ASSESSING ENTOMOLOGICAL AND PARASITAEMIA PREVALENCE TO MONITOR A MALARIA CONTROL PROGRAMME IN ZAMBÉZIA, MOZAMBIQUE

By

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ABSTRACT

Malaria vector control interventions in Africa are currently being scaled up in order to attain universal access and appropriate coverage. The aim is to reduce or interrupt disease transmission, and to reach long term goals of malaria elimination and eradication. Currently, in Mozambique the main methods of vector control are indoor residual spraying and long-lasting insecticidal nets. The selection of insecticide resistance is a major concern for the national malaria control programme and its partners, as both these interventions rely on insecticides. This study was designed to evaluate the impact of IRS and ITN programmes implemented in Zambézia province, Mozambique on malaria transmission, through monitoring the vector species abundance, sporozoite rate and insecticide susceptibility. The impact on malaria was measured through parasite prevalence studies.

Anopheles gambiae complex and *An. funestus* group mosquitoes were collected in 23 sentinel sites established in 7 districts in Zambézia province, using windows exit traps. These collections were used to assess abundance, sporozoite rate and transmission index and mouth aspiration catches of females were used for insecticide resistance assays. Identification of *An. gambiae s.l* and *An. funestus s.l* and sporozoite detection from the head/thorax were performed using polymerase chain reaction. *Plasmodium falciparum* prevalence in children aged between 1 and 15 years was assessed using ICT™ malaria rapid diagnostic tests. Annual prevalence was calculated the changes in susceptibility and malaria prevalence in subsequent survey periods compared.

Anopheles gambiae s.s, An. arabiensis and *An. funestus* were the only known malaria vectors found in the study area. *Anopheles gambiae* and *An. funestus* were most abundant. *Anopheles gambiae s.s* and *An. funestus* were confirmed to be vectors of malaria transmission in the area and the sporozoite rate for both were ranging from 4.1 % and 2.3 % in 2006 to 2007 to 1 % to 0 % in 2009-2010 respectively. No infected *An. arabiensis* were detected.

After several IRS rounds with DDT from 2006 to 2009 there was a significant reduction in mosquito abundance and sporozoite rates ($P < 0.001$), and a reduction in malaria infection as reflected in the overall drop in prevalence from 50% to 32% ($P < 0.001$).

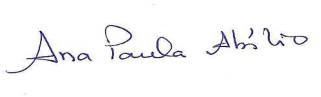
Insecticide resistance assays were carried out using WHO adult diagnostic tests for DDT (4%), lambda-cyhalothrin (0.05%), permethrin (0.75%) and bendiocarb (0.1%). Resistance to carbamate and pyrethroids was first detected in 2010 where high levels of pyrethroid resistance were detected in Mocuba district (76.2 % to lambda-cyhalothrin, 93.5% bendiocarb) and Milange district (lambda-cyhalothrin 82.9% and bendiocarb 84.5%). No DDT resistance was detected during this study.

With the change from DDT to lambda-cyhalothrin use for IRS in Zambézia and throughout the country, these findings of insecticide resistance and previously reported data for Mozambique suggest an increased risk to a currently successful malaria control campaign.

PREFACE

The field work described in this thesis was carried out in Zambézia province, northern Mozambique, from October 2006 to April 2010, while the laboratory analyses of mosquito specimens was carried out in Quelimane, Zambézia, and at Medical Research Council Durban, of South Africa.

These studies represent original work by the author and have not otherwise been submitted in any form for a degree or a diploma to any University. Where use has been made of the work of others, it is duly acknowledge in the text.

Signed: 

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To My Family

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CHAPTER 1

INTRODUCTION

The World Health Organization estimates there are 3 billion people at risk of malaria infection in malarious countries and territories with around 250 million cases annually, leading to approximately 1 million deaths. The vast majority of cases (86%) were in the African region, followed by the South-East Asian (9%) and Eastern Mediterranean regions (3%) (WHO 2008a). The percentage of cases in Africa that are due to *Plasmodium falciparum* is approximately 90 % (Snow *et al.,* 2005, WHO 2008a, WHO 2009, Guerra *et al.,* 2008).

Malaria is transmitted by *Anopheles* mosquitoes. The major malaria vectors in sub-Saharan Africa are *Anopheles gambiae s.s*. Giles, *Anopheles arabiensis* Patton and *Anopheles funestus* Giles (Diptera: Culicidae) (Gillies and De Meillon 1968, Gillies and Coetzee 1987, White 1974).

1.1. **The *Anopheles gambiae* complex**

The sequences of events leading to the discovery of the *An. gambiae* complex in the early 1960's have been well described (Paterson 1964, 1968). A vast amount of research has shown it to be a complex of at least six morphologically indistinguishable species showing pronounced ecological and behaviour diversity (Ribbands 1944, Muirhead-Thomson 1948, Service 1993). Three of these sibling species are adapted to fresh-water breeding sites: *An. gambiae s.s.*, *An. arabiensis* and *An. quadriannulatus*; two are salt-water breeding: *An. merus* and *An. melas* and one to mineral water: *An. bwambae* (White 1974, White 1985, Gillies and Coetzee 1987, Service 1993).

Anopheles gambiae s.s. is an efficient vector of malaria and filariasis in Africa. Females of this species show a high degree of anthropophily (White 1974).

The taxonomy of *An. gambiae s.s.* is complicated as this species seems to be in the process of further speciation in West Africa. Non-stochastic association and frequencies of some polymorphic chromosomal inversion arrangements in *An. gambiae s.s.* indicate the existence in West Africa of five chromosomal forms designated using non-Linnean nomenclature, as Bamako, Bissau, Forest, Mopti and Savanna (Touré and Petrarca 1983, Coluzzi *et al.*, 1985).

Further molecular analyses of the intergenic spacer (IGS) and the internal transcribed spacer (ITS) region of rDNA, revealed nucleotide substitution which differentiated two forms within *An. gambiae s.s.* designated as S and M forms in the case of IGS (Della *et al.*, 2001), and Types I and Type II in the case of ITS (Gentile *et al.*, 2001).

Anopheles arabiensis is the primary vector of malaria in many parts of Africa. This species was originally classified as endophagic with partial or complete endophily (White 1974). However, studies have shown this species to have mixed feeding and resting behavior, biting and resting both indoors and outdoors and feeding on both man/bovine (Sharp and Le Sueur 1990).

In general *An. gambiae s.s.* predominates in humid situations, whereas *An. arabiensis* predominantes in arid zones (White 1974).

Anopheles quadriannulatus is less widespread in its distribution. Its occurs in widely separated areas: Zanzibar (Odetoyinbo & Davidson 1968 [cited by (White 1974)]), Ethiopia (Turner 1972 [cited by (White 1974)]), and extensively in southern Africa (Paterson 1963). In Zanzibar and Southern Africa, *An. quadriannulatus* was found to be almost completely exophilic while it tends to be endophilic at high altitudes in Ethiopia (White 1974, Coluzzi *et al.*, 1979). This species feeds principally on animals other than

man (Mahon *et al.,* 1976). Hunt *et al.,* (1998), found that the Ethiopian population of *An. quadriannulatus* is a different species and designated it *An. quadriannulatus* B.

Anopheles merus is confined to the east coast of Africa and adjacent inland areas, coastal islands and at inland localities in association with salt pans (Muirhead-Thomson 1951, Gillies and De Meillon 1968, White 1974, Mosha and Petrarca 1983, Sharp 1983, Gillies and Coetzee 1987, Le Sueur and Sharp 1988, Sharp and Le Sueur 1990, Service 1993) This species is predominantly zoophagic (White 1974) and for low rate of malaria transmission (Muirhead-Thomson 1951) and efficient filariasis transmission in Tanzania (Bushrod 1981) and Kenya (Mosha and Petrarca 1983). *Anopheles merus* plays an important role in malaria transmission in coastal Tanzania (Temu *et al.,* 1998).

Anopheles melas is a malaria vector in West Africa (White 1974, Davidson 1977, Gillies and Coetzee 1987, Service 1993) this species is the sole member of the complex known to feed readily on goats and sheep (White 1974). Muirhead-Thomson (Muirhead-Thomson 1948) considered that they probably do not discriminate between man, cow, pig and goat.

Anopheles bwambae is known only from the Semliki forest area of the Uganda/DRC border, where breeding is apparently confined to mineral water swamps, vegetated principally with *Cyperus laevigatus* sedge, and formed by geothermal activity in the Rift valley (White 1974). This species is a local vector of malaria and filariasis in the Bwamba County (White 1985).

In southern Africa four members of the *An. gambiae* complex occur: *An. gambiae s.s., An. arabiensis, An. quadriannulatus* and *An. merus* (Shelley 1973, Petrarca *et al.,* 1984, Sharp *et al.,* 1987, Le Sueur and Sharp 1988, Sharp and Le Sueur 1990, Paskewitz *et al.,* 1993, Coetzee *et al.,* 1999).

1.1.1 Species identification

Since the recognition of the complex in 1962, precise identification of each species has been carried out using various methods, as the morphological characteristics used for primary identification of members of the *An. gambiae* complex have limited value (Coluzzi 1964, Davidson *et al.,* 1967, Gillies and Coetzee 1987).

There are two morphological characters of saltwater species, which are useful in separating them from the freshwater species. The eggs of both *An. melas* and *An. merus* are characterized by being longer and the opening on the dorsal surface is broader than that of Fresh Water *An. gambiae s.l.* This characteristic was used with success by Muirhead-Thomson (Muirhead-Thomson 1945, Muirhead-Thomson 1948).

Muirhead-Thomson (Muirhead-Thomson 1951) introduced a physiological method for identification of *An. merus* and *An. melas*. This method is based on differential response to saline waters, which distinguishes the first instars larvae of the three freshwater-breeding species, from those of the saltwater-breeding forms. Sharp (Sharp 1983) using colonized *An. merus*, *An. arabiensis* and *An. gambiae s.s.* extended this test in the laboratory to include all instars.

Cross-breeding is a technique that has been used with much success in elucidating cryptic species. Here species identification is based on hybrid sterility of site-specific hybrids (Paterson 1964 [cited by (Sharp and Le Sueur 1990)]). This technique is not practical for the routine identification of field samples and was largely superseded by genetic (Coluzzi 1968, Coluzzi and Sabatini 1967, Green 1972, Hunt 1973), electrophoretic (Mahon *et al.,* 1976) and most recently PCR-based techniques (Collins *et al.,* 1988, Scott *et al.,* 1993).

Carlson and Service (Carlson and Service 1979) investigated the possibility of identifying adults of both sexes of *An. gambiae* and *An. arabiensis* by extracting and analyzing their cuticular hydrocarbons. The preliminary results of this study merit more

detailed appraisal of these non-volatile and chemically inert cuticular hydrocarbons for the separation of *An. gambiae* and *An. arabiensis* and other species within the gambiae complex.

Bushrod (Bushrod 1981) successfully separated *An. merus* from the fresh water species of the *An. gambiae* complex (*An. gambiae s.s.* and *An. arabiensis*) in Tanzania by plotting the number of coeloconic sensilla against the palpal ratio.

Coetzee (Coetzee *et al.,* 1982) and Coetzee (Coetzee 1986), showed that *An. gambiae* and *An. arabiensis* could be distinguished from *An. merus* and *An. quadriannulatus* by the width of the pale band at the apex of hind tarsus three and the base of hind tarsus four. Sharp and others (Sharp *et al.,* 1989) evaluated the effectiveness of this method of identification of *An. gambiae s.l.* species in Natal. This resulted in only 56% correct identification.

More recently, molecular methods have been devised which use differences in DNA polymorphisms to distinguish species by the polymerase chain reaction (PCR) (Paskewitz and Collins 1990, Scott *et al.,* 1993, Bredenkamp and Sharp 1993, Flavia *et al.,* 1997, Fettene *et al.,* 2002, Fanello *et al.,* 2002).

The PCR assay developed by Paskewitz & Collins (Paskewitz and Collins 1990) and Scott and others (Scott *et al.,* 1993) is based on species-specific fixed differences in the ribosomal DNA (rDNA) region, which includes part of the 28S coding region and part of the intergenic spacer (IGS). The method uses a universal (UN)21 primer that anneals to a sequence shared by all members of the complex, in combination with specific reverse primers AR, GA, QD and MR that bind to unique sequences of each sibling species, *An. arabiensis*, *An. gambiae s.s.*, *An. quadriannulatus* and *An. merus* or *An. melas*, respectively.

A method involving new primers to identify the two molecular M and S forms within *An. gambiae s.s.* has been developed (Flavia *et al.,* 1997).

Fettene and others developed a PCR to distinguish between species A and B of *An. quadriannulatus s.l.* as well as other members of the *An. gambiae* complex (Fettene *et al.*, 2002).

Fanello and others (Fanello *et al.*, 2002) proposed a new method for differential identification of sibling species in the *An. gambiae* complex, including simultaneous separation of M and S forms within *An. gambiae s.s.* This method is a combination of the protocols established by Scott and others (Scott *et al.*, 1993) and Flavia and others (Flavia *et al.*, 1997).

To clarify if the speciation processes are ongoing within *Anopheles gambiae s.s.*, further analysis has been done on the insertion polymorphism of a 200 bp Short Interspersed Elements (SINE200) within genome areas of high differentiation (i.e. "speciation islands"). This resulted in the development of a new easy-to-use PCR for analysis of genetic differentiation between M and S forms (Santolamazza *et al.*, 2008).

1.2. **The *Anopheles funestus* group**

Anopheles funestus is an important malaria vector, in some cases playing a more important role than *An. gambiae* or *An. arabiensis* (Fontenille *et al.*, 1997).
The *An. funestus* group is comprised of at least ten members: *An. funestus*, *An. vaneedeni* Gillies & Coetzee, *An. parensis* Gillies, *An. aruni* Sobti, *An. confusus* Evans & Leeson, *An. rivulorum* Leeson, *An. fuscivenosus* Leeson, *An. leesoni Evans*, and *An. brucei* Service (Gillies and Coetzee 1987, Koekemoer *et al.*, 1999, Hargreaves *et al.*, 2000, Brooke *et al.*, 2001, Kamau and Hunt 2002). Recently in Malawi, based on combined molecular, cytogenetic and cross-mating studies a new species provisionally designed *Anopheles funestus like* was indentified (Spillings *et al.*, 2009). Only two species within this group are implicated in malaria transmission: *An. funestus* and *An. rivulorum*. The latter has been implicated as a minor vector in Tanzania (Wilkes *et al.*, 1996).

Within this group, *An. funestus* is the most abundant and widespread in southern Africa, and is highly endophilic, endophagic and anthropophilic. The other species are typically more limited in abundance and distribution, and mainly bite animals outdoors (Bruce-Chwatt 1954, Hackett *et al.,* 2000). However they avidly bite humans outdoors in the absence of other hosts (Gillies and De Meillon 1968). *Anopheles rivulorum* is the second most abundant and widespread species in the funestus group (Hackett *et al.,* 2000).

1.2.1. Species identification

Differentiation of species comprising the funestus group is difficult using traditional taxonomic measures (Hackett *et al.,* 2000). However four members of this group: *An. brucei, An. confusus, An. leesoni* and *An. rivulorum,* can be identified from characteristics of the egg and larval morphology (Gillies and Coetzee 1987).

Cytogenetic methods have been used to identify female adults of two species, *An. parensis* and *An. funestus* (Green and Hunt 1980). This method uses half-gravid females. However, *An. vaneedeni* and *An. funestus* are homo-sequential species, which complicates identification.

Koekemoer and others developed a PCR -SSCP assay, which discriminates between four members of the funestus group: *An. funestus, An. vaneedeni, An. rivulorum* and *An. leesoni* (Koekemoer *et al.,* 1999).

The standard PCR assay used agarose gel electrophoresis today was developed by Koekemoer end others (Koekemoer *et al.,* 2002). Hackett and others developed a second PCR to identify *An. funestus* and *An. rivulorum* using the second ribosomal DNA internal transcribed spacer (Hackett *et al.,* 2000).

1.3. Distribution of the *Anopheles gambiae* complex and *Anopheles funestus* group in Mozambique

Little is known about the distribution of malaria vectors and their behavioural status as it relates to malaria transmission in Mozambique. A survey by Petrarca and others (Petrarca *et al.*, 1984) showed that four species of the *An. gambiae* complex occur in Mozambique: *An. gambiae s.s.*, *An. arabiensis*, *An. merus* and *An. quadriannulatus*. *An. gambiae s.s.* occurs in the central-northern regions (north of the Save river) from the coast to the western mountains. On the coast its distribution is often sympatric with that of *An. merus*.

Anopheles arabiensis is the most widely distributed species of the *An. gambiae* complex within the country, while *An. merus* is confined to the coastal region inner areas where the rivers are tidal and brackish and/or the soil is saline. *Anopheles quadriannulatus* was only found in a southern locality, Bela-Vista-Maputo area (Petrarca *et al.*, 1984).

De Meillon (De Meillon 1941) showed that the *An. funestus* group is widely distributed within the country. Mendis and others (Mendis *et al.*, 2000) demonstrated that *An. arabiensis* and *An. funestus* are equally important vectors of malaria in Matola, a coastal suburb of Maputo, southern Mozambique. *Anopheles merus* collected after the floods in 2000, was found with sporozoite showing their contribution in malaria transmission in Mozambique (Cuamba and Mendis 2009).

1.4. Insecticide resistance

Insecticide resistance in malaria vector mosquitoes reduces the efficacy of insecticide mode of action and is therefore of major concern in regard to effective malaria vector control by insecticides. According to the Seventh report of the WHO (World Health Organization) Expert Committee on Insecticides, "Resistance to insecticides is the development of an ability in a strain of insects to tolerate doses of toxicants which would prove lethal to the majority of individuals in a normal population of the same species" (WHO 1992).

Insecticides have been used against *Anopheles* mosquitoes in malaria control programmes for over 60 years, with varying success [Park Ross 1936 cited by (Hargreaves *et al.,* 2000)]. Between the two World Wars, anti-larval operations were the traditional malaria control measure applied on a relative large scale in Africa. During this period, pyrethrum was also used on small scale as an aerosol (Kouznetsov 1977). Dichloro-diphenyl-trichloroethane (DDT) and dieldrin with their superior residual effects replaced pyrethrum for indoor vector control shortly after the second World War (Sharp and Le Sueur 1990). The common and widespread use of insecticide in Africa in public health and agriculture subsequently resulted in the emergence of resistant strains of malaria vector mosquitoes (Coetzee *et al.,* 1999).

Pyrethroids were first developed in the 1970s and have now largely replaced DDT for indoor residual spraying (IRS) in malaria control programmes. Pyrethroids are currently the only insecticides available for insecticide-treated nets due to their low mammalian toxicity (Curtis 1990, Zaim *et al.,* 2000, Najera and Zaim 2001).

Vector control is a central element of most antimalarial campaigns. In the mid 1980s only a few countries of Africa, (Botswana, Ethiopia, Namibia, South Africa, and Zimbabwe) had achieved sustained vector control using IRS (WHO 1985). This number has recently been expanded to include Angola, Cape Verde, Equatorial Guinea, Eritrea, Kenya, Mauritius, Madagascar, Sao Tomé and Principe, Swaziland, Tanzania and

Zambia, who have adopted IRS as a major component of their malaria control strategy (WHO 2007c, 2008b) .

In Europe, IRS for vector control is a dominant method with 8 of 9 endemic countries adopting this strategy, with the greatest number of houses sprayed in Azerbaijan, Tajikistan and Turkey. In South-East Asia 6 of 10, in America 12 of 22 and in Western Pacific just 2 of 10 endemic countries use IRS as a strategy for vector control (WHO 2008b).

The efficacy of chemical control measures depends on a number of factors: the vector species; the insecticide in use and particularly the degree to which the local vector species have acquired resistance (WHO 1997).

The evolution of vector resistance to DDT and other insecticides is a major problem for vector control (WHO 1986). Resistance to organochlorines was first observed in *Aedes* mosquitoes in the late 1940s (Brown 1986, WHO 1997). In 1946, WHO reports indicated that only two species of *Anopheles* were resistant to DDT (WHO 1992), but by 1986, 106 species of mosquitoes worldwide were known to have developed resistance to organochlorine insecticides. In addition, 38 species had developed resistance to organophosphorus insecticides and 17 species were resistant to carbamates (Brown 1986, WHO 1997).

In Africa, the first case of insecticide resistance involving *An. gambiae s.s.* was reported in 1967 in Bobo Dioulasso (Burkina Faso) and attributed to the use of DDT against cotton pests (Chandre *et al.,* 1999). Pyrethroid resistance was detected in *An. gambiae s.s.* from both West and East Africa (Elissa *et al.,* 1993, Vulule *et al.,* 1994, Vulule *et al.,* 1996, Darriet *et al.,* 1997, Martinez-Torres *et al.,* 1998). The primary resistance mechanism in these populations is of the kdr-type, which gives broad-spectrum resistance to all pyrethroids and DDT.

A broad spectrum resistance pattern covering organophosphates and carbamates is rare but has been described in some populations of *An. albimanus*, *An atroparvus* and *An. sacharovi*. The resistance is due to an altered target site. This mechanism was detected more recently in *An. gambiae* from West Africa. This is predominantly due to the widespread use of a cocktail of insecticides against agricultural pests in neighbouring areas and severe contamination of anopheline breeding sites (Onori *et al.,* 1993). During the last decade a number of reports incriminated the extensive use of the pesticides in agriculture as leading to widespread insecticide resistance in mosquito vector populations (Akogbeto *et al.,* 2006, Djouaka *et al.,* 2007, Chouaibou *et al.,* 2008).

In southern Africa, the first case of pyrethroid resistance in *An. funestus* was reported in 1999 from the Kwazulu-Natal province of South Africa (Hargreaves *et al.,* 2000). In Mozambique the first evidence of resistance to pyrethroids and carbamate in the *An. funestus* s.s population was seen in 2000 from Beluluane in southern Mozambique (Brooke *et al.,* 2001). Studies carried out as part of the Lubombo Spatial Development Initiative (LSDI) confirmed the resistance of *An. funestus* s.s to the pyrethroids, lambda-cyhalothrin and deltamethrin and low level of resistance to the carbamate, bendiocarb. The same survey reported evidence of resistance in *An. arabiensis* to pyrethroid and carbamate (Casimiro *et al.,* 2006a, Casimiro *et al.,* 2006b).

Later, *An. gambiae s.s.* and *An. arabiensis* collected as part of the LSDI monitoring initiative in southern Mozambique showed resistance to lambda-cyhalothrin and bendiocarb (Coleman *et al.,* 2008). Recently, a study carried out in Chokwe a southern district of Mozambique observed very high levels of pyrethroid resistance in *An. funestus* (Wondji *et al.,* 2010).

1.5. Insecticide classification and modes of action

Insecticides are chemicals that are used to control insect pests. There are several classifications of insecticides, however, for public health insect control the insecticide can be divided into four major groups: organochlorines, organophosphates, carbamates and pyrethroids (WHO 1997).

1.5.1. Organochlorines

The organochlorines are insecticides that contain carbon, hydrogen, and chlorine. They are also known by other names: chlorinated hydrocarbons, chlorinated organics, chlorinated insecticides, and chlorinated synthetics. The organochlorines are now primarily of historic interest, since few survive in today's arsenal. There are four groups of organochlorines: Diphenyl aliphatics, HCH (Hexa chlorocyclohexane), cyclodienes and polychloroterpenes but only DDT is used routinely for vector control.

1.5.1.1. Diphenyl aliphatics

This is the oldest group of the organochlorines. In this group the insecticide used in public health is DDT which was originally synthesized in 1854. The first trials of DDT took place during the second World War (Onori *et al.,* 1993). DDT is probably the best known and most notorious insecticide of the 20th century. It acts on the sodium channel to cause "leakage" of sodium ions (Ware and Whitacre 2009). The target site of DDT is the Na+ channel proteins (Sawicki 1987).

1.5.1.2. Cyclodienes

This group of organochlorines appeared after World War II: In this group dieldrin was the insecticide employed in public health. Most cyclodienes are persistent organic pollutants and are stable in soil and relatively stable to exposure to ultraviolet in

sunlight. Unlike DDT and HCH, the cyclodienes have a positive temperature correlation-their toxicity increases with increases in the surrounding temperature. The target site of cyclodienes are the altered gamma amino butyric (GABA) gated chloride channels (Ffrench-Constant *et al.*, 1991, Ware and Whitacre 2009).

1.5.1.3. Polychloroterpenes

The polychloroterpenes: toxaphene was developed in 1947, and strobane in 1951. Toxaphene was widely used in agriculture while strobane was used very little. Toxaphene is rather easily metabolized by mammals and birds, and is not stored in body fat to the same extent as DDT, HCH or the cyclodienes (Ware and Whitacre 2009).

1.5.2. Organophosphates

This group of insecticides, was widely used in agriculture and subsequently commonly employed in public health, mainly because of resistance to other insecticides. Their mode of action is inhibition of the enzyme acetylcholinesterase (Onori *et al.*, 1993).

The organophosphates (OPs) are generally divided into three groups: aliphatic, phenil and heterocyclic derivates. The most common used in public health OPs belonging to the aliphatic group. Malathion was introduced in 1950 (Cecchine *et al.*, 2000). The phenil OPs are generally more stable than the aliphatics. Fenitrothion is one of the most common phenil used in public health (Ware and Whitacre 2009).

1.5.3. Carbamates

Carbamates were originally extracted from the Calabar bean, which grows in West Africa. Extracts from this bean contain physostigmine, a methylcarbamate ester (Baron 1991). Carbamates are derivatives of carbamic acid. Like the organophosphates, carbamates as a class are not generally persistent in the environment. Their mode of action is inhibition of acetylcholinesterase. The first successful carbamate insecticide, carbaryl (Sevin®), was introduced in 1956. Propoxur and bendiocarb are the carbamates most commonly used in public health, although propoxur was recently withdrawn for commercial reasons.

1.5.4. Pyrethroids

The pyrethroid insecticides are typically esters of chrysanthemic acid having a high degree of lipophilicity (fat solubility). The pyrethroids were derived from the natural pyrethrins, which were isolated from the flowers of Chrysanthemum (Bloomquist 2009).

The pyrethroids have an interesting evolution, which can be divided into four generations: the insecticide used in public health belonging to the last generation, include lambda-cyhalothrin, cypermethrin, cyfluthrin and deltamethrin (Ware and Whitacre 2009).

Pyrethrins and pyrethroids are contact nerve poisons. Most of the pyrethrins are unstable being quickly oxidized in the presence of air and water. They have low toxicity to humans (WHO 1997). The target site of this group of insecticide are Na+ channel proteins (Martinez-Torres *et al.,* 1998).

1.6. Insecticide resistance mechanisms

Resistance arises as result of mutations, which alter a normal physiological, morphological or behavioural attribute of a species. The identification of resistance mechanisms is very important because it helps to determine the cross-resistance spectrum, facilitates the choice of alternative insecticides, and allows detailed mapping of areas with resistant populations (Beaty and Marcquardt 1996).

The resistance mechanisms selected in insects can broadly be classified as follows:

- cuticular (reduced penetration),
- behavioural,
- metabolic and
- altered target site resistance.

Of these four categories the last two metabolic and target site are by far the most important (WHO 1998, Pasteur and Raymond 1996, Beaty and Marcquardt 1996). Although resistance due to changes in behaviour (Drobozina *et al.*, 1988, Stanczyk *et al.*, 2010) and decreased penetration (Beaty and Marcquardt 1996) have been described in mosquitoes, the genetics are unknown.

Metabolic resistance involves qualitative or quantitative changes in the enzymes which metabolize or sequester the insecticides before they reach their target sites. There are three groups of enzymes involved: esterases, glutathione S-transferase and monooxygenases (Brogdon and McAllister 1998).

1.6.1. Esterases

This is a common resistance mechanism in some insects. It involves modified levels or activities of esterase enzymes that metabolize a wide range of insecticides (Brogdon and McAllister 1998). Resistance can occur through quantitative and qualitative changes in esterases. When increased quantities occur, sequestration is generally the primary mechanism. Qualitatively changed carboxylesterases can hydrolyze insecticides at faster rates than their counterparts in susceptible insects (Karunaratne *et al.,* 1998).

Changes in carboxylesterase activity have been associated with resistance to organophosphate insecticides in *Culex* mosquitoes, aphids, blowflies and houseflies (Claudianos *et al.,* 1999). Esterase levels can be elevated by either gene amplification or altered gene expression (Scott 1995) or a combination of both (Hawkes and Hemingway Unpublished data).

1.6.2. Glutathione S-transferase

Glutathione S-transferases (GSTs) are a major family of detoxification enzymes found in most organisms. All eukaryotes possess multiple GSTs with different substrate specificities to accommodate the wide range of catalytic functions of this enzyme family (Ranson *et al.,* 2002). They catalyze the nucleophilic attack of the endogenous tripeptide glutathione on a variety of reactive substrates. In early literature a subset of GSTs are referred to as DDT dehydrochlorinases (DDTases) because of their involvement in dehydrochlorination of DDT to DDE (Prapanthadara *et al.,* 1995).

In mosquitoes, GSTs commonly confer resistance to DDT (Prapanthadara and Ketterman 1993). Insect GSTs are now classified into five classes, but previously only two such classes were recognized (Ranson *et al.,* 2002). Class I GSTs are most closely related at the amino acid level to mammalian theta class GSTs, while class II GSTs are related to the pi class. This relationship between insect and mammalian classes does not

extend to their substrate specificities (Hemingway and Ranson 2000). In *An. gambiae* seven GSTs have been partially purified which possess 100% of the DDTase activity (Prapanthadara *et al.,* 1995).

1.6.3. Monooxygenases

The monooxygenases, also termed cytochrome P450 oxidases or MFOs (mixed function oxidases) metabolize insecticides through O-, S-, and N-alkyl hydroxylation, aliphatic hydroxylation and epoxidation, aromatic hydroxylation, ester-oxidation, and nitrogen and thioether oxidation [(Wilkinson 1976 cited by (Brogdon and McAllister 1998)]).

The cytochrome P450s belongs to a vast super family of enzymes. There are 62 families of P450s recognized in animals and plants. The *An. gambiae* genome has over 90 P450s genes (Ranson *et al.,* 2002). The insect P450s responsible for resistance primarily belong to family six, which, like esterases, occur in Diptera as a cluster of genes (Maitra *et al.,* 1996). The cytochrome P450 monooxygenases are involved in many cases of resistance of insects to insecticides.

Mutations that confer metabolic resistance are considered to be rare and unique events (Pasteur and Raymond 1996).

1.6.4. Altered target sites

In this category of resistance mechanism, an alteration in the target-site prevents the insecticide interaction with the target. These changes must be highly specific, so that the normal physiological functions of the target site are largely unaffected. Most of these changes are due to a substitution of a single amino acid in the protein sequence of the target site. There are three target sites: AChE (acetylcholinesterase), Na+ channel

proteins and GABA (gamma amino butyric acid) receptors for the four main insecticide families used for vector control (Karunaratne *et al.,* 1998).

- **Altered acetylcholinesterase**

Acetylcholinesterase is the target site for organophosphate and carbamate insecticides and point mutations in the Ace gene are associated with resistance in *Drosophila melanogaster* and *Musca domestica* (Nabeshima *et al.,* 2004).

Acetylcholinesterase catalyses the hydrolysis of the neurotransmitter, acetylcholine, thereby ending transmission of nerve impulses at synapses of cholinergic neurones in central and peripheral nervous systems (Baxter and Barker 1999). Quantitative and qualitative changes in AChE confer resistance to insecticides (Fournier *et al.,* 1992). In resistant insects the enzyme has reduced sensitivity to insecticide inhibition while maintaining its normal function at levels at least adequate for survival (Raymond *et al.,* 1985).

Vaughan and others, demonstrated that the same mutations that cause insecticide resistance in *D. melanogaster* AChE also confer resistance in *Aedes aegypti* (Vaughan *et al.,* 1997).

- **Altered gamma amino butyric acid receptors (GABA)**

Altered gamma amino butyric acid receptors are the primary target of cyclodiene insecticides (Scott 1995). All recorded cases of cyclodiene resistance ere due to decreased sensitivity of the GABA subtype A receptor (Ffrench-Constant *et al.,* 1991).

- **Altered Na+ channel proteins**

The Na+ channel proteins in the insect nervous system are the target site for pyrethroids and DDT. Insects with altered Na+ channel proteins are resistant to the rapid knock-down effect of pyrethroids and are called "*kdr*" (knock-down resistance) or "super *kdr*" (highly resistant). These mechanisms have been observed in houseflies (*M. domestica*) (Rossignol 1988, Grubs *et al.,* 1988) and *A. aegypti* (Malcolm and Wood 1982, Hemingway *et al.,* 1989) and many other insects. In *An. gambiae s.s., kdr* has been reported throughout West Africa (Martinez-Torres *et al.,* 1998, Chandre *et al.,* 1999, Coleman *et al.,* 2006) and Kenya (Ranson *et al.,* 2000)

.

Pasteur & Raymond (1996) suggest migration and selection as the two major factors in the evolution of resistance in natural populations. Their work showed that the passive migration of *Culex pipiens* associated with commercial transport plays an important role in the dispersal of resistance-associated mutations (Pasteur and Raymond 1996).

Resistance may decline with the age of the mosquitoes, for example, DDT resistance in *An. gambiae s.s.* (Lines and Nassor 1991). Mixed-age wild samples of both fed and unfed, DDT resistant *An. gambiae s.s.* from Kikobweni, Zanzibar, gave a higher mortality after exposure to 5% DDT than newly emerged insects.

If mosquitoes lose their tolerance to DDT so quickly with age, it will still be possible to control resistant populations with DDT to prevent them reaching the age where they can transmit malaria (Rowland and Hemingway 1987). This may explain reports that DDT is still useful even against highly resistant populations (Sharma *et al.,* 1986).

Unfortunately, this argument may not always be reliable in practice for three reasons. Firstly, we do not know how mortality in a laboratory test relates to that in sprayed houses. Secondly, it may be that tolerance to DDT declines with age in susceptible as well as resistant insects, so that resistance improves the probability of survival at all ages. Thirdly, we know that the efficiency of house-spraying for vector control depends

on the mosquitoes having a repeated chance of being killed each time they enter a house to feed; it follows that even resistance which is restricted to younger insects is likely to have significant epidemiological impact (Lines and Nassor 1991). This emphasizes the recommendation of Davidson and Zahar (Davidson and Zahar 1973) that the decision whether or not to switch from DDT to an alternative, possibly more expensive, insecticide should be based mainly on epidemiological evidence for the continued success of control.

1.7. Insecticide resistance management

In malaria control, spraying of insecticides inside human habitation severely restricts the number of suitable compounds which can be used by control programmes. The use of an insecticide until resistance becomes a limiting factor is rapidly eroding the number of suitable insecticides for malaria control. A better management strategy may be the use of compounds in rotational or mosaic alternation (Mellon and Georghiou 1984, Curtis 1985, Hemingway *et al.,* 1997) . Numerous mathematical models have been produced to determine the optimal strategies for resistance management (Greever and Georghiou 1979, Georghiou 1980, Curtis 1985, Tabashnik 1989). These models have been tested under laboratory but not field conditions due to the practical difficulties of accurately assessing the changes in resistance gene frequencies associated with different patterns of insecticide use in large-scale field populations of insects (Taylor *et al.,* 1983). With the advent of more sophisticated biochemical and molecular assays for resistance detection, it is now practical to analyze accurately large numbers of insects individually for a range of insecticide resistance genes and to monitor their changes over time (Hemingway 1983, Hemingway *et al.,* 1995).

1.8. History of malaria control in Mozambique

1.8.1. Indoor Residual Spraying

In Mozambique, malaria control was initiated in 1946 with IRS using DDT and partially benzene hexachloride (BHC) in the southern part of the country in the semi-urban area of Maputo city and in the rural area of Limpopo Valley (Soeiro 1956). Prior to the introduction of malaria control in southern Mozambique, between 1937 and 1938 an overall parasite and spleen rate of 92.1% and 65.3% was recorded in children one to five years old (Ferreira 1958). The main malaria vectors *An. gambiae s.l.* and *An. funestus* were also widespread and found in high abundance indoors throughout the malarious areas.

Following the introduction of malaria control by house spraying with DDT in Maputo the capital of Mozambique, malaria admissions dropped dramatically from 733 cases in 1944 to 328 after 1946 to a low of 214 and 94 in 1952 and 1954, respectively, following the extension of residual spraying in 1950 (Figure 1.1). In the Limpopo Valley after the introduction of malaria control (IRS) parasite and spleen rates in children under a year old declined from 62.7% and 59.4% respectively in 1953 to 23.6% and 21% in 1954 and to 17% and 1% in 1955 (Figure 1.2).

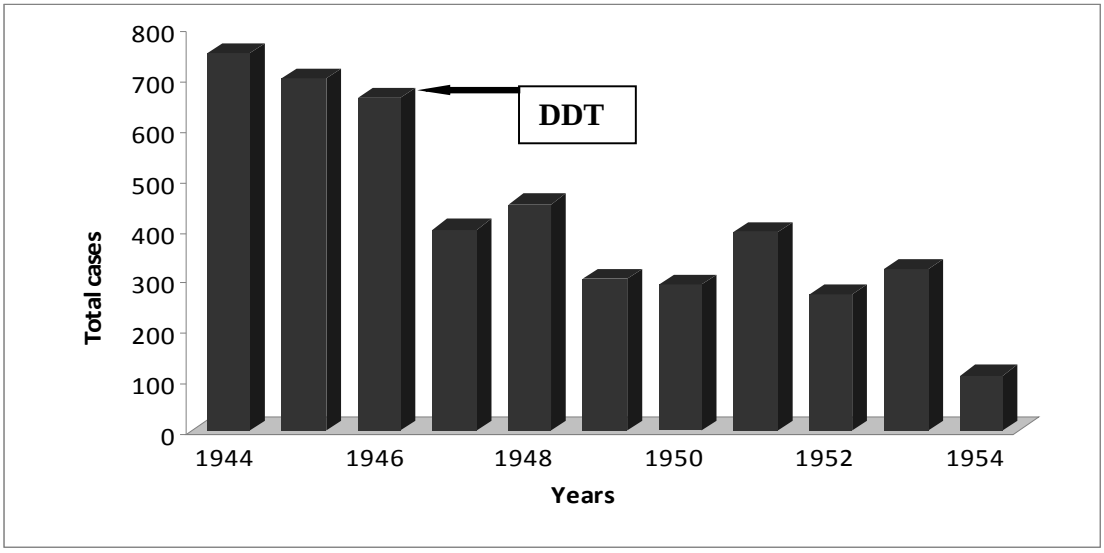


Figure 1.1. Annual totals of clinical malaria cases admitted to the Maputo Central Hospital, for the period 1944-1954 (Soeiro 1956).

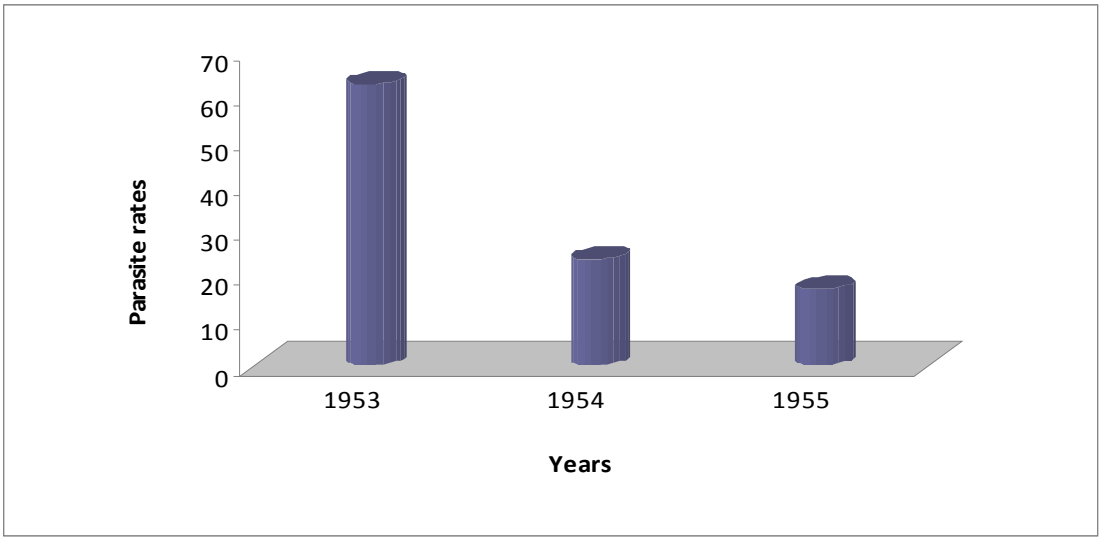


Figure 1.2. Malaria infection in children 0-1 year old for the period 1953-1955 in the Limpopo Valley, Southern Mozambique

The introduction of residual insecticides also had a marked impact on the abundance of indoor resting mosquitoes of *An. gambiae s.l.* and *An. funestus* in sprayed houses (Soeiro 1956). Between 1946 and 1956 good control was achieved with overall parasite ratios

being kept consistently below 10% for the ten year period (Soeiro 1956). After that the plan was to establish anti-malarial centres in each city and municipality of the country, however, due to shortage of trained manpower and financial resources vector control efforts were abandoned in 1956 (Soeiro 1959).

In 1960 following an agreement between the government and the World Health Organization (WHO), a malaria eradication pilot project was initiated in the southern parts of Mozambique. The objective was to determine whether malaria transmission could be interrupted by applying DDT house spraying combined with surveillance and therapeutic measures. Although malaria transmission was never fully interrupted, dramatic reductions in malaria prevalence were achieved between 1961 and 1971. Reductions, were mainly in the southern parts of the country where malaria control activities had been carried out since 1946 (Schwalbach and De La Maza 1985, Martinenko 1992), (Figure 1.3). Failure to fully interrupt transmission was attributed mainly to population movements from other parts of the country, which made it impossible to prevent constant renewal of the reservoir of infection.

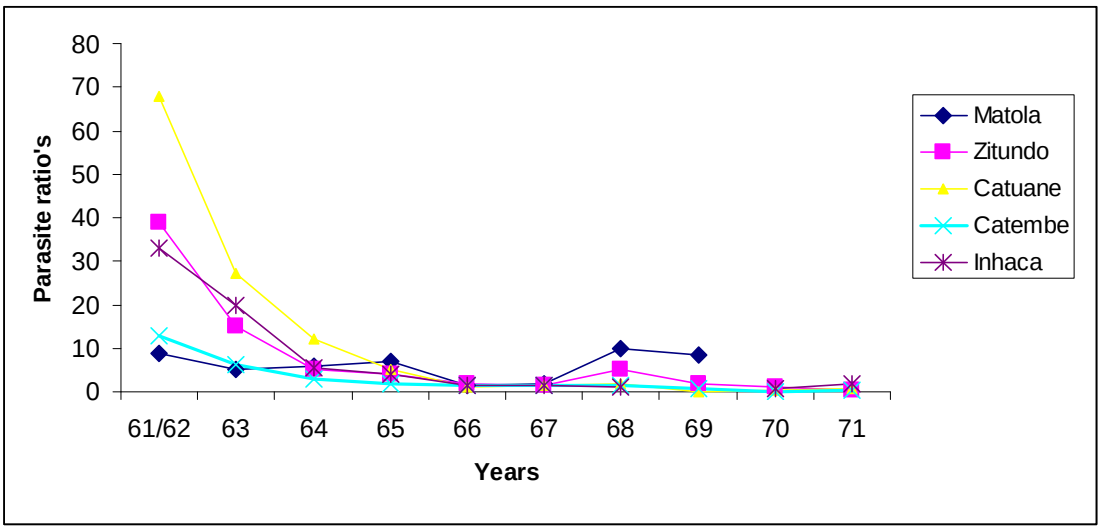


Figure 1.3. Annual parasite ratio's (all ages) for the period 1961-1971 following the introduction of the malaria eradication activities in 1960 in Mozambique (Schwalbach & De La Maza 1985).

The escalation of civil war in the late 1970's led to a complete breakdown of control measures. As a result, in the mid 1970s, after the eradication campaign, house spraying was confined to regions of the Limpopo Valley, Maputo City, Quelimane and Nampula and by the 1980s control operations were restricted to just the Maputo area (Martinenko *et al.,* 1989).

During the 1990s some alternative insecticides were tested for IRS and insecticide treated nets (ITNs) in Mozambique, to assess product efficacy and impact, safety and other factors under local circumstances. Large-scale operational IRS field trials of two pyrethroids: lambda-cyhalothrin 10% wettable powder, (Icon® 10 WP) (Cuamba and Dambo 1994, Franco 1994) and cyfluthrin 10% wettable powder, (Baythroid® 10 WP) (Martinenko *et al.,* 1995), were evaluated entomologically and epidemiologically for malaria control by the Instituto Nacional de Saúde in conjunction with the Programa Nacional do Controlo da Malária.

In 2000 the LSDI (Lubombo Spatial Development Initiative) malaria control programme, a regional initiative between South Africa, Swaziland and Mozambique aimed at protecting communities from malaria and to create a suitable environment for economic development and promotion of eco-tourism in the area. The programme introduced IRS in Maputo province using carbamate (bendiocarb) until 2005, due to the discovery of pyrethroid resistance in *An. funestus* population in the area and with evidence-based DDT was reintroduced for IRS programme and increasingly was becoming the main insecticide used for malaria vector control in Mozambique until on 2009 (Casimiro *et al.,* 2006, Casimiro *et al.,* 2006, Casimiro *et al.,* 2007, Sharp *et al.,* 2007, Coleman *et al.,* 2008).

Currently Mozambique's vector control is through a combination of annual IRS with pyrethroids combined with the use of insecticide treated nets.

1.8.2. Insecticide Treated Nets in Mozambique

An insecticide-treated net (ITN) is a mosquito net that repels, disables and/or kills mosquitoes coming into contact with insecticide on the netting material. There are two categories of ITNs: Conventionally treated nets and long-lasting insecticidal nets:

A conventional net is a mosquito net that has been treated by dipping in a WHO-recommended insecticide. To ensure its continued insecticidal effect, the net should be re-treated after three washes, or at least once a year.

A long-lasting insecticidal net (LLIN) is a factory-treated mosquito net made with netting material that has insecticide incorporated with or bound around the fibres. The net must retain its effective biological activity without re-treatment for at least 20 WHO standard washes under laboratory conditions and three years of recommended use under field conditions (WHO 2009).

In Mozambique the first pilot project of ITNs was carried out in Boane district between 1994 and 1998. ITNs were not extensively used in the country until 2000 when two communities based malaria prevention and control initiatives were successfully started in Zambézia and Gaza provinces.

In 2000, as part of the efforts to assist families affected by severe floods, the Ministry of Health and UNICEF distributed more than 200,000 mosquitoes nets nationally, spearheading large scale net distribution projects particularly in Gaza and Zambézia provinces, followed later by projects of varying sizes. An estimate suggests that between 1999 to 2005 approximately 1,650,000 ITNs were distributed across the country. This includes the 400,000 LLIN, funded by the Canadian International Development Agency and distributed in Sofala and Manica provinces in December 2005, in collaboration with the Mozambican Red Cross. Gaza, Inhambane, Tete, Zambézia and Cabo Delgado provinces have projects that are well established though Ante Natal Care and children. In many provinces this distribution is being carried out in collaboration with UNICEF.

In 2005, the Spanish Agency for International Cooperation (SAIC) with funding from UNICEF purchased approximately 140,000 LLINs for free distribution to pregnant women and children under five in Niassa province. In the same year, the Department for International Development (DfID) was financing a £8 million project to establish a sustainable ITN market in Mozambique, being implemented by the non-government organization, the Malaria Consortium. This project was launched in Inhambane province in 2005 and expanded in 2006 to Nampula and Cabo Delgado and in 2008 expanded to Zambézia province. Many other non-government organizations (NGOs) have been selling mosquitoes nets in Mozambique communities.

Despite these efforts the actual coverage in Mozambique with ITNs or LLINs, remains very low: an estimated 15.8% of all the households owned at least one insecticide-treated net and about 18.5% of those households with a pregnant woman and/or child under 5 years owned at least one ITN or LLIN (Mabunda *et al.*, 2007).

1.8.3. Chemoprophylaxis and Chemotherapy

Every successful malaria control programme is a combination of effective vector control and treatment (WHO 2006). Following independence in 1975, a campaign of chloroquine chemoprophylaxis was instituted throughout Mozambique with the objective of reducing the morbidity due to malaria. Chloroquine was distributed to school children and villages once a week. The targeted population coverage was not reached because of logistical problems. In 1983, resistance was detected in *P. falciparum* to chloroquine and the chemoprophylaxis campaign stopped (Martinenko 1992). By 1999, resistance of *P. falciparum* to anti-malarial drugs, especially to chloroquine, the first-line treatment for non-complicated malaria, varied from 15% to 40% (Ministério da Saúde. 2001).

Due to rising resistance a policy change was made in 2002 (MISAU 2002) to:
1st line treatment: Amodiaquine (AQ) + Sulfadoxine-Pyrimethamine (SP)
2nd line treatment: Artemether + Lumefantrine
3rd line treatment: Quinine

It should be noted that despite chloroquine resistance, the policy still advises its use for malaria treatment at the community level (Mabunda *et al.,* 2007).

Given the debate around AQ and the fact that the drug was banned by WHO due to alleged severe side effects, together with its similarities to chloroquine and potential cross-resistance between the two drugs, meant that in 2002 senior MISAU management were already considering a new first-line treatment based on a combination containing artemisin (ACT) derivatives. Towards the end of 2004, the Ministry of Heath authorised another change to the first-line malaria treatment, with artesumate (AS) + AQ replacing AQ+SP as the first line treatment.

1.8.4. Intermittent Presumptive treatment (IPT)

Malaria infection during pregnancy is an enormous public health problem, with substantial risks for the mother and her foetus. In areas of low transmission of *P. falciparum*, where levels of acquired immunity are low, women are susceptible to episodes of severe malaria, which can result in stillbirths or spontaneous abortion and possible death of the mother (Luxemburger *et al.,* 1997). In areas of high transmission of *P. falciparum*, where levels of acquired immunity tend to be high, women are susceptible to asymptomatic infection, which can result in maternal anaemia and placental parasitaemia, both of which can subsequently lead to low birth weight (Steketee *et al.,* 1996). There is evidence that *P. vivax* may also cause anaemia and low birth weight (Nosten *et al.,* 1999). Low birth weight is an important contribution to infant mortality (McCormick 1985; McDermott *et al.,* 1996). It has been estimated that

malaria during pregnancy contributes to 75 000 to 200 000 infant deaths each year (Steketee *et al.*, 2001).

The WHO currently recommends a package of interventions for controlling malaria during pregnancy in areas with stable (high) transmission of *P. falciparum* (WHO 2004, WHO 2006, WHO 2007b), which includes the use of ITNs, intermittent preventive treatment (IPT) and effective case management of malaria and anaemia (WHO 2007b).

In Mozambique following approval for the introduction of IPT in 2004 with SP drugs during pregnancy there were long delays in implementation. It was only in the first quarter of 2006 that plans for introducing the strategy in the country were finalized.

This strategy was officially implemented nationwide in May 2006, although the levels of implementation vary from province to province (MOH 2006). Recent surveys show that only 20.3% of the women who completed a pregnancy during 2006 received two or more doses of IPT during that pregnancy, and only 23.3% attended an antenatal consultation more than once.

1.9. Malaria in Zambézia province

In the central-north part of the country, Zambézia province is mainly a rural community and malaria is transmitted all year round. Malaria control in the province is currently based on the combination of IRS, ITNs and IPT in line with the national malaria strategic plan.

From 1995 to 2003 vector control in Zambézia was based on IRS and fogging with ICON (lambda-cyhalothrin) in three (Mocuba, Morrumbala and Quelimane) of sixteen districts in the province. In 2004 there were insufficient funds for vector control in the province, this contributed to the increase in levels of morbidity and mortality when compared to malaria rates over the previous five years (DPSZ, 2003-2005).

Because of the success achieved in reducing parasite prevalence in children in Maputo province, by the LSDI programme (Sharp *et al.*, 2007), the Ministry of Health initiated a pilot project to scale up IRS in rural areas in Zambézia province. An IRS programme with DDT commenced again in 2005 in Quelimane and two new districts, Nicoadala and Namacurra. Mocuba and Morrumbala were sprayed with ICON remaining from the 2003 spray campaign (DPSZ, 2005).

From 2006 to 2008 the IRS expanded again in partnership with the Presidents Malaria Initiative (PMI) to cover six districts Milange, Mocuba, Morrumbala, Namacurra, Nicoadala and Quelimane, (DPSZ, 2006-2008). In 2009 IRS reverted its policy back to pyrethroids due to the pressure from environmentalists and funders on the MOH after finding DDT in some markets and on some farms in Mozambique. The final stocks of DDT were used in Mocuba district, while all other districts were sprayed with lambda-cyhalothrin. This district was chosen due to its high levels of malaria transmission.

The first study for assessing the insecticide resistance of malaria vectors to the insecticides used in the Zambézia province was performed in 2000-2002 through pre-implementation of the LSDI malaria control programme. The results showed that *An.*

gambiae s.s., An. arabiensis and *An. funestus* s.s in Quelimane were 100% susceptible to pyrethroids, carbamates and DDT (Casimiro *et al.,* 2006a, Casimiro *et al.,* 2006b, Coleman *et al.,* 2008).

1.10. Malaria transmission

Humans become infected with malaria as a result of their exposure to blood-feeding infectious *Anopheles* mosquitoes. The mosquito is infectious when the sporozoites released from mature oocysts are present in the salivary gland of the mosquito (Baton and Ranford-Cartwright 2005). Sporozoite-stage parasites inoculated by even a single infectious mosquito can cause human malaria infection and life threatening disease (Chege and Beier 1994, Beier *et al.,* 1994, Trape and Rogier 1996). To fight malaria successfully, control programmes must use current tools effectively and measure the impact of these tools on transmission (Shaukat *et al.,* 2010). The intensity of malaria transmission is the main tool that affects most aspects of malaria epidemiology and control (Snow *et al.,* 1994, Snow *et al.,* 1997, Snow and Marsh 2002, Struik *et al.,* 2004, Reyburn *et al.,* 2005). A basic understanding of relationships between malaria transmission by the vector population of mosquitoes and the outcomes is to measure the transmission intensity in terms of malaria prevalence, malaria incidence, the incidence of severe disease, and mortality (Githeko *et al.,* 2006, Beier *et al.,* 1999). The entomological correlates of epidemiological impacts are vectorial capacity, entomological inoculation rates and the basic reproductive number all of which have a bearing on the vector species abundance and infectivity (Githeko 2006, Smith *et al.,* 2007).

An important concept in the epidemiology of disease is the basic reproduction rate (Ro), which is the average number of secondary cases of a disease (e.g. malaria) arising from each primary infection in a defined population of susceptible individual hosts. Ro represents the maximum reproductive rate per generation, leaving aside complications such as host immunity and super-infection. If Ro > 1, the number of people infected by

the parasite increase and the disease is maintained, with the level of transmission depending on the size of Ro but if Ro < 1, the number of people infected declines consequently the disease decreases and will eventually disappear from the population (Smith *et al.,* 2007, Silver 2008).

Vectorial capacity is the entomological component of the basic reproduction rate of malaria. It is an average number of inoculations from a single case of malaria in unit time, usually a day, that the vector population transmits to man, where all vectors biting an infected person become infective. Reduced vectorial capacity means reduced Ro. The basic formula for vectorial capacity (VC) (Garret-Jones and Shidrawi 1969, Silver 2008) is:

$$VC = \frac{Ma^2 px}{-\ln p}$$

where, M = man-biting rate or vector density in relation to man, a = the daily man–biting rate, p = daily survival rate, x = duration of the sporogonic cycle. Expectation of the life span of a vector = 1/-log p, and Expectation of the infective life span = px/-log p. However vectorial capacity is an indirect method of estimating transmission rate by a vector.

The intensity of malaria transmission can be measured in several ways: Parasite rate, annual parasite index, spleen rate and the entomological inoculation rate (EIR) (Killeen *et al.*, 2002, Warrell and Gillies 2002, Fontenille and Simard 2004, Killeen *et al.*, 2000, Smith and McKenzie 2004, Smith *et al.*, 2007). Most of these indices, derived from field and theoretical data, are calculated using assumptions and are generally not used for evaluating control programmes (Shaukat *et al.*, 2010). Good estimates of malaria transmission intensity are therefore necessary to compare and interpret malaria interventions conducted in different places and times and to objectively evaluate options for malaria control (Smith *et al.*, 2007). A more direct way to measure the intensity of

malaria parasite transmission by anophelines vectors and vector control interventions as the only tools currently considered able to interrupt malaria is to use the entomological inoculation rate (EIR) or infective biting rate (IBR), or as it is often called the inoculation rate (h) because it quantifies the parasite-infected mosquito pool and its propensity to transmit infectious parasites to the human population (Shaukat *et al.*, 2010).

The EIR is the number of infectious bites per person per unit time, usually measured or expressed per year. It can be estimated as the product of the human reservoir infectiousness (k), the life-time transmission potential of individual mosquitoes (L) and the rate at which they emerge from larval breeding sites (E) relative to human population size (E/Nh) (Killeen *et al.*, 2000):

$$EIR = k\ L\ E/Nh$$

Alternatively, EIR can be expressed as a product of the human biting rate and the sporozoite rate:

$$EIR = MaS$$

The human biting rate (Ma) is the number of vectors biting an individual over a fixed period of time. M equals the number of *Anopheles* per person and a equals the average number of persons bitten by one *Anopheles* in one day. The sporozoite rate (S) is the fraction of vector mosquitoes present and biting that are considered infectious, i.e. *Anopheles* with sporozoites in their salivary glands (Warrell and Gillies 2002, Snow and Marsh 2002). The structure of this equation directly implies that measures which reduce the value of any of these contributors will amplify each other's effects when combined and thus decrease the EIR. These three contributors are also discreet targets for transmission control that are reduced by quite different interventions (Killeen *et al.*, 2000). The only intervention envisioned which could usefully reduce k, and which is likely to be available in the foreseeable future is a malaria vaccine (Miller and Hoffman

1998) and widespread use of transmission-blocking drugs. Tools for the reduction of L include IRS, ITNs, and zooprophylaxis (Snow *et al.,* 1999, Rozendal 2007, Lengeler *et al.,* 1998), whereas source reduction and other forms of larval control represent well established methods for controlling E/Nh (Shousha 1948, Kitron and Spielman 1989).

The EIR values are used to quantify the impact of IRS, ITNs and source reduction (SR) on malaria transmission. This analysis is extended by evaluating available vector control tools globally. Numerous factors influence the EIR, including temperature, altitude, rainfall, and urbanization (Warrell and Gillies 2002). In general the EIR is directly proportional to temperature because heat accelerates the sporogonic cycle, the time necessary for ingested gametocytes to develop into infectious sporozoites. The optimal temperature for malaria transmission is 25-27°C and an average monthly relative humidity above 60% (Pampana 1969). For the same reason, the EIR is inversely proportional to altitude because temperature decreases as altitude increases. The EIR is directly proportional to rainfall because female *Anopheles* mosquitoes lay their eggs in water. Generally, the EIR is inversely proportional to urbanization because with urbanization comes fewer bodies of water and greater pollution of water sources (Robert *et al.,* 2003). Therefore, tropical areas with warm temperature, heavy rainfall, high humidity, and efficient *Anopheles* vectors are ideal for malaria transmission (Breman 2001). These factors explain a large part of the variability in the EIRs across Africa.

An adult mosquitoe's lifespan is particularly important in the transmission of malaria. The mosquito must survive long enough for the parasite to complete sporogonic development from the point where gametocytes are ingested with the blood meal to the time when infectious sporozoites appear in the salivary glands. This process typically takes 10 days for *P. falciparum* (Killeen *et al.,* 2002). Therefore, decreasing the lifespan of mosquitoes substantially decreases the EIR.

In Africa, many studies have demonstrated that standard vector control measures are useful for controlling and even eliminating malaria in certain areas where transmission levels are marginal (Mouchet *et al.,* 1998). A foundation of malaria vector control is that

actions to decrease vector-host contact through methods including larval habitat modification, insecticide treatment of larval habitats, spraying insides of houses with residual insecticides, insecticide-treated bed nets, or the use of repellents will have correspondingly beneficial outcomes in terms of reduction in morbidity and mortality. Effective vector control measures decrease the incidence of malaria infections because there is a linear relationship between EIRs and malaria incidence (Beier *et al.,* 1994). In fact, studies in Saradidi in western Kenya showed that 74% of the variation in *P. falciparum* incidence is explained by EIR (Beier *et al.,* 1994).

EIR is more useful than either vectorial capacity or reproductive number because this parameter is more meaningful as an epidemiologic predictor and is testable by measuring EIR directly (Killeen *et al.,* 2000). Based on the assumption that an EIR below one is needed to interrupt malaria transmission (Shaukat *et al.,* 2010), many errors emerge in estimating both the human biting rate and sporozoite rate. These result from variation in methods used, attraction of mosquitoes to the capturer, diligence of the technical teams (Fontenille *et al.,* 2001) and lack of consistently used standard EIR protocols, thus making it not easy to calculate reliable EIR values. Several methods are used to measure the human biting rate, including using "capturers" (human landing catches), pyrethrum spray catches, exit trap collections, and CDC light traps (WHO 1975). Human landing catches are the gold standard proxy of human-biting rates but because of the logistical difficulties coupled with ethical issues, EIR is not a readily available tool for control programmes. However, measuring the impact of specific interventions on the vector population, sporozoite rates or infectious reservoir has been observed in the field as alternative to measure EIR because of their linear correlation (Macdonald 1957, Saul 1993, Molineaux 1997, Charlwood *et al.,* 1998, Beier *et al.,* 1999, Killeen *et al.,* 2000, Shaukat *et al.,* 2010).

1.11. General objective

The main objective of this study was to evaluate the impact of IRS and ITN programmes implemented in Zambézia province, Mozambique on malaria transmission. This was to be achieved through monitoring the vector species abundance, infectiousness and insecticide resistance. The impact on malaria was measured through annual parasite prevalence studies.

CHAPTER 2

METHODOLOGY

2.1. Location

Mozambique is located in southern Africa bordering Malawi, South Africa, Swaziland Tanzania, Zambia and Zimbabwe, with the Indian Ocean on the East. The land mass of Mozambique covers 784 090 Km^2 with an estimated population of 20 million persons. The climate is tropical humid with average temperatures of 24^o-25^oC. There are two distinct seasons, winter is the dry season from April to October and the summer is characterized by rain and warm temperatures from October to March.

This study was carried out in Zambézia province, central-northern region of Mozambique. 23 sentinel sites were established in seven of the seventeen districts Namacurra, Nicoadala, Maganja da Costa, Milange, Mocuba, Morrumbala and Quelimane (Figure 2.4) from October 2006 to April 2010.

Figure 2.4. Map of study area districts from Zambézia province central-northern Mozambique.

Sentinel sites were located in 6 districts where IRS with DDT for vector control was to be carried out and one district Maganja da Costa where ITNs were distributed. IRS was carried out in October and November in each year from 2006 to 2008. Initially IRS was carried out with the insecticide DDT. In 2009 there was a policy change and the pyrethroid lambda-cyhalothrin was used for IRS in all districts except Mocuba that was sprayed with the remaining DDT stock. IRS coverage was estimated at over 80% in each year and 95% of the families in Maganja da Costa had one or more ITN or LLIN since 2008 (DPSZ 2008). The extensive distribution of the sentinel sites throughout the province facilitated localized monitoring of malaria vectors. These data are useful for the evaluation of the effectiveness of IRS and ITNs or LLINs and for long term planning of the malaria control programme.

2.2. Mosquito collections

There are various techniques available for sampling vector mosquitoes (Service 1976), and the choice is determined by the entomological investigation under consideration. This study collected adult mosquitoes in two ways for monitoring insecticide resistance, species abundance and *Plasmodium* infections rate.

a) Insecticide resistance collections

Adult female *Anopheles* mosquitoes were collected monthly in 10 different houses from each sentinel site early in the morning (06.00-10.00 hours), using an aspirator and torch, during the period October 2006 to March 2010. Insecticide resistance assays were initially (2002-08) carried out on wild caught mosquitoes due to the lack of an insectary. Mosquitoes from 2002-2005 were collected thorough the LSDI programme. From 2010 onwards blood fed female mosquitoes were induced to lay eggs and subsequent F1 generations were reared through to adults for testing at 26+/-2oC and 70%-80% humidity in a new local insectary established at the Direcção Provincial de Saúde da Zambézia (DPSZ).

b) For species abundance

Window traps were initially installed in Namacurra, Nicoadala, Maganja da Costa, Mocuba, Morrumbala and Quelimane districts in 2006, the first year of initiation of vector control interventions. These traps were removed in 2008 due to logistics issues at the DPSZ and the lack of entomological resources in the malaria control programme. This project reactivated the window exit traps in February 2009 to monitor the impact of vector control on the local malaria vector mosquitoes. As the IRS programme has extended to Milange district, 4 additional sentinel sites were included (Figure 2.5).

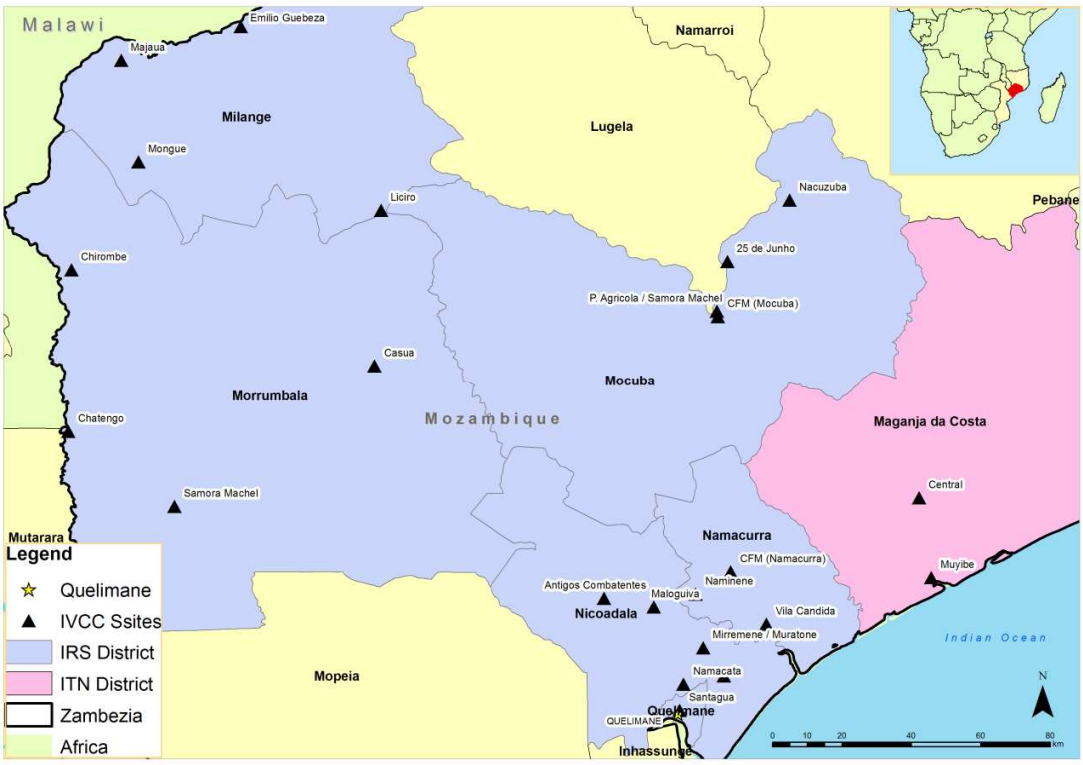


Figure 2.5. Map of sentinel sites in the IRS and ITNs districts of Zambézia province in central-northern Mozambique.

Six households were randomly selected in each sentinel site and window exit traps were attached with the homeowners consent. Houses were selected with the following criteria: traditional houses, with a window in the room used to sleep in. Home owners were trained to collect mosquitoes from window exit traps daily and store samples in pre-labelled specimen jars containing isopropanol. A checklist specifying nights for which traps were operating was also completed. Specimen jars were collected and replaced at four week intervals. All culicines caught were recorded to ensure that in the absence of anopheline catches, the traps were being successfully operated (Sharp *et al.*, 2007).

Due to timing issues mosquitoes were not collected prior to the onset of the programme, which did not allow for comparison between pre- and post-intervention periods. However, comparisons between each spray round were made. The number of

mosquitoes caught was compared over time, between sentinel sites and with respect to resistance to insecticides, species composition and infection rate.

Number of mosquitoes per trap per night were calculate for each vector species within subsequent rounds for IRS and pre- and post-ITNs, based on day of capture of the specimen in relation to spray or ITNs coverage status of the site in which the window traps was located.

2.3. Mosquito species identification

Initially mosquitoes were identified using morphological keys (Gillies and De Meillon 1968, Gillies and Coetzee 1987). *Anopheles gambiae* complex and *An. funestus* group were subsequently identified to species level by Polymerase Chain Reaction (PCR) (Scott *et al.,* 1993, Koekemoer *et al.,* 2002) at the MRC laboratory, Durban, South Africa.

2.4. Insecticide resistance assays

Wild caught and F1 mosquitoes were subdivided for susceptibility testing to avoid genetic bias. All insecticide resistance assays were carried out following the standard WHO diagnostic assays (WHO 1998).

Insecticides tested include;
Organochlorine-DDT (4%)
Pyrethroids- lambda-cyhalothrin (0.05%) and permethrin (0.75%)
Carbamate- bendiocarb (0.1%)

In brief;

Between 5 and 25 adult F1 mosquitoes, one to three day old, non-blood fed, or wild caught before the insectary facility was available, were exposed to an insecticide impregnated paper, supplied by WHO. The exposure tubes were placed in vertical position and the mosquitoes were exposed for one hour. After this time they were transferred to a holding tube and maintained for 23 hours. A 10% sugar solution was made available during the holding period. A control was carried out for each experiment where mosquitoes were exposed to WHO control papers.

Chi Square analysis was used to compare the change in susceptibility of malaria vectors from each spray intervention period to the three groups of insecticide. The *P* value $\leq$ 0.05 was considered to be statistically significant.

2.5. Sporozoite detection

Sporozoite detection was carried out on wild caught female mosquitoes from window exit traps using the TaqMan assay (Bass *et al.,* 2008). In brief, DNA was extracted from the head and thorax of single mosquitoes using the Livak method (Collins *et al.,* 1987). A PCR reaction (20µl) contained 1 µl of genomic DNA, 10 µl of SensiMix DNA (Quantance), 800 nM of each primer and 300 nM of probe PlasF and 200 nM probe OVM+. Reactions were run on a Rotor-Gene 6000TM (Corbett Research) using the temperature cycling conditions of: 10 minutes at 95oC followed by 40 cycles of 95oC for 10 seconds and 60oC for 45 seconds. The increase in VIC and FAM fluorescence was monitored in real time by acquiring each cycle on the yellow (330 nm excitation and 555 emission) and green channel (470 nm excitation and 510 emission) of the Rotor-Gene respectively.

2.6. Transmission index

Using the species specific estimated sporozoite rate, the number of infective mosquitoes per trap per night, by species was calculated; the ratio of infective number per trap per night first spray, relative to subsequent spray, was defined as the relative transmission index according to the equations below:

$$\text{Sporozoite rate (SR)} = \frac{\text{Number of infected mosquitoes per trap per night}}{\text{Total mosquitoes tested}}$$

$$\text{Transmission index} = [(\text{Total mosquitoes per trap per night}) \times \text{SR}]$$

$$\text{Relative transmission index post spray} = \frac{\text{Transmission index post spray}}{\text{Transmission index pre- spray}}$$

2.7. Malaria prevalence in human population

Cross-sectional household and prevalence surveys were carried out at each sentinel site on a random sample of 140 individuals >1 and <15 years of age. Sentinel sites were divided into four localities from which participants were selected to ensure as much geographical spread as possible. Rapid diagnostic tests with ICTTM malaria rapid test (ICT, Global Diagnostics, Cape Town, South Africa) were used to assess the prevalence of *P. falciparum* infection. Participants who tested positive were offered treatment with Coartem® (Novartis) (artemether and lumefantrine) in line with Mozambique National Guidelines.

Prevalence surveys were carried out prior to the initiation of IRS in October 2006 and subsequently in the same month in 2007 and 2008 at all sentinel sites except those in Milange district. The sentinel site specific sample size was determined to allow detection of a significant change at the 5% significance level, assuming a reduction in *P. falciparum* prevalence of at least 20% following intervention (Sharp *et al.*, 2007).

Prevalence was calculated annually for each sentinel site and 95% Confidence Intervals (CI) were calculated using variance estimates that took account of clustering by sentinel site (Rao and Scott 1981) as implemented in the statistical software package STATA (StataCorp 2003).

Sentinel sites were considered the primary sampling unit. Logistic regression, allowing for complex survey designs, was performed to estimate the mean effect of the intervention on prevalence compared to baseline prevalence of infection in different years.

Note: Analysis of this work was carried out by Dr. Immo Kleinschmidt, London School of Hygiene and Tropical Medicine.

Ethics was approved for this work by the Ministry of Health, Mozambique Reg: 3622/IMS-2/DNS/06.

CHAPTER 3

RESULTS

The initial IRS in five districts was undertaken by the MOH in October 2006 and at the same time the window exit traps were installed in the first 19 sentinel sites in six districts. IRS was subsequently supported by the PMI initiative from 2007 to 2010. The entomology survey was stopped in 2008 due to logistics issues at the DPSZ and in the same year ITNs were distributed in Maganja da Costa district. In 2009 the lack of entomological resources in the malaria control programme was corrected and surveys were reactivated, including the window exit traps from January 2009 to April 2010, to monitor the impact of vector control on the local malaria vector mosquitoes. The PMI programme was expanded to include IRS in Milange district and 4 more sentinel sites were established. Prevalence surveys were carried out in the same period of the year (October each year), in 2006, 2007 and 2008 respectively.

3.1. Insecticide resistance

Three vector species are common in Mozambique, *An. funestus, An. arabiensis* and *An. gambiae s.s.* and both *An. funestus* and *An. gambiae s.l.* were represented in the live catches. A total of 3664 *Anopheles* were tested during the study of which 1516 were wild caught adult mosquitoes prior to the establishment of an insectary and 2148 were F1 generation mosquitoes from 99 *An. funestus* and 6 *An. gambiae s.l* families tested after the insectary was established. A total of 594 *An. gambiae s.l.* and 3070 *An. funestus* were tested. Following the successful implementation of IRS in the province by the PMI initiative the number of mosquitoes collected in the field was significantly reduced.

No resistance was detected in the province until 2010 when resistance to the carbamate bendiocarb and pyrethroids was detected in *An. funestus* from Majaua and Mugeba (Table 3.1). High levels of pyrethroid resistance were detected in Mugeba (76.2 % mortality to lambda-cyhalothrin) in Mocuba district when compared to previous years this was a significant difference ($X2 = 26.38$; $P < 0.001$) (Table 3.2). Low levels of resistance were also detected to the carbamate, bendiocarb (93.5 % mortality) but there was no resistance to pyrethroid permethrin and DDT. Resistance was also detected in Majaua, Milange district to lambda-cyhalothrin (82.9 % mortality) and bendiocarb (84.5 % mortality).

Table 3.1. WHO susceptibility test result on wild caught adult mosquitoes from 2002 to 2008 and one-three days old F1 generation from 2010, both for *Anopheles funestus* and *Anopheles gambiae s.l.* from different localities, in the Zambézia province of Mozambique.

Locality	2002 to 2008										2010							
	Bendiocarb (0.01%)		DDT (4%)		Deltamethrim (0.05%)		Lambacyhalothrim (0.05%)		Permethrim (0.75%)		Bendiocarb (0.01%)		DDT (4%)		Lambacyhalothrim (0.05%)		Permethrim (0.75%)	
	% M	n	% M	n	% M	n	%M	n	%M	n	% M	n	%M	n	%M	n	% M	n
An. funestus																		
Quelimane					100	28	100	50										
A. combatentes			100	25	100	25												
Namacurra			100	20														
Maganja da Costa	100	22	100	60	100	85	100	74										
Muibi	100	28	100	45	100	5	100	11										
CFM- Mocuba			100	59	100	78												
Posto Agricola			100	209	100	121												
25 de Junho			100	79	100	31												
Mugeba											93.5	229	100	224	76.2	234	99.4	197
Majaua											84.5	207	100	193	82.9	159	100	193
An. gambiae s.l																		
Quelimane	100	91			100	15	100	130	100	4								
Nicoadala	100	60					100	56										
Central			100	30	100	30												
Muibi													100	29	100	14		
Nacuzuba													100	44	100	43	100	20

Table 3.2. WHO susceptibility test result on wild caught adult mosquitoes from 2002 to 2008 and one-three days old F1 generation from 2010, both for *Anopheles funestus* and *Anopheles gambiae s.l.* from each district bioassayed, in the Zambézia province of Mozambique.

Districts	**2002 to 2008**						**2010**							
	Bendiocarb (0.01%)		DDT (4%)		Pyrethroids		Bendiocarb (0.01%)		DDT (4%)			Pyrethroids		
	% M	n	% M	n	% M	n	%M	n	% M	n	p	% M	n	*p*
An. funestus s.s.														
Quelimane					100	78								
Nicoadala			100	25	100	25								
Namacurra			100	20										
Maganja	100	50	100	105	100	175								
Mocuba			100	347	100	230	93.5	229	100	224	> 0.05	89	431	<0.001
Milange							84.5	207	100	193		95	347	
An. gambiae s.l														
Quelimane	100	91			100	149								
Nicoadala	100	60			100	56								
Maganja			100	30	100	30			100	29	> 0.05	100	14	> 0.05
Mocuba									100	44		100	63	

Chi-square tests were carried out at districts where data were collected at both time points and *P* values are given.

3.2. Impact of IRS and ITNs on vector abundance and transmission

3.2.1. Vector species identification

During the study, which comprised 788 trapping nights a total of 6622 anophelines were collected from 114 window traps. Three thousand seven hundred and sixty nine were morphologically identified as *An. gambiae s.l.* and 2853 as *An. funestus s.l.* Of these, 905 *An. gambiae s.l.* and 946 *An. funestus s.l.* were further identified to species level using PCR (Table 3.3). *Anopheles gambiae s.s.* and *An. arabiensis* were the two members of *An. gambiae* complex and *An. funestus s.s.* was the only member of the *An. funestus* group to be identified. *Anopheles gambiae s.s.* mosquitoes were the most predominant species in all spray round periods from the windows traps (Table 3.3).

In the first year of spraying, *An. arabiensis*, *An. funestus* and *An. gambiae s.s.* were identified from six, fourteen and eighteen sentinel sites respectively and after the fourth spray round the same species were identified from eleven, ten and all nineteen sentinel sites respectively. The same combinations of the three species were found in all of sentinel sites with the exception of the Central sentinel site of Maganja da Costa district where *An. gambiae s.s.* was the predominant vector.

In the first period during the window exit trap survey of 2006 to 2007 the proportion of *An. gambiae s.s.* and *An. arabiensis* was 91 % and 5 % respectively, and in the second period of 2009 to 2010, the proportion of *An. gambiae s.s.* decreased to 63 % ($P = 0.02$) and the proportion of *An. arabiensis* increased to 22% ($P = 0.001$). The changes were statistically significant for both species (Table 3.3).

Three rainy seasons (main transmission season) were used for comparison of abundance, sporozoite rate and transmission index (Table 3.4): The results showed that after the first year of IRS (2006) the proportion of *An. gambiae s.s.* in the rainy season (January to April 2007) was 92 % of the *An. gambiae s.l.* captured. After the third spray round of IRS (2009) the proportion was reduced to 76 %, which was not statistically significant ($P = 0.2$), but after the fourth round the proportion was reduced further to just 8 % which was statistically significant ($P < 0.001$). The proportion of *An. arabiensis* caught in the same period increased from 6 % to 10 % ($P = 0.317$) and after fourth round spray increased again to 74 %. Again this was statistically significant ($P < 0.001$).

3.2.2. Species abundance

While the average numbers of *An. gambiae s.s.* and *An. funestus* per window trap per 100 nights fell (from 1 to 0.5 and 1.59 to 0.19 respectively) during the period of IRS and ITN deployment, the estimated number of *An. arabiensis* showed an increase in number (from 0.058 to 0.2) (Table 3.3).

This decline in *An. gambiae s.s.* is best seen when looking at the rainy season (January to April) which is also the main transmission season, following each round of IRS (Table 3.4). Note that the estimated number of *An. gambiae s.s.* falls from 2.33 in 2007 to 1.27 in 2009 and 0.06 in 2010. *Anopheles funestus* follows a similar pattern, falling from 1.17 to 0.205 to 0.117 over the same time period. The increase in *An. arabiensis* is most marked in 2010 as it goes from 0.15 to 0.16 to 0.54 after the three spray rounds although this difference is not statistically significant ($P = 1$ and $P = 0.6$ respectively).

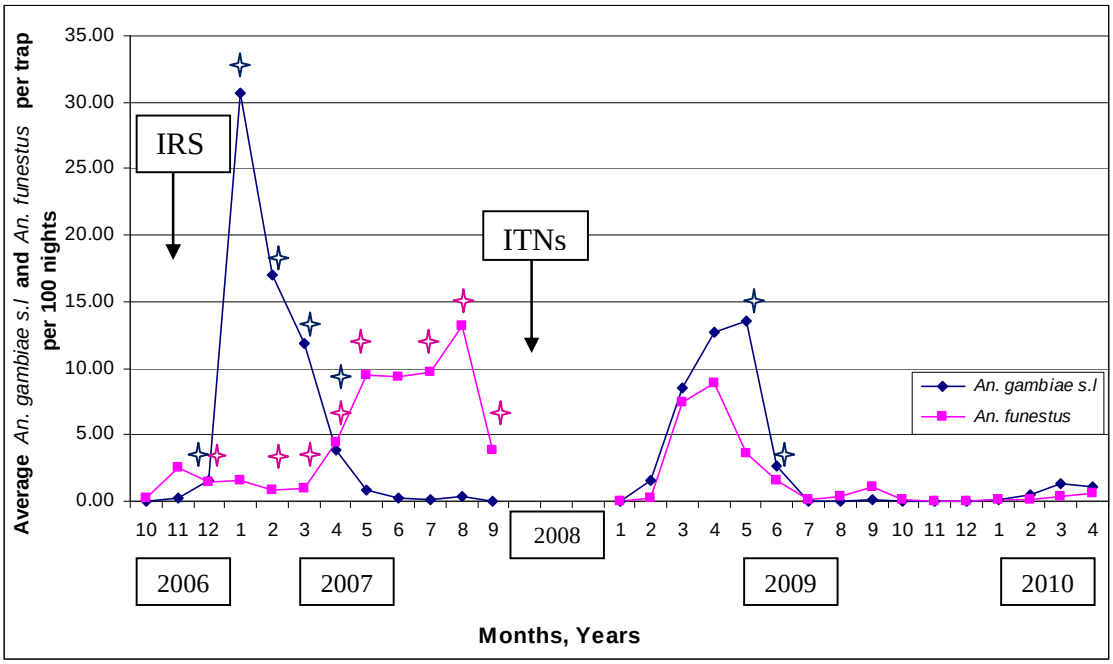
The abundance of *An. gambiae s.l.* and *An. funestus* populations per month in the first period of window exit trap surveys from 2006 to 2007 and the second period from 2009 to 2010 showed a reduction trend in all sentinels sites for each intervention (Figure 3.6).

In general, the abundance of *Anopheles* mosquitoes appeared to be dependent on rainfall, increasing with the rains in late October and early November, reaching a peak around January to March (rainy season) in the case of the *An. gambiae* complex. A second peak occurs in May to July when vegetation is available in the breeding places (beginning of dry season) where the peak is predominantly for the *An. funestus* group. While *An. gambiae* populations decrease with the end of the rains, *An. funestus* populations decrease later in the dry season.

Both groups of *Anopheles* species collected in study area showed an increase in their population density with the start of the rainy seasons in 2006 and 2007 (Figures 3.7 and 3.8). However, *An. gambiae s.l.* numbers increased first followed by the *An. funestus* populations. *An. gambiae s.l.* was the first species to reach its peak between January and March, 2007 in the rainy season, and secondly in August in the beginning of dry season of the same year was *An. funestus* (Figures 3.7 and 3.8).

In the IRS sentinel sites after the second (2007) and third (2008) spray rounds, *An. funestus* and *An. gambiae s.l.* populations had a peak of 10 and 15 mosquitoes per window trap per 100 nights in April 2009 and May 2010 respectively (end of rainy season), but after the fourth spray round in 2009 very low numbers of *An. gambiae s.l.* and *An. funestus* were found, ranging from 1.18 to 1.3 and 0.4 to 0.7 per window trap per 100 nights respectively (Figure 3.7).

Prior to ITN distribution in 2007, the abundance of *An. gambiae s.l.* and *An. funestus* was 52.2 and 88.6 per window trap per 100 nights respectively. This number was reduced dramatically to 2.7 in 2009 and 1.6 in 2010 for *An. gambiae s.l.* and zero for *An. funestus* for both 2009 and 2010 (Figure 3.8). The changes for both anopheline species pre- and post-intervention were statistically significant ($P < 0.001$) and this suggests an instant impact on mosquito population densities.



Month in which *An. gambiae s.l.* sporozoite were detected

Month in which *An. funestus* sporozoite were detected

Figure 3.6. Average number of *An. gambiae s.l.* and *An. funestus* per window trap per night at 17 IRS and 2 ITN sites and months with sporozoite detection, Zambézia October 2006 –April 2010.

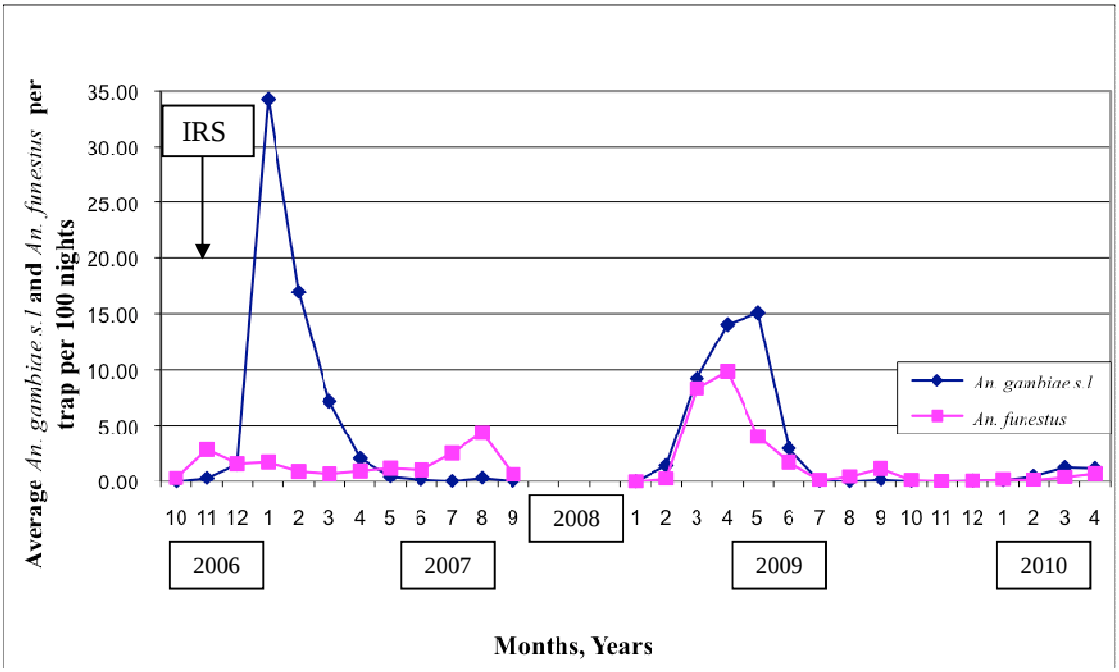


Figure 3.7. Average number of *An. gambiae s.l.* and *An. funestus* per window trap per night at 17 IRS sites, Zambézia, October 2006 –April 2010.

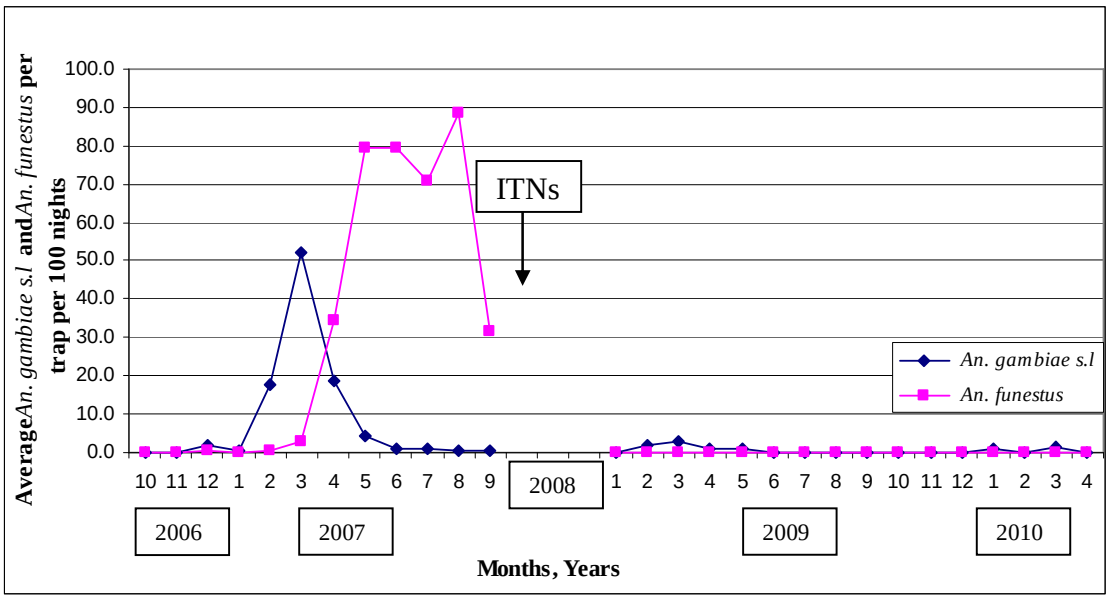


Figure 3.8. Average number of *An. gambiae s.l.* and *An. funestus* per window trap per night at 2 ITN sites, Zambézia, October 2006 –April 2010.

3.2.3. Sporozoite rates

In the period of October, 2006 to September, 2007 sporozoite rates were 4.1 % and 2.3 % for *An. gambiae s.s.* and *An. funestus* respectively. After four rounds of IRS this was reduced to 1 % for *An. gambiae s.s.* and to no infective *An. funestus* mosquitoes in the January, 2009 to April, 2010 period (Figure 3.6; Table 3.3). No infective *An. arabiensis* were identified during this study.

In the rainy season the sporozoite rates were 3.1 % and 1.9 % for *An. gambiae s.s.* and *An. funestus* respectively in 2007. In the subsequent rainy seasons in 2009 and 2010, no infective mosquitoes of either species were detected (Figure 3.6; Table 3.4).

3.2.4. Transmission index

The transmission index is defined as anopheline infective density, or average number of female *Anopheles* found with sporozoites in the salivary glands per room per day [Christopheles 1949 cited by (Garret-Jones and Shidrawi 1969)]. In this case the transmission index is calculated per window trap per 100 nights.

The transmission indices in 2006 to 2007 were 0.049 and 0.036 for *An. gambiae s.s.* and *An. funestus* respectively and these were reduced to 0.0062 for *An. gambiae s.s.* in 2009 to 2010. The relative transmission index in 2006 and 2007 was 0.13 for *An. gambiae s.s.* and after three spray rounds no infected *An. funestus* s.s were identified, so no transmission index could be calculated (Table 3.3).

In the rainy season the transmission index was 0.073 and 0.022 for *An. gambiae s.s.* and *An. funestus* respectively for 2007. No sporozoite rates were calculated for 2009 and 2010, consequently no transmission index could be calculated (Table 3.4).

Table 3.3. Abundance, sporozoite rate and transmission index of specimens collected during two years at all sentinel sites in Zambézia province, 2006-2010.

	Period 1	Period 2
	2006 to 2007	2009 to 2010
An. gambiae s.l		
No Caught	2304	1465
No analyzed for species identification	456	449
Proportion of *An. gambiae s.s* (%)	91	63
Proportion of *An. arabiensis* (%)	5	22
An. arabiensis		
No estimated	115	322
No per trap per 100 nights	0.058	0.2
Sporozoite rate (%)	0 (n=24)	0 (n=98)
Transmission index	0	0
Transmission index relative to baseline	1	0
An. gambiae s.s		
No estimated	2097	923
No per trap per 100 nights	0.997	0.59
Sporozoite rate (%)	4.096 (n=415)	1.053 (n=285)
Transmission index	0.0486	0.0062
Transmission index relative to baseline	1	0.13
An. funestus s.s		
No Caught	1997	856
No per trap per 100 nights	1.59	0.19
Sporozoite rate (%)	2.272 (n=660)	0 (n=272)
Transmission index	0.036	0
Transmission index relative to baseline	1	0

Table 3.4. Abundance, sporozoite rate and transmission index of specimens collected during period January to April for each of three years from 2007, 2009 and 2010 at all sentinel sites in Zambézia province.

	Post spray1	Post spray3	Post spray4
	2007	2009	2010
An. gambiae s.l			
No Caught	2188	783	102
No analyzed for species identification	348	230	100
Proportion *An. gambiae s.s* (%)	92	76	8
Proportion *An. arabiensis* (%)	6	10	74
An. arabiensis			
No estimated	131	78	75
No per trap per 100 nights	0.154	0.161	0.541
Sporozoite rate (%)	0 (n=21)	0 (n=22)	0 (n=74)
Transmission index	0	0	0
Transmission index relative to baseline	1	0	0
An. gambiae s.s			
No estimated	2013	595	8
No per trap per 100 nights	2.3339	1.2719	0.0585
Sporozoite rate (%)	3.125 (n=320)	0 (n=174)	0 (n=8)
Transmission index	0.073	0	0
Transmission index relative to baseline	1	0	0
An. funestus s.s			
No Caught	260	572	44
No per trap per 100 nights	1.17	0.205	0.117
Sporozoite rate (%)	1.875 (n=160)	0 (n=101)	0 (n=40)
Transmission index	0.022	0	0
Transmission index relative to baseline	1	0	0

3.3. Impact of IRS and ITNs on malaria prevalence in human population

A total of 4,864 children from 1 to < 15 year olds were surveyed in 2006 for parasitaemia using the ICTTM diagnosis test. A follow-up survey was carried out in 2007, with 5,314 children from households at the same sentinel sites and a third survey was carried out in 2008 and 5,258 children where included (Table 3.5).

Overall prevalence of infection with *P. falciparum* across all six districts between 2006 and 2008 is given in table 3.5. Prevalence reduced from 50 % in 2006/7 to 32 % in 2008 across the combined age groups.

In the second survey in 2007 all districts and ages with the exception of children from 1 to < 5 years old from Mocuba, Namacurra and including 1 to < 15 for Morrumbala districts showed an increase of prevalence. These changes were statistically significant for Maganja da Costa and Nicoadala districts for the three ages assessed with the exception of children from 1 to < 15 years old from Nicoadala district (Table 3.6). However, in the 2008 survey five of the six districts showed a reduction in prevalence which was statistically significant for all ages (*P* values ranging from < 0.001 to < 0.06) (Figure 3.9, 3.10 and 3.11; Table 3.5).

The highest prevalence in 2006 and in the subsequent survey periods was recorded in Morrumbala district with 78.7 % (95 % CI = 54.9 - 91.8 %) in children from 1 to < 5 years old and 79.3 % (95 % CI = 74.1 - 83.7 %) in children from 5 to < 15 years old respectively. However, in the 2008 survey the highest prevalence was recorded in Maganja da Costa district with 54.4 % (95 % CI = 21.9 - 83.5 %) in children from 1 to < 5 years old (Figure 3.10 and 3.11; Table 3.5).

The greatest decline in prevalence in all surveyed periods and ages was in Quelimane district, with a substantial reduction in age group 1 to < 5 from 34.8 % (95 % CI = 22.39 - 49.64 %) to 4.9 % (95 % CI = 1.18 - 18.09 %) in 2007 and 2008 respectively. The change was statistically significant (*P* < 0.001) (Figure 3.9).

Table 3.5. Prevalence of infection with *P. falciparum* in children 1 to <5, 5 to <15 and 1 to <15 years of age, by districts, observed during household surveys in 2006, 2007 and 2008 on Zambézia province of Mozambique.

Sentinel site	October 2006		October 2007			October 2008		
	Prevalence of infection % (n)	95% Confidence interval	Prevalence of infection % (n)	95% Confidence interval	*P*	Prevalence of infection % (n)	95% Confidence interval	*P*
1 to < 5 years								
Maganja da Costa	31.1 (45)	[18.4-47.5]	76.7 (103)	[61.9-85.7]	<0.001	54.4 (103)	[21.9-83.5]	0.052*
Mocuba	56.8 (183)	[26.5-82.8]	50 (192)	[23.2-76.8]	0.511	48.3 (201)	[21.6-76.0]	0.865
Morrumbala	78.7 (183)	[54.9-91.8]	71.2 (205)	[67.8-74.5]	0.54	32.5 (194)	[19.2-49.3]	<0.001
Namacurra	43.8 (160)	[31.3-57.0]	40.1 (167)	[17.4-68.1]	0.686	16.3 (141)	[9.0-27.7]	0.002*
Nicoadala	38.1 (218)	[33.0-43.4]	45.8 (225)	[31.5-60.8]	0.04*	15 (274)	[8.5-24.9]	<0.001
Quelimane	25 (36)	[12.14-44.56]	34.8 (46)	[22.39-49.64]	0.205	4.9 (41)	[1.18,18.09]	<0.001
All	51.4	[39.26-63.37]	53.9	[42.52-64.97]	0.808	29.6	[19.23,42.51]	0.008*
5 to <15 years								
Maganja da Costa	42.1 (114)	[23.8-62.8]	76.3 (186)	[61.6-86.6]	0.002*	51.4 (177)	[21.2-80.6]	0.028*
Mocuba	45.9 (368)	[20.2-74.0]	58.4 (377)	[34.9-78.6]	0.221	46.5 (357)	[22.7-72.1]	0.245
Morrumbala	76.4 (382)	[48.7-91.7]	79.3 (352)	[74.1-83.7]	0.816	37.9 (335)	[24.0-54.2]	<0.001
Namacurra	46.1 (228)	[31.9-60.9]	51.8 (253)	[34.2-68.9]	0.564	24.8 (278)	[14.6-39.0]	0.002*
Nicoadala	48.9 (438)	[40.0-57.8]	64.4 (452)	[49.7-76.8]	0.14	21.9 (434)	[16.9-27.8]	<0.001
Quelimane	24.7 (77)	[14.0-39.8]	37.4 (99)	[25.0-51.6]	0.107	10.6 (94)	[5.0-21.2]	<0.001
All	52.2	[40.51-63.7]	64.1	[54.6-72.58]	0.27	50.1	[41.28-58.81]	0.19
1 to <15 years								
Maganja da Costa	39 (159)	[22.7-58.2]	76.1 (289)	[61.8-86.3]	<0.001	52.5 (280)	[21.4-81.8]	0.037*
Mocuba	49.5 (551)	[22.3-77.1]	55.5 (569)	[31.0-77.7]	0.558	47.1 (558)	[22.6-73.1]	0.407
Morrumbala	77.2 (565)	[50.7-91.7]	76.3 (557)	[72.3-79.9]	0.944	35.9 (529)	[22.3-52.3]	<0.001
Namacurra	45.1(388)	[33.4-57.4]	47.1 (420)	[26.8-68.5]	0.836	22 (419)	[13.0-34.6]	0.003*
Nicoadala	45.3 (656)	[39.3-51.3]	58.2 (677)	[44.0-71.2]	0.205	19.2 (708)	[14.3-25.3]	<0.001
Quelimane	24.8 (113)	[15.34-37.46]	36.6 (145)	[25.72-48.94]	0.132	8.9 (135)	[4.58-16.55]	<0.001
All	52.3	[40.62-63.66]	60.4	[50.54-69.56]	0.445	32	[22.52-43.13]	0.003*

* Change since 2006 was statistically significant.

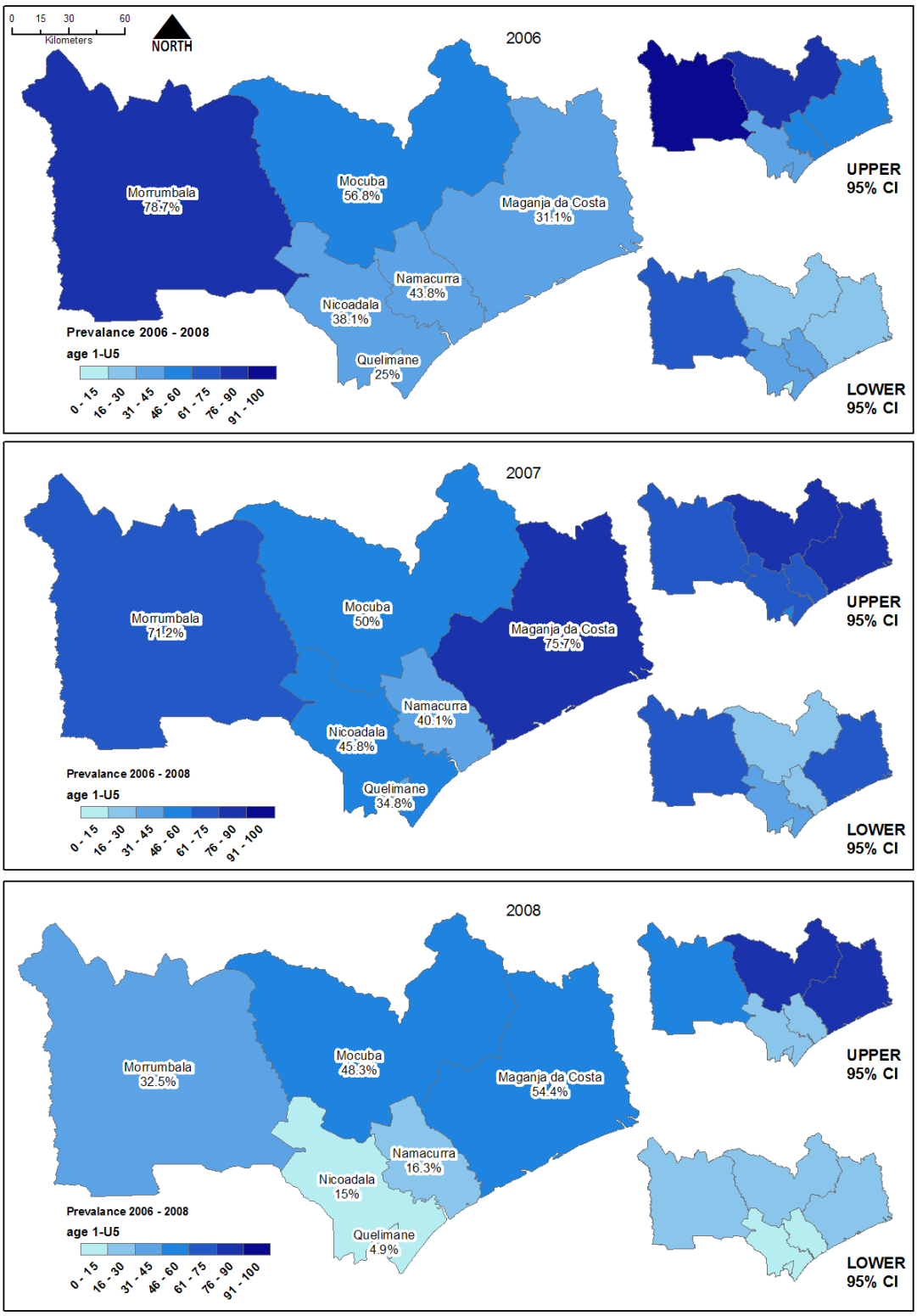


Figure 3.9. Estimated prevalence (%) *Plasmodium falciparum* in children 1 to < 5 years of age, by surveillance area and survey year. CI = Confidence Interval.

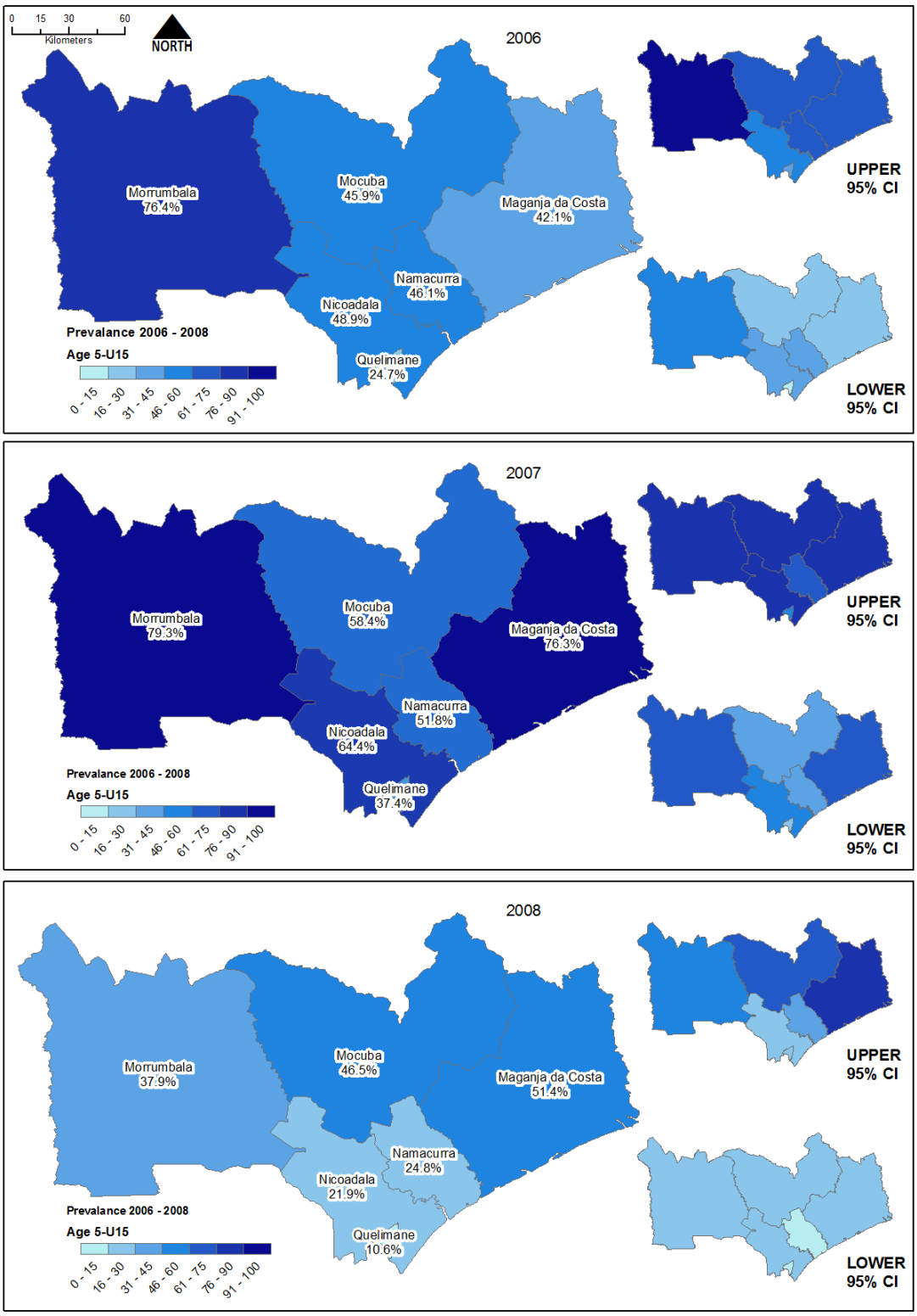


Figure 3.10. Estimated prevalence (%) *Plasmodium falciparum* in children 5 to < 15 years of age, by surveillance area and survey year. CI = Confidence Interval.

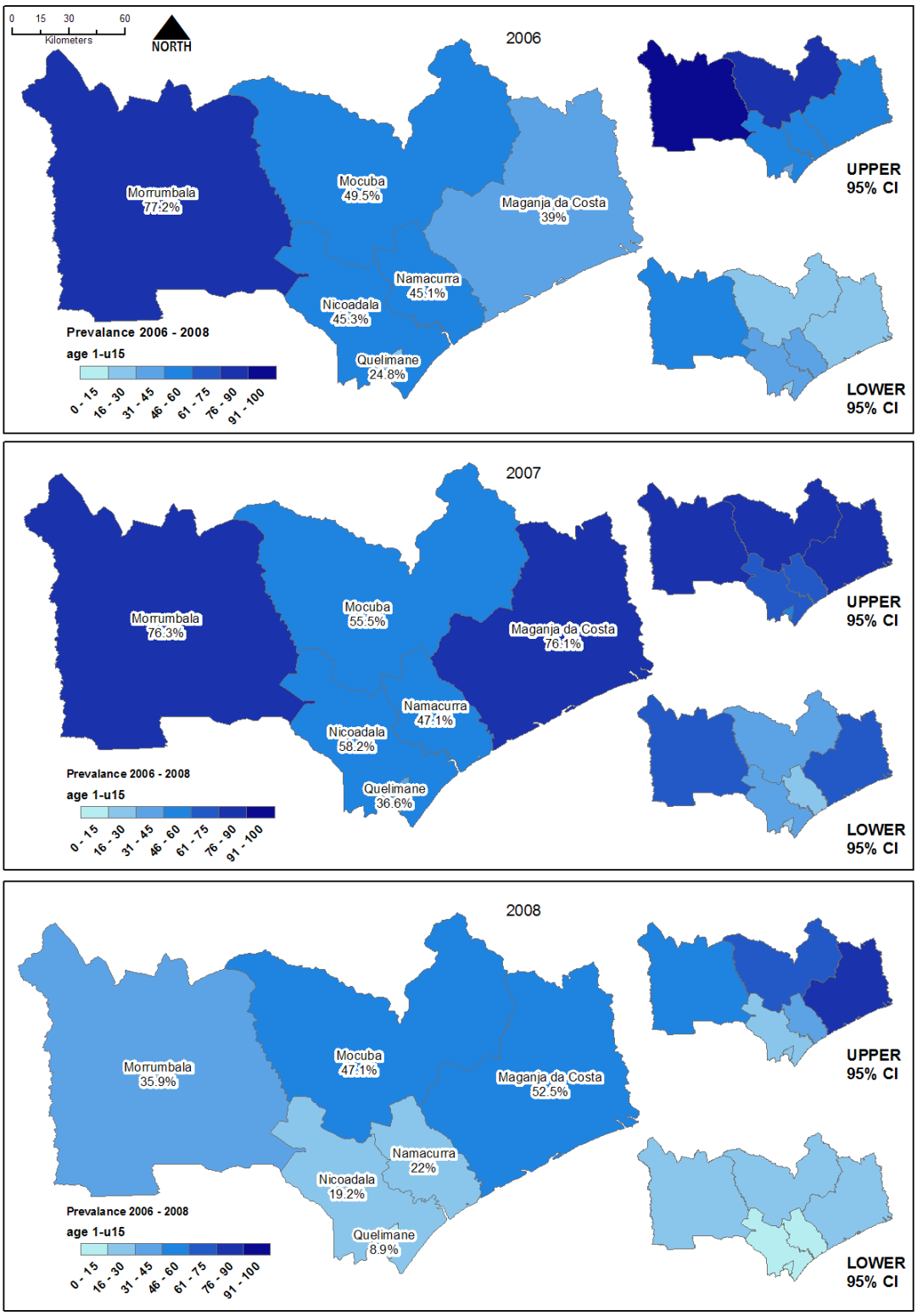


Figure 3.11. Estimated prevalence (%) *Plasmodium falciparum* in children 1 to < 15 years of age, by surveillance area and survey year. CI = Confidence Interval.

CHAPTER 4

DISCUSSION

The success of LSDI in southern Mozambique and the upsurge of malaria in the northern part of the country led to the MOH and the National Malaria Control Programme (NMCP) re-initiating a comprehensive vector control programme in Zambézia province. This project is embedded in this programme to evaluate the interventions against malaria vectors and monitor malaria prevalence as an impact indicator of progress. Prior to 2006 vector control in the province was sporadic and uneven due to limited funding. Control was focused in just three districts and only in the capital villages, where high malaria cases had previously been recorded, namely Mocuba, Morrumbala and Quelimane. ITNs were distributed to pregnant woman and fogging with pyrethroid insecticide was carried out between 1995 and 2005 with a break in 2004 due to no funding. This and other factors, including rising *P. falciparum* resistance to chloroquine, the first line treatment drug (MOH 2006) and flooding (2000-2001), led to a severe increase in mortality and morbidity by malaria in the province (DPSZ 2003, DPSZ 2004, MOH 2006). The high number of cases registered from 2000-2005 was undoubtedly a consequence of the high anthropophilic and endophilic behavior of *An. gambiae s.s.* and *An. funestus*.

In 2006, the first year of IRS scale up with DDT in five districts of the province, *An. gambiae s.s.* and *An. funestus* were shown to be the dominant malaria vector species in Zambézia province, where *An. gambiae s.s.* was the most predominant. This correlates with the previous studies of distribution of anophelines in northern Mozambique (De Meillon 1941, Petrarca *et al.,* 1984). With a tropical climate, warm temperature, heavy rainfall and high humidity, this area is ideal for these malaria vectors (Breman 2001, Coetzee *et al.,* 1999, Hay *et al.,* 2005). *Anopheles arabiensis* was detected in the area but in low abundance.

Mosquito species abundance goes through a natural cycle depending on climatic variables (Gillies and De Meillon 1968, Gillies and Coetzee 1987). Both *An. gambiae s.l.* and *An. funestus* were present throughout the year, but with seasonal variation in abundance. *Anopheles gambiae s.l.* reached the peak around January to May and *An funestus* around April to August. As species cohabit in the same environment (Mbogo *et al.,* 2003), but with different breeding habitats and different seasonal peaks in abundance, the transmission season of malaria is prolonged (Gillies and De Meillon 1968). Generally, *An. gambiae s.l.* is most abundant during the rainy season and *An. funestus* is predominant at the end of the rainy season and beginning of the dry season (Gillies and De Meillon 1968, Coetzee *et al.,* 1999).

Over three rounds of IRS with the insecticide DDT showed a dramatic impact on local vector populations. Following this, very low levels of abundance remained during the fourth round of IRS with the pyrethroid lambda-cyhalothrin. Similar results were found in the south of Mozambique after the IRS program, when the three major vectors including *An. arabiensis* were radically reduced (Casimiro *et al.,* 2006a, Casimiro *et al.,* 2006b, Casimiro *et al.,* 2007, Sharp *et al.,* 2007, Coleman *et al.,* 2008) and also in Bioko Island, Equatorial Guinea (Sharp *et al.,* 2007) when an effective insecticide was applied. In this study, the abundance of *An. arabiensis* increased overall but the total numbers of mosquitoes collected were very low. The possible increase of the density of the *An. arabiensis* population may be coupled with their resting and feeding behavior. Monitoring this increase is important as *An. arabiensis* can be an effective malaria vector (White 1974, Mendis *et al.,* 2000, Tirados *et al.,* 2006, Mahande *et al.,* 2007, Kweka *et al.,* 2009).

The trends in decline of species abundance could be supported by the feeding and behavioral characteristics of the vectors, where *An. gambiae s.s.* and *An. funestus* females are highly anthropophilic and endophilic making them especially vulnerable to control by IRS. *Anopheles arabiensis* is more exophagic and exophilic and the houses sprayed by DDT promote an excito-repellency effect on this species (Sharp *et al.,* 1993, Crook and Baptista 1993, Mendis *et al.,* 2000).

Anopheles gambiae s.s. and *An. funestus* as vectors of malaria in this area was confirmed by the detection of sporozoites in the head/thorax, classifying them as infectious. *Anopheles gambiae s.s.* had a higher sporozoite rate prevalence than *An. funestus* populations during the time period studied, suggesting that this is the more important vector in the region.

Previous studies of malaria transmission in Mozambique have been limited to the southern part of the country. Comparisons of the present data show similarities in *An. funestus* composition but differences with the *An. gambiae* complex composition. In the south of the country the malaria vectors from this complex found are *An. arabiensis* and *An. merus*, while *An. gambiae s.s.* is found in the northern parts of the country (Petrarca *et al.*, 1984, Thompson *et al.*, 1997, Mendis *et al.*, 2000). This study, clearly show that *An. gambiae s.s.* is an important malaria vector in the central-northern part of the country. The absence of sporozoites in the few *An. arabiensis* specimens caught in window exit traps in this study suggests this species plays only a minor role, if any, in disease transmission in this area but this might be due to sample sizes. Elsewhere in Africa a large proportion of *An. arabiensis* populations feed on cattle and rest outdoors (White *et al.*, 1972, Mendis *et al.*, 2000, Tirados *et al.*, 2006, Mahande *et al.*, 2007, Kweka *et al.*, 2009).

After three rounds of IRS with DDT from 2009 to 2010 the reduction in sporozoite rate of the both *An. gambiae s.s.* and *An. funestus* was significant. This correlates with a marked reduction in prevalence of parasites in the human population. Indoor-based methods of control, such IRS and LLINs with residual insecticides, are highly effective against *An. gambiae s.s.* and *An. funestus*, both of which mainly feed indoors on humans and rest there once fed.

If in Zambézia province any role in malaria transmission is played by *An. arabiensis*, the degree to which this species feeds on non-human hosts and rests outdoors (White *et al.*, 1972, Mendis *et al.*, 2000, Tirados *et al.*, 2006, Mahande *et al.*, 2007, Kweka *et al.*, 2009) could reduce the efficacy of Zambézia control efforts. This also offers

opportunities for other approaches, and future investigation is needed to monitor the dynamics of *An. arabiensis* populations including their behavior related to finding alternative hosts.

In 2010 this project detected resistance to pyrethroid and carbamate insecticides in *An. funestus* populations from Mocuba and Milange districts in Zambézia. Compared with previous data this was a significant increase (Chi Square $P < 0.001$). This comparison was made by aggregating data for different pyrethroids due to the lack of testing the same insecticide at the same locality. This has limitations as resistance to different pyrethroids may vary in the same populations (Casimiro *et al.,* 2006b, Coleman *et al.,* 2006, Coleman *et al.,* 2008). However, as only wild caught mosquitoes had been tested before, there are a number of factors that may have influenced previous results, including insecticide pre-exposure and age which can mask resistance (WHO 1998, Hemingway *et al.,* 1997). The WHO diagnostic dose is set at twice that which kills 100% of a susceptible population, hence, it is feasible that these assays can hide a 2 to 10 fold resistance. All previous tests on *An. gambiae s.s.*, *An. arabiensis* and *An. funestus* were 100% susceptible to the insecticides bendiocarb, DDT, deltamethrin, lambda-cyhalothrin and permethrin prior to 2002 (Casimiro *et al.,* 2006a, Casimiro *et al.,* 2006b Coleman *et al.,* 2008).

The pattern of resistance detected here in *An. funestus* is similar to that found in southern Mozambique, where pyrethroid resistance was attributed to elevated levels of monooxygenase P450 enzymes (Brooke *et al.,* 2001, Casimiro *et al.,* 2006b, Casimiro *et al.,* 2007, Wondji *et al.,* 2010). Monooxygenase enzymes are associated with pyrethroid resistance and can also give cross resistance to carbamate insecticides (Brogdon and McAllister 1998, Hemingway and Ranson 2000).

Following the detection of high pyrethroid and low carbamate resistance in southern Mozambique, a policy change was made to use the carbamate bendiocarb for IRS. Continual monitoring of the programme showed increased resistance to carbamates and biochemical assays of *An. funestus* showed increased levels of altered

acetylcholinesterase (AChE) (Casimiro *et al.,* 2006b, Casimiro *et al.,* 2007, Coleman *et al.,* 2008) which is a major resistance mechanism for this class of insecticides (Brogdon and McAllister 1998, Hemingway and Ranson 2000). This led to another policy change in insecticide in September 2006 to DDT to avoid selection of high levels of carbamate and organophosphate resistance through the insensitive AChE resistance mechanism (Coleman *et al.,* 2008). Recently, in 2009 there has been another policy change back to the pyrethroid, lambda-cyhalothrin in for the whole country.

Unfortunately, it was not possible to carry out the biochemical assays in this study because of the logistics regarding the lack of a structured cold chain to move samples to the nearest laboratory where the biochemical assays could be carried out. New techniques to look at monooxygenase levels through cytochrome P450 molecular analysis (Wondji *et al.,* 2009) will make this work feasible in the future. Along with monitoring P450's, all other potential resistance mechanisms should be monitored in all vector species to allow for an insecticide resistance management programme (Hemingway *et al.*, 1997, Penilla *et al.*, 1998).

The policy change to pyrethroids for IRS in Zambézia was based on data collected prior to 2010 that showed all species susceptible. The policy change was driven by pressure from environmentalists on the Ministry of Health as a result of finding DDT in some of the sellers markets and on some farms in the province (DPSZ 2009, Mendis, personal communication). The effect of spraying with lambda-cyhalothrin was immediately made evident by an increase in the number of malaria cases reported in the health services including the provincial hospital from Zambézia (DPSZ 2010) and *An. funestus* was found resting in the house sprayed with lambda-cyhalothrin (field observations).

The distribution of ITNs or LLIN in Maganja da Costa district had a positive impact on *An. funestus* and *An. gambiae* abundance as populations were reduced in number to the point where zero mosquitoes where being found in exit traps and huts when doing collection for mosquitoes assays.

While ITNs are known to be effective vector control methods (Noor *et al.,* 2009), this dramatic impact is suspicious if compared to others studies (Lengeler 2004, Kleinschmidt *et al.,* 2006, Kleinschmidt *et al.,* 2009). Confounding factors may include excessively low rainfall. Also if exit traps did not collect mosquitoes regularly, compliance by homeowners to check the traps becomes low. Surrounding huts were checked monthly for mosquitoes for insecticide resistance assays and only four *An. gambiae s.l.* were found in Muibi in 2009-10 of which F1 progenies were 100% susceptible to DDT, lambda-cyhalothrin and permethrin.

In the first year of the survey (2006-2007) rainfall was much higher in Maganja da Costa district and rice cultivation was the main crop. In 2008 to 2010 there was no rice cultivation as it was too dry. An alteration in agricultural land use is often closely associated with a change in abundance of a vector-borne disease as breeding sites are added or removed (Lacey and Lacey 1990). Climate analyses have also found positive correlations between rainfall and the abundance of the *An. gambiae s.l.* and *An. funestus* populations (Mbogo *et al.,* 2003, Kelly-Hope *et al.,* 2009). These two factors would account for the dramatic decline of abundance of the vectors observed in this ITN area.

Development of insecticide resistance in insect species of medical importance is a serious problem in controlling disease. More than 90% of all insecticides produced have been used for agricultural purposes (Roberts and Andre 1994). Currently insecticides recommended for public health are also used for agrochemical purposes particularly in the cotton, rice and maize cultivations in Zambézia province; this includes DDT, despite its banned use in agriculture, due to leakage from the malaria control programme. This will have added selection pressure to the emergence of resistance detected here and will continue to facilitate the spread of resistance.

Insecticide resistance in *An. funestus* is likely to have arisen from the indiscriminate and widespread use of pyrethroids in agriculture and not only from public health usage of insecticide. *Anopheles funestus* group larvae prefer well vegetated permanent water bodies (Molina *et al.,* 1993, Mendis *et al.,* 2000) where insecticides might accumulate in

small amounts leading to the development or enhancement of resistance in the larvae (Brooke *et al.*, 2001).

The detection of pyrethroid resistance in this study has severe implications for the malaria control programme in this region. As both the IRS and ITN programmes now rely on this class of insecticides.

Pyrethroid and DDT resistance in *An. gambiae* in Côte d'Ivoire is believed to have evolved from the early use of DDT and later use of pyrethroids in agriculture (Chandre *et al.*, 1999). Extended insecticide use in public health and the agriculture sector may lead to an increase in insecticide resistance in malaria vectors, and this may constitute an obstacle for future success of malaria control programmes. More studies are required to assess the impact of the breeding site environment on resistance in adult mosquito populations.

Prevalence surveys in non-immune persons such as children give a good indication of the reservoir of infection in a population, and thus of transmission potential. Such surveys are relatively simple to conduct, particularly after the advent of rapid diagnostic test devices (Craig *et al.*, 2002). In Zambézia province the parasite prevalence surveys in children from 1 to < 15 years were carried out prior to IRS in 2006 and subsequently in 2007 and 2008.

In the second household prevalence survey in 2007 all districts and ages, with the exception of children from 1 to < 5 years old from Mocuba, Namacurra and including 1 to < 15 for Morrumbala, showed an increase in prevalence compared to the pre IRS survey in 2006. This was statistically significant for Maganja da Costa and Nicoadala districts for all age groups. A small reduction occurred in 1 to < 5 year olds from Mocuba, Namacurra and 1 to < 15 for Morrumbala compared to the baseline. These districts had the highest parasitaemia in the province ranging from 44 % to 79 %. This is consistent with results from Bioko Island where two areas with the highest levels of

infection at baseline, non-metro Malabo and Riaba, had the weakest intervention effect (Kleinschmidt *et al.*, 2006).

There was no decrease in overall prevalence in Maganja da Costa. This is not surprising, as the district only received vector control by ITNs on a large scale in 2008 (DPSZ 2008). However, the overall increase in prevalence following 2006 IRS can in part be explained by excessive flooding in the area, increasing vector abundance. However, the IRS in 2006 was carried out only with DPSZ resources that were not sufficient and coverage was less than 50 % of targeted houses in each district. There was also a lack of resources that led to poor quality supervision of spray teams, reducing the quality of IRS that was completed.

In the 2007 spray season the Presidents Malaria Initiative (PMI) began to support the IRS campaign in Zambézia and IRS coverage increased to > 80 % of targeted houses with good quality supervision. The impact of this is seen in the third annual prevalence survey, conducted in 2008, where a significant impact on prevalence was observed across all age ranges in all IRS districts ($P < 0.05$).

The changes in prevalence correlate well with a reduction in vector abundance and reduced transmission index. At high transmission intensity, prevalence of infection is more or less insensitive to moderate changes in transmission intensity. Only once transmission intensity (as measured by the transmission index) is reduced below a threshold value will there be corresponding reductions in prevalence (Beier *et al.*, 1999). In areas of high prevalence in Zambézia province the reduction in transmission intensity has reduced enough to result in significant reductions in prevalence.

Our results show that children < five years of age are more vulnerable to malaria than children from 5 to <15 age when assessed for parasitaemia, with an increased risk that was 4.4 times higher. This is expected in a hyper-endemic area (Beier *et al.*, 1999).

This corresponds with previous studies carried out in Mozambique, (Saute *et al.,* 2003) where in Manhiça district in the south of the country the prevalence of infection increased with age. In this instance the maximum age of prevalence was seven. A separate survey carried out in 24 districts from Mozambique found that the parasite density increased during the first year of life reaching the peak in children from 12 to 23 months (Mabunda *et al.,* 2008) and with the widely held assumption that the children from 1 to < 5 group were at highest risk (Baird *et al.,* 1998, Snow *et al.,* 1999, WHO 2003, Kleinschmidt *et al.,* 2001). The significantly high prevalence in children less than five years of age (data from Zambézia) indicates that these children may be more at risk than their older counterparts, because of their low immunological development. It is known that, during the first 6 to 12 months of life infants obtain a degree of immunity from the mother. For this reason, children less than 1 year old were excluded from the study. The risk of the infection first increases with age and then decreases when the individual achieves a degree of immunity due to numerous contacts with the parasite (Gilles and Warrell 1993).

The greatest decline in prevalence in all surveyed periods and ages was reached in Quelimane district with a substantial reduction in the age group 1 to < 5 in 2007 and 2008. The change was statistically significant ($P < 0.001$). Quelimane is the capital of the Zambézia province and as such has had some previous degree of continual malaria control. It has been involved on and off in IRS and there has been a good historical usage of ITNs, both purchased and distributed free. Quelimane has developed to the stage that most of the mosquitoes caught are not malaria vectors, but "nuisance" mosquitoes especially *Culex*. Quelimane has a large provincial hospital that has been assisted by Non-Government Organizations (NGOs) to definitively diagnose and treat malaria patients for several years, which may have contributed significantly to the reduction observed.

High spray coverage in an area (80% plus) (WHO 2007) benefits the whole community, including those in unsprayed houses. ITNs are seen as a much more personal protection measure, but with high enough coverage and usage they too can offer a community

effect (Curtis *et al.,* 2003, Kleinschmidt *et al.,* 2009). Work carried out looking at the combination of both ITNs and IRS in Zambézia clearly showed that there was a combined effect if both interventions were used (Kleinschmidt *et al.,* 2009). As well as the need to scale up interventions across the whole of Zambézia province, there is a need in the long term to consider a combination approach to reach much higher levels of sustained control.

This study clearly demonstrates that an effective programme with DDT that was initiated in Zambézia province, had an impact on transmission and parasite prevalence. These results are similar to those achieved in southern Mozambique (Soeiro 1956, Soeiro 1959, Schwalbach and De La Maza 1985, Sharp *et al.,* 2007) and other IRS programmes with DDT in South Africa (Vaughan Williams 2003, Sharp *et al.,* 2007), Madagascar (Romi *et al.,* 2001) and Zambia (Sharp *et al.,* 2002). However, the recent policy change back to pyrethroids is a potential disaster for the control programme in Mozambique.

In South Africa a significant increase in the number of malaria cases was detected in KwaZulu-Natal following the discontinuation of DDT use in 1996 in favour of a pyrethroid. This was largely due to the reintroduction of *An. funestus* from Mozambique into KwaZulu-Natal where they had previously been excluded by DDT IRS (Sharp *et al.,* 1988, Hargreaves *et al.,* 2000, Maharaj *et al.,* 2005, Sharp *et al.,* 2007). A higher than expected number of malaria cases was the first indicator, similar to that seen in Zambézia.

With known pyrethroid resistance in *An. funestus* in Zambézia there is the potential for this vector to increase to the same abundance and with the same transmission rate as that prior to the IRS campaign. A similar result may also be seen in southern Mozambique where *An. funestus* and *An. arabiensis* are pyrethroid resistant (Casimiro *et al.,* 2007, Coleman *et al.,* 2008, Wondji *et al.,* 2010) and a similar profile in vectors and environment is observed (Sharp *et al.,* 2007).

Continual monitoring of the vector and indicators of disease are required in order to detect any failure in the malaria control programme and to take corrective measures. One measure would be to revert back to DDT, a strategy that was successful in South Africa (Hargreaves *et al.,* 2000, Vaughan Williams 2003, Maharaj *et al.,* 2005, Sharp *et al.,* 2007), and combined with effective drug treatment (Barnes *et al.,* 2005) gave a rapid decline in the numbers of malaria cases reported.

The national malaria control programme in Mozambique is based on vector control by IRS and LLINs in combination with early laboratory diagnosis and effective treatment and health education (MOH 2006). Although all component strategies of the programme are important for control of the disease, overall success is dependent on the reduction of transmission brought about by the control of vector mosquitoes. This in turn is dependent on the availability of effective and safe insecticides that can be used in close association with the human population at risk.

The use of an insecticide until resistance becomes a limiting factor is rapidly eroding the number of suitable insecticides for malaria control (Curtis *et al.,* 1993). In some countries, where more resources have become available, malaria control programmes have deployed both IRS and LLINs in the same malaria risk area. The reason for this combined approach is to reduce transmission and hence the malaria burden more rapidly than may be feasible with one method alone; to increase overall coverage of vector control protection, for example when full IRS or LLINs coverage is difficult to sustain (Beier 2008) and to delay insecticide resistance (Hemingway *et al.,* 1997) by using different classes of insecticide for IRS.

In Mozambique a rotational method of spraying, using different insecticides could be an alternative strategy for implementing IRS (Hemingway *et al.,* 1997) or a combination of IRS with non-pyrethroids and LLINs are possible alternatives. The aim of insecticide rotation strategies is to reduce the selection pressure caused by a single insecticide alone to ensure that it can be reused in the future. This could be possible using organophosphates, but the vector resistance status is unknown in Mozambique. It may

not be possible to use pyrethroids and carbamates, as resistance to these insecticides is still increasing in the southern part of the country and *An. funestus* resistance to those compounds has now been detected through this study in central-northern areas of the country.

This work demonstrates the positive effect that a well implemented vector control programme can have on disease transmission. However, it is essential that entomological and epidemiological monitoring continues in not only Zambézia province but all of Mozambique especially with the policy change to an insecticide to which one of the major vectors is highly resistant. As this resistance is to pyrethroids, it is essential that all vector control methods are monitored closely as currently ITNs are reliant on this class of insecticide.

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