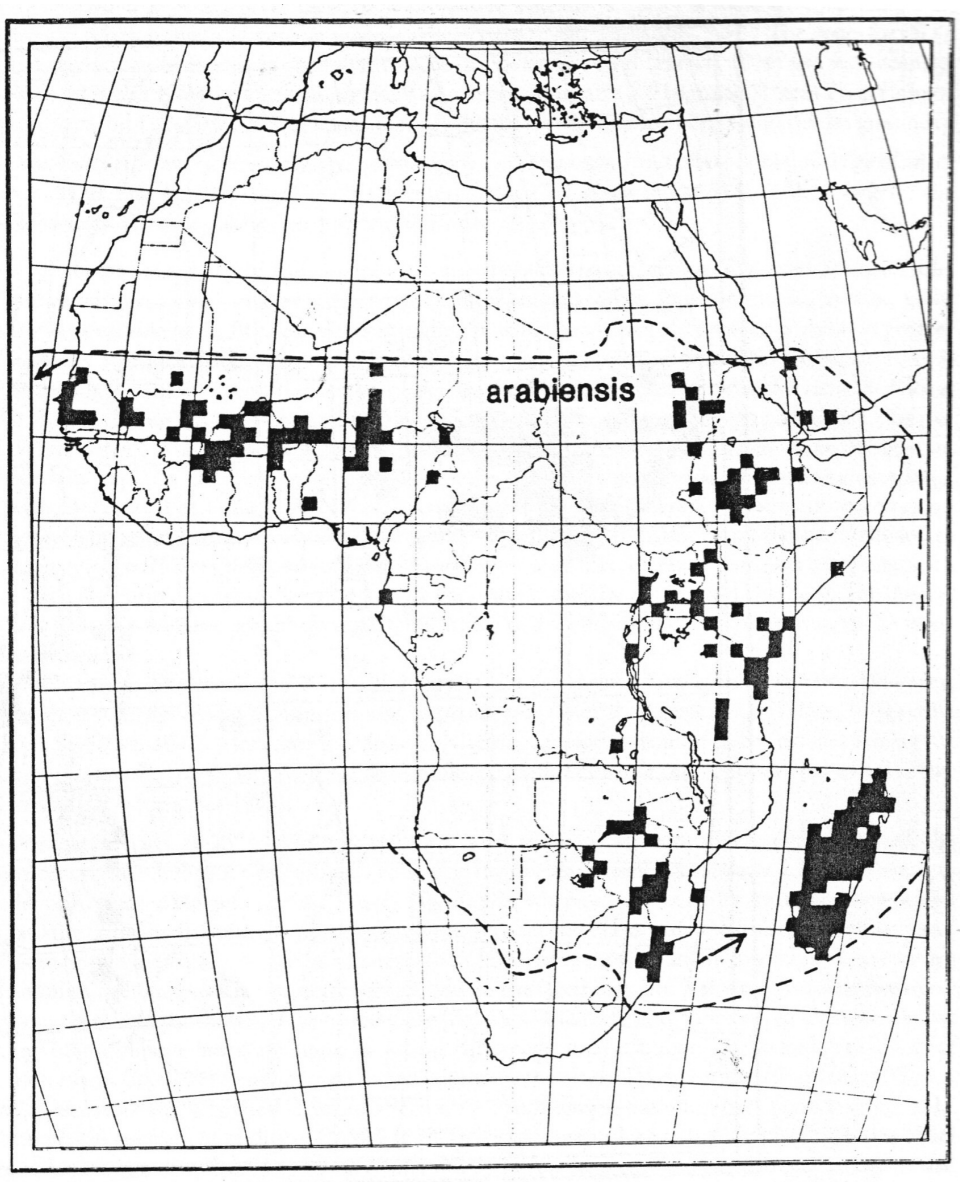
Appendix 6: Distribution of Dominant or Potentially Important Malaria Vectors in Major Regions of the World (Source: Kiszewksi *et al*., 2004).



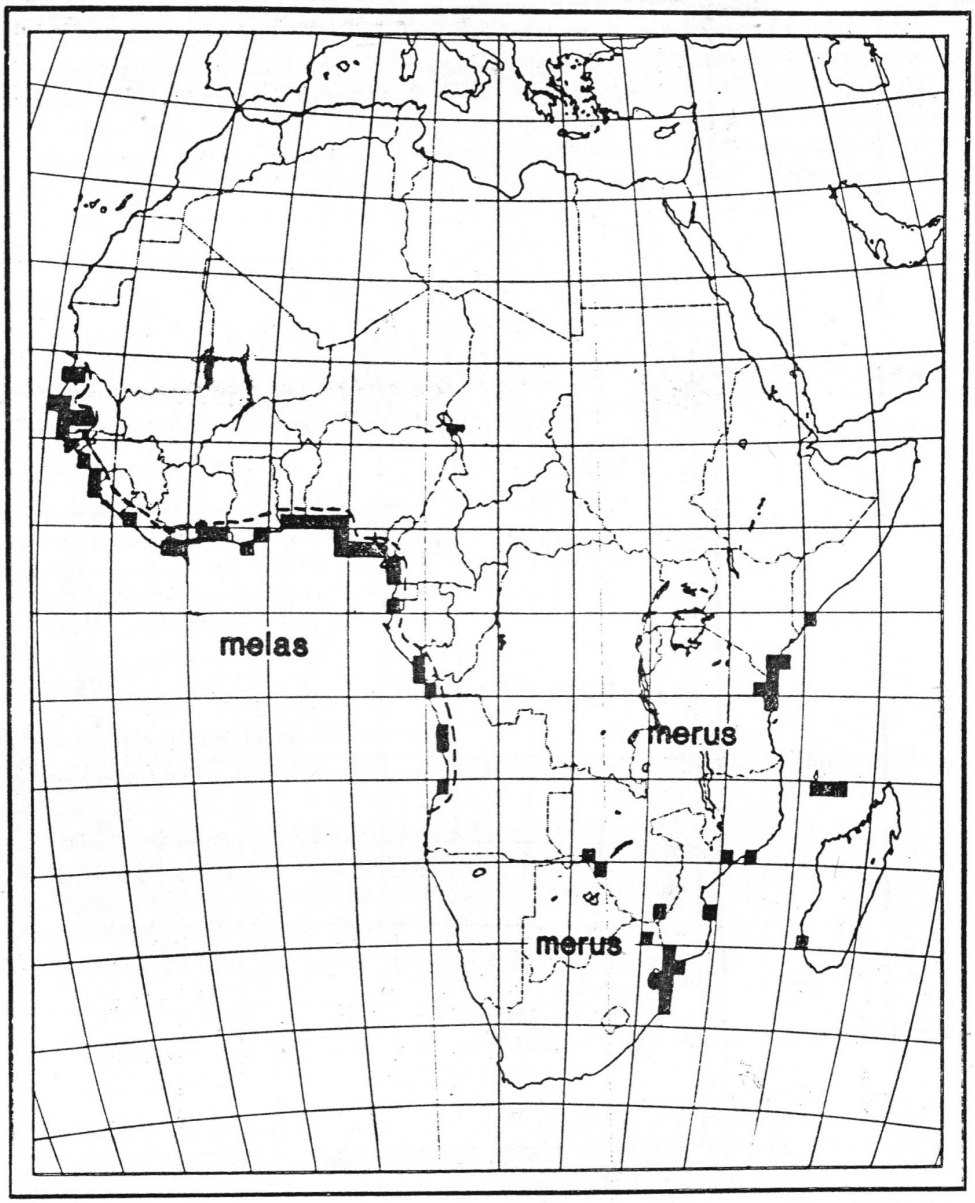
Appendix 7: Distribution of the *Anopheles gambiae* s.s. in Africa (Adopted from Gillies and Coetzee, 1987).



Appendix 8: Distribution of the *Anopheles arabiensis* in Africa (Adopted from Gillies and Coetzee, 1987).

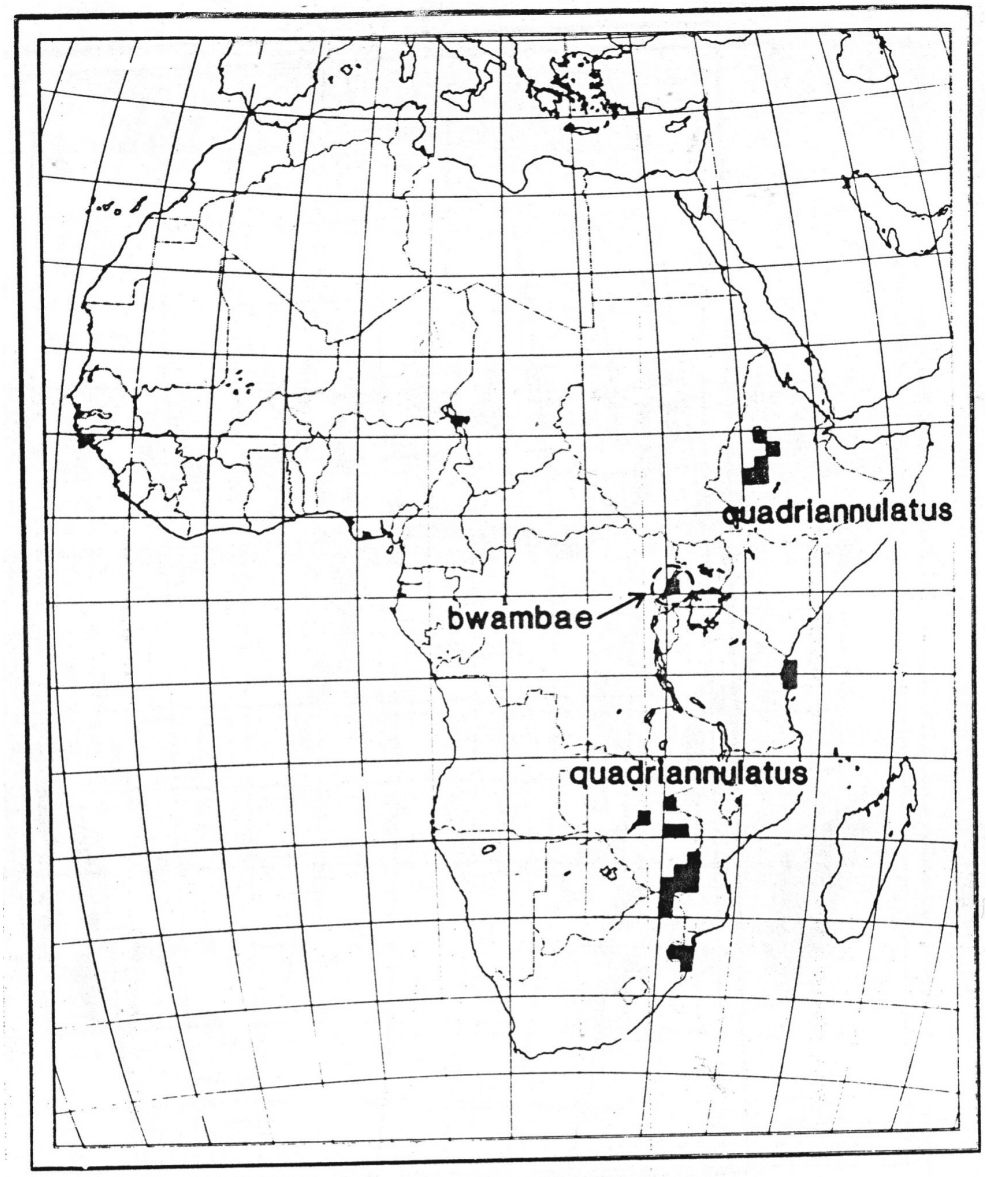


Appendix 9: Distribution of the *Anopheles melas* and *Anopheles merus* (Adopted from Gillies and Coetzee, 1987).



Appendix 10: Distribution of the *Anopheles quadriannulatus* and *Anopheles bwambae*

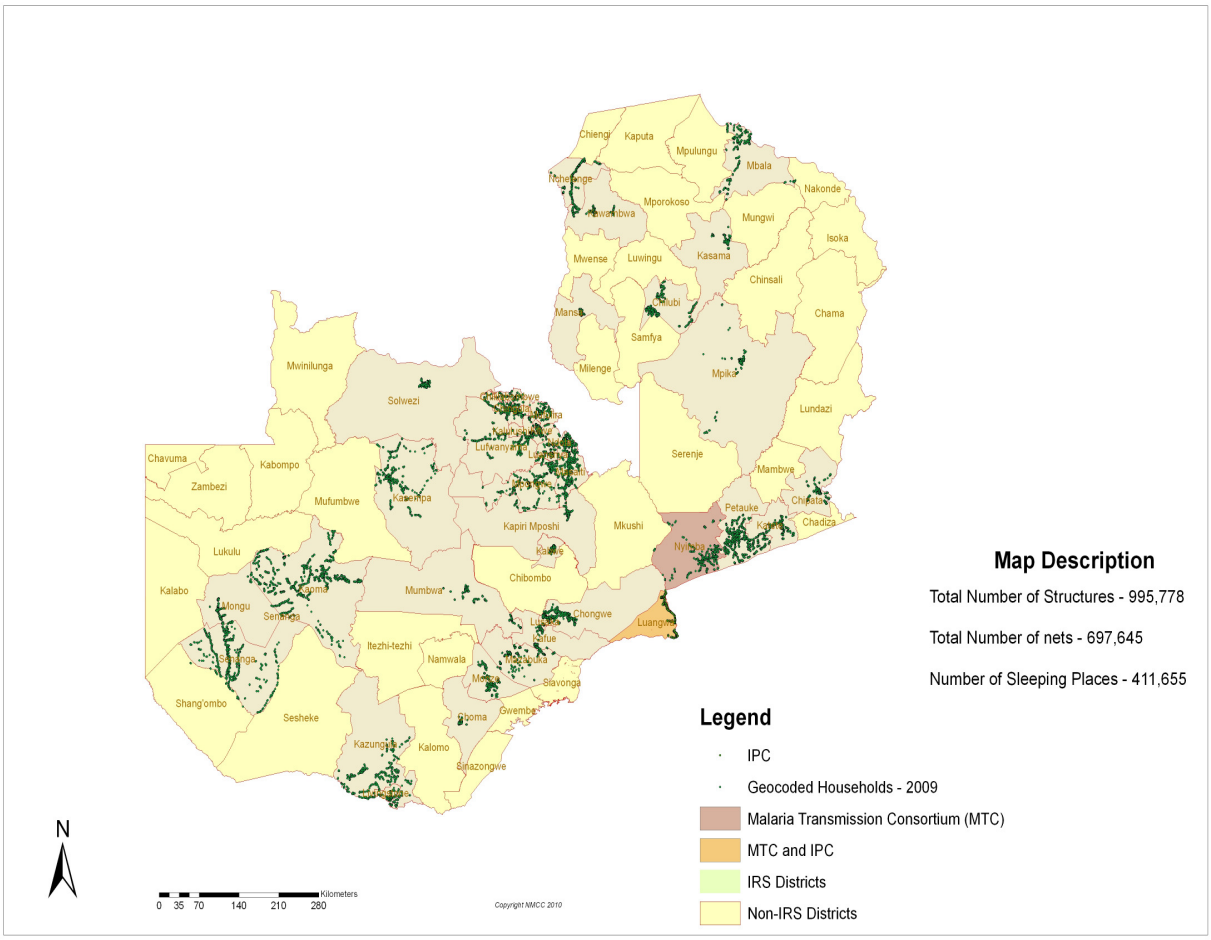
(Adopted from Gillies and Coetzee, 1987).



Appendix 11a: Names of Indoor Resting House Spraying (IRHS) operational districts in Zambia (NMCC, 2009).

No.	District	Target population
1	Chililabombwe	77,000
2	Chilubi	93,500
3	Chingola	69,300
4	Chipata	247,500
5	Choma	93,500
6	Chongwe	176,550
7	Kabwe	232,650
8	Kafue	195,800
9	Kalulushi	93,500
10	Kaoma	143,000
11	Kapiri Mposhi	99,000
12	Kasama	132,000
13	Kasempa	55,000
14	Katete	137,500
15	Kawambwa	66,000
16	Kazungula	154,000
17	Kitwe	456,500
18	Livingstone	135,300
19	Luanshya	140,250
20	Lufwanyama	75,900
21	Lusaka	1,650,000
22	Mansa	110,550
23	Masaiti	113,190
24	Mazabuka	115,500
25	Mbala	110,000
26	Mongu	145,750
27	Monze	66,000
28	Mpika	71,500
29	Mpongwe	72,600
30	Mufulira	180,400
31	Mumbwa	100,925
32	Nchelenge	137,500
33	Ndola	401,500
34	Petauke	165,000
35	Senanga	69,603
36	Solwezi	115,500
	Total	**6,499,268**

Appendix 11b: Location of Indoor Residual House Spraying (IRHS) operational districts in Zambia (NMCC, 2007).



Note: MTC and IPC indicated on the legend are organizations partnering with the Ministry of Health of Zambia in the IRHS Programme in Luangwa and Nyimba districts in the Eastern Province of Zambia.