MALARIA SITUATION IN NAMIBIA: A STUDY OF VECTOR SPECIES AND EFFECTIVENESS OF THE PAST AND CURRENT CONTROL STRATEGIES IN SELECTED PARTS OF NAMIBIA

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ABSTRACT

The distribution of malaria in Namibia is mainly confined to the northern parts of Namibia. Although the malaria vectors were presumed to be *Anopheles arabiensis*, a member of the *Anopheles gambiae* group of morphologically similar species, no scientific study had been undertaken to identify the species in Namibia. Given the variable behaviour of this species, in an area where the mainstay for vector control has been indoor residual house spraying (IRS) with 75% DDT wettable powder since the 1960's, reassessment of the vector species composition and its resting and biting behaviour are absolutely important. In addition to insecticide house spraying, the malaria control included case management with Chloroquine, as the first line treatment for uncomplicated malaria.

The general objective of the study was to determine the vector species in malaria transmission, seasonal abundance, behaviour of malaria vectors and efficiency of the diagnostic and treatment procedures and the overall malaria control scenario in Namibia.

Two sites were selected for this study based on the demographic, epidemiological and climatic conditions that are believed to represent the various malaria endemic areas in northern Namibia.

Malaria vector species were collected from Kalimbeza village, Katima Mulilo district in northeast of Namibia by means of exit window traps of the Muirhead-Thomson design for a year. In addition, Pyrethrum Spray Catches (PSC) were used to collect mosquitoes in Calueque, southern Angola and northern Namibia. Species identification was by means of Polymerase Chain Reaction (PCR). Thick and thin blood smears and body temperatures were collected from suspected malaria patients in Bukalo and Mahenene health centres in order to determine malaria parasite prevalence and malaria episodes caused by *Plasmodium falciparum*. The therapeutic efficacy study was carried out over 28 days' follow-up period, in line with the World Health Organization (WHO). Retrospective malaria data for inpatient and outpatient were obtained from the Ministry of Health and Social Services. They were analysed and graphically presented on a map.

Analysis of the retrospective malaria statistics in Namibia from 1995 to 2003 showed that, children under the age of five years were more affected by malaria as compared to those above five years. This pattern is similar in all malaria endemic areas because of lack of immunity among the children under the age of five years. Immunity due to malaria is known to build up with age. In non-malaria endemic areas on the other hand, there is usually no significant difference between the different age groups. The highest mean incidence ratio of malaria among the two age groups was observed in Kavango (3.4:1) and Caprivi (3:1) regions. Furthermore, the overall malaria morbidity and mortality rates were exceptionally higher in Kavango regions followed by Omusati and Ohangwena regions. There are two explanations for this finding. Firstly the areas receiving more rains and/or

are in proximity to rivers have more malaria than others. The same is true if the areas border with neighbouring countries where there are no prevention interventions in place as a result of either a damaged health system or general lack of resources. This was the case for the three regions. It is therefore important for neighbouring countries to strengthen border coordination for malaria control and prevention as malaria knows no borders.

The identification of mosquito specimen by means of Polymerase Chain Reaction (PCR) revealed that the most common vector species in northern Namibia is *An. arabiensis* (89%). This was the only member of the *An. gambiae* group of species reported from the present study. Other malaria vector species included *An. funestus,* which accounted for 1% and the remaining 10% comprised of non-malaria vector species. The study further revealed that there are more *An. funestus* (36%) in unsprayed areas of Calueque, in southern Angola than *An. arabiensis* which only accounted for 30%. This confirmed the high endophilic tendency (preference for indoor-resting) of *An. funestus*, which makes it more amenable to vector control interventions such as indoor residual house-spraying and/or insecticide-treated bednets (ITNs).

More *An. arabiensis* (77%) were identified during the wet season in Andara and Kalimbeza villages than during the dry season (13%). To the contrary, *An. funestus* species were more abundant during the dry season (6%) compared to 3% collected during the wet season. A chi-square statistic of 67.3, P = 0.000 with *2 degrees of freedom* (n = 444) indicate that the two

variables (season and species) are not independent. The seasonal distribution of *An*. *arabiensis* therefore coincided with the malaria peak season which is during the wet season. Any vector control intervention must therefore be applied just before the wet season to have an impact on the malaria transmission.

Out of a total of 1294 mosquitoes belonging to *An*. *arabiensis* caught in window traps, only 31% were fully blood fed. These results suggest that some mosquitoes could penetrate into huts but could not rest on walls or roofs of sprayed huts to find sufficient time to land on humans for a blood meal. The possible explanations for this are variable. It could possibly be that the insecticide on the walls (DDT) irritated and/or inhibited feeding of the mosquitoes. A high proportion of unfed female mosquitoes could also be due to lack of insemination during mating before they entered the sprayed huts. Answers to these questions however, were not part of the initial objectives of the present study.

The results of the relationship between fevers and clinical malaria on the other hand, revealed no correlation between fevers and clinical malaria. The correlation coefficient between having fever and having a positive blood smear was 0.10 ($p = 0.71$), indicating that the difference is not statistically significant. Moreover, the Chi-square statistic for reports of fevers and a positive blood smear was also not statistically significant different. For example 72% of the patients who had fever were in fact not having malaria. This means that the statistical test reveals that most patients that were

diagnosed to have fever and hence given malarial treatment did not have clinical malaria on the basis of microscopic examination (clinical malaria).

The current study therefore indicates that health facilities in Namibia, like elsewhere are wasting a lot of meagre resources through over-diagnosis of patients as malaria. The situation is made worse when patients have to be treated with the more expensive Artemisnin-based combination therapies (ACTs) due to chloroquine and supposedly Sulfadoxine Pyrimethamine failures. This finding calls for the need to use microscopic examination to improve malaria diagnosis in health facilities so that only those positive for malaria are treated with antimalarial drugs.

The results of the chloroquine efficacy study demonstrated that Treatment Failure rate was not significantly lower than 25%, which is the cut-off point for detecting resistance. With these results it is clear that chloroquine can no longer be used as a first line drug for the treatment of uncomplicated malaria in Namibia.

Based on the findings of the current study and its implications to improve the vector control and case management interventions employed by the National Vector-borne Disease Control Programme in Namibia, the following recommendations are pertinent:

1. There is a need to further explore the presence of *An. funestus* along the border of Kavango and Angola and that of Caprivi and Zambia. Further investigation is needed to establish whether or not this species

is indigenous. The possibility of introduction from Angola and Zambia cannot be completely ruled out thus calling for closer malaria border coordination activities.

2. The proportion of surviving adult blood fed mosquitoes caught in exit window traps is worrying as they can still transmit malaria even in the presence of insecticide residues. The challenge remains for the National Vector-borne Disease Control Programme to further monitor the proportion of vectors resting in-doors and out-doors and based on the respective findings, be able to respond appropriately.

3. The insecticides currently in use (DDT, deltamethrin and permethrin) by the National Vector-borne Disease Control Programme are effective against the local malaria vectors - *An. arabiensis* and *An. funestus* group. To ascertain their continued use in Namibia would require the strengthening of the control programme current capacity to monitor insecticide resistance in well chosen sentinel sites across the malaria endemic regions of the country.

4. Malaria is highly over-diagnosed. The MoHSS needs to seriously consider introducing diagnostic facilities at peripheral health facilities to minimize over-diagnosis and wastages of drugs - especially as the current trend is to move towards the more expensive artemisinin-based combination therapy (ACT) as first line treatment for uncomplicated malaria.

In non-sprayed villages of southern Angola malaria prevalence is higher than in the neighbouring villages of Namibia where spraying was done. It is important that border coordination is strengthened with neighbouring countries – including the area of vector control.

5. Because of the reduced efficacy of chloroquine, the need to change the treatment policy to combination-based therapies is more imminent than ever before for reasons of efficacy and compliance.

6. Analysis of the retrospective malaria data in the country demonstrated that malaria incidence was highest in Kavango region followed by Omusati, Ohangwena, Oshikoto, Caprivi, Kunene, Otjozondjupa and Oshana respectively. On the other hand, the analysis of mortality data revealed that Kavango, Omusati, Oshikoto, Ohangwena, Oshana and Caprivi regions experience the highest rates in descending order. The data also revealed that the under five-age group in Kavango and Caprivi were three times more frequently affected than the five years and above age groups. This is an indication that malaria is relatively more stable in Kavango and Caprivi regions as compared to other malaria endemic regions.

7. Appraising the role of vector control as one of the key strategies for malaria control in Namibia, it is important that the national capacity to plan, implement, monitor and evaluate the different vector control interventions is strengthened by allocating adequate resources for capacity in entomology and vector control and resources for physical

infrastructures and for operations.

8. With the general trend of most malaria endemic countries to decentralize their health services to the lowest administrative levels, it will be necessary for the MoHSS to determine the administrative level to which vector control services could be devolved while ensuring that the impact of key vector control interventions are not compromised.

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DEDICATION

This work is dedicated to

My late beloved Mother and Dad, Indeed to my late brother,

Shylock David Samunzala Kamwi, who

While in exile fighting against the apartheid colonial rule of then South Africa

Died from malaria

In Affection and Gratitude

DECLARATION

This dissertation is my original work. It has not been submitted for a degree elsewhere. Therefore, no part of this dissertation may be reproduced, stored in any retrieval system, or transmitted in any other form, or by any means (e.g. electronic mechanical, photocopying, recording or otherwise) without my written permission or that of the University of Namibia.

CHAPTER ONE: INTRODUCTION

1.1 General Introduction

Malaria is one of the most important parasitic diseases of man. This human disease is a protozoan infection of red blood cells transmitted by the bite of a blood-feeding female *Anopheline* mosquito. Malaria, or ague as it was commonly known, has been described since antiquity (Cook, 1996). Hippocrates is usually credited with the first clear description amongst occidental writers: in *Epidemics* he distinguished different patterns of fever, and in his *Aphorisms* he describes the regular paroxysms of intermittent fever. In Europe seasonal periodic fevers were particularly common in marshy areas, and were frequently referred to as 'paludial' (Cook, 1996). Malaria was thought by Italian writers to be caused by the offensive vapours emanating from the Tiberian marshes (Bruce-Chwatt, 1988). The word 'malaria' comes from the Italian, and means literally 'bad air'. Indeed the cause of the seasonal periodic fevers was a continuous source of debate until the late nineteenth century (Smith *et al.*, 1988). In 1897 Ronald Ross reported the presence of pigmented bodies in the gut of a certain species of mosquito fed on patients with malaria (Ross, 1897). He speculated that these might represent the parasite stage in the mosquito (he was in fact describing the oocysts) but because of difficulties in obtaining these 'unusual' mosquitoes and his transfer to Culcutta, he was unable to characterise the complete life cycle, i.e. transmission from human to mosquito to human. Ronald Ross identified the dapple-winged or *Anopheles* mosquito as the vector of human malaria.

1.2 Biology and ecology of malaria

There are four generally recognized species of malaria parasites of humans: *Plasmodiu malariae* (Laveran, 1881), *P. vivax* (Grassi and Feletti, 1890), *P. falciparum* (Welch, 1897), *P. ovale* (Stephens, 1922), of which only *P. malariae* may naturally infect non-human primates (Gilles *et al*., 1993).

In their human host, malaria parasites have an asexual intracellular cycle of development called schizogony. The parasites live and multiply, first in the cells of the liver and then in the red blood cells.

The forms of the parasite, which rapture from the red blood cells, infect new red blood cells. Some of these, instead of repeating red blood cells schizogony, develop into gametocytes, which are the asexual forms of the parasite by which it is transmitted to the mosquito to continue its life cycle.

In the mosquito, a sexual extracellular cycle of development called sporogony occurs. In this, male and female gametes are formed, fertilization occurs, and sporozoites are produced which are infective to humans. Transmission occurs when the sporozoites are injected by an infective female *Anopheles* mosquito when it takes a blood meal from humans (Cheesbrough, 1991).

Malaria is endemic in the northern regions of Namibia. For example in the year 2000, there was an average malaria incidence of about 240-cases/1,000 population for the whole country (MoHSS, 2002). The sharp increase in malaria incidence could have potentially severe consequences not only for

public health but also for tourism and economic development in the northern regions of the country. Some 1,090,000 people live in malarious areas in Namibia (Obeid *et al*, 2001; Mabaso *et al.*, 2004). Malaria is the most important cause of outpatient consultations and hospital admissions in Namibia. It is the leading cause of illness and death amongst children under the age of five years, and the third important cause of death among adults after HIV/AIDS and tuberculosis. The mortality rate increased from 30/100,000 in 1998 to 45/100,000 in 2000 respectively (MoHSS, 2002).

Seasonal climatic conditions generate high transmission risk, mainly between January and June in the north-eastern and north-western regions of the country. The main vector is presumed to belong to *Anopheles gambiae group* of which *An. arabiensis* is one of them (White, 1974). Malaria control relies heavily on case management using chloroquine and residual spraying with Dichloro-diphenyl-trichlorethane (DDT) for the past three decades (Kamwi, personal experience). Overall, there has been a general complaint from medical practitioners within health facilities in Namibia that a possibility of chloroquine resistance in the malarious regions exists as many of the patients were not responding to treatment. No studies, however, have been carried out to investigate the efficacy of Chloroquine for malaria treatment in Namibia.

While DDT is recommended by the World Health Organization (WHO) for public health use only and strictly for indoor residual house-spraying (Curtis, 1994; WHO, 1997), the global trend is to ultimately phase out its use by replacing it with appropriate alternatives (UNEP, 2004). Because of the continued use of DDT in Namibia for many years, there was need to

evaluate the efficacy of potential alternative insecticides to DDT for malaria vector control (Sharp, 1993; UNEP, 2004).

Like elsewhere, there is increasing evidence that malaria control measures are becoming less effective (WHO, 2004). This is partly due to the increasing problem of resistance of *P. falciparum* to antimalarial drugs; the development and spread of resistance of the *Anopheles* vectors to insecticides; and also weak health infrastructures (WHO, 2003).

1.3 Literature Review

Malaria remains one of the most serious public health problems in Africa. According to WHO, malaria affects the lives of all people living in the area of Africa defined by the southern fringes of the Sahara Desert in the north, and latitude of about 28^0 in the south (WHO, 1995; 1997; 2000; 2003). Similarly, Brundtland (WHO, 1998) in her inaugural speech stated that malaria was the single largest disease in Africa and a primary cause of poverty, citing that about 3,000 children die from malaria everyday. However, many authors do not agree with this estimated number of deaths of children. For example, Marsh *et al.* (1999) estimated that a million children die of malaria each year in Africa. The same view is held by Ayala *et al.* (1998). Greenwood (1990) and others maintain that *P. falciparum* causes 300 million to 500 million cases of clinical illness and an estimated half a million African children die each year of malaria (Greenwood, 1990; Snow *et al.*, 1994; Salako, 1999; WHO, 2003). According to the American Association for the Advancement of Science (AAAS, 1991), the disease afflicts pregnant women, young children and migratory populations severely because of their low or non-existent immunity to the disease.

It is estimated that malaria causes from 300 to 500 million clinical cases, and 1.5 to 2.7 million deaths world-wide each year, with 80 to 90% of the clinical cases and one million deaths occurring in Africa alone (WHO, 1997; 1998; Phillips, 1997; Teklehaimanot, 1999; WHO, 2001; Obeid *et al* 2001, WHO, 2003). As part of the WHO programme for the accelerated implementation of malaria control in 34 countries of Africa, health facility surveys were carried out in selected districts of these countries to obtain baseline information and to monitor the impact of the interventions. As an example, data from the district hospital of Winneba, in Ghana, showed that in 1997, 46.1% of outpatient consultations (out of a total of 11,460 consultations) were due to clinical malaria (Teklehaimanot, 1999). A report by WHO (1995) on the status of malaria control in the African Region and prospects for intensified support estimated that malaria accounts for about 10% of hospital admissions and 20-30% of outpatient consultations. It further stated that among the cases referred to hospitals with severe malaria, 10-30% of the patients would eventually die. These estimates are in line with those reported by Mnzava and others from Tanzania (Mnzava *et al.*, 1991). As is the case for most African countries (WHO, 1990), malaria is a major cause of hospital attendants and admissions. Roll Back Malaria (WHO, 2000) reports that in Angola, malaria represents 50% of outpatient attendance and 20% of admissions. About 93% of Angola's population lives in malaria endemic areas whilst the remainder lives in areas without malaria or in areas with a very low risk (WHO, 1998; 2000).

Throughout most of Africa south of the Sahara, malaria shows a high endemicity with low epidemic potential (Gilles *et al.*, 1993). WHO (1997) estimated that of all the patients who die in hospitals, 17% to 30% die from

malaria in the African Region. Patients admitted to hospitals in Africa with severe malaria may have up to 40% risk of dying. Meanwhile, Snow *et al.* (1994) stated that the epidemiology of malaria as a potentially fatal disease is poorly understood. There is need to increase understanding of the relationship between transmission of *P. falciparum* malaria and its ensuing morbidity and mortality.

1.3.1 Epidemiological stratification

The highly endemic malaria areas cover most of Africa south of the Sahara and include ecosystems of equatorial tropical forests and savannas (Giles *et al.*1993; WHO, 1992; 1996). In this "area of optimum", the level of transmission of malaria is extremely high, much higher than elsewhere in the world (Stanley *et al.* 1991; WHO, 1996). Transmission is usually perennial often with seasonal fluctuations, rising during the rainy season and soon thereafter. *Plasmodium falciparum* is the most common species found in these areas and is the most pervasive and malignant human parasite (Bruce-Chwatt, 1991; Cheesebrough, 1991; Knell, 1991; Gilles *et al*., 1993). Only a few countries in the Region (Lesotho, the Seychelles, St Helena, the southern part of South Africa and mountainous areas with an altitude above 2000m in Ethiopia, Kenya, Tanzania, Burundi and Rwanda) are naturally free from malaria (Beales *et al*. 1991; Demas, 1993; WHO, 1996). According to Lindsay *et al.* (1998), it is considered that those areas higher than 1500 m have little or no malaria for reasons that the environment is not conducive for breeding of malaria vector species.

In Botswana, Swaziland, South Africa and Zimbabwe, transmission is seasonal and endemicity ranges from hypo- to meso-endemic levels in only relatively small areas in each country (Demas, 1993). In Namibia, malaria is endemic in the northern part where 60% of the total population is at risk of the disease due to low immunity because of the seasonality of transmission (Teklehaimanot *et al.,* 1990; Service, 1994; Ameneshewa *et al.,* 2000). Morbidity may extend into adult age groups and does vary greatly from year to year. There is the possibility of recurrent epidemics of malaria when meteorological conditions especially rainfall are favourable for transmission (Teklehaimanot *et al.,* 1990; Service, 1993; Renshaw, 1996).

In between the areas of intensive and stable malaria and those in which malaria does not exist, there are transition belts of unstable malaria that are characterised by seasonal or irregular interruptions of transmission (sometimes for several years). Desert and highland fringes and low mountains serve as good examples (Lindsay and Martens, 1998). Marked seasonality and quasi-cyclic occurrence of heavy rains lead occasionally to epidemics or serious exacerbations of endemicity, as was the case with Botswana, Namibia, Rwanda, Swaziland, Zambia and Zimbabwe during 1996 and Mozambique during 2000 (Sharp & le Sueur, 1996; Sharp *et al*., 2002).

1.3.2 Malaria epidemics in southern Africa and associated factors

For many of the world's poorest countries, and in particular those of sub-Saharan Africa, epidemic malaria remains a major threat, placing all too heavy a burden on overstretched health services, and posing a major constraint to economic development (OAU, 1997). Although, there is no single and universal definition of an epidemic, Molineaux (1988) defined it as the incidence of new cases clearly in excess of the expected. In a recent epidemic response plan document for Namibia, a malaria epidemic was defined as an increase in the monthly number of malaria cases to a level that exceeds the mean plus two standard deviations of the mean monthly cases reported for at least the preceding three years (Renshaw, 1996). This definition has extensively been used in Thailand (Cullen *et al*., 1984). Connor *et al.* (1999) on the other hand, defined malaria epidemic as "an increase in the disease beyond that normally experienced".

Epidemics may occur as a result of a breakdown of control in areas protected for a long time (Najera, 1999). For example, in Madagascar, cessation of malaria control programmes resulted in the deadly epidemic of 1987-88 (Hargreves *et al.,* 2000, Sharp *et al.,* 2000). The same situation was observed in Swaziland in 1984-85 (Mouchet *et al.,* 1998; Sharp, *et al.,* 2000). This hypothesis agrees with the findings of Teklehaimanot *et al.* (1990), who assessed the cause of the malaria outbreak of 1990 in north-west Namibia. He associated this epidemic with a breakdown of vector control measures just before independence. In the 1950s vector control programmes in Madagascar led to the eradication of *Anopheles funestus* – a highly anthropophilic/endophilic vector in the central highland plateau and

that malaria was almost eradicated (Lepers *et al.*, 1988). Since then there has been a progressive increase in malaria transmission due to the collapse of the spraying programme (Fontanel *et al.*, 1990). Similarly, towns in the highlands of Zambia where malaria was once rare, experienced a substantial resurgence as a result of the cessation of vector control activities (Fisher, 1985). A similar situation repeated itself in Katima Mulilo and Andara in Mukwe Constituency during 1996 when the town was not sprayed for the preceding three years. This was also observed during 1990 in the four regions of Oshana, Ohangwena, Omusati and Oshikoto for reason that there had been some interruption of residual insecticidal spraying during 1999 (Kamwi, personal experience).

An epidemic may also occur as a result of a single event, which exposes susceptible populations to high malaria transmission, such as:

- displacement of a susceptible population into an endemic area,
- invasion of a hypo- or meso-endemic area by a highly efficient vector, and
- introduction of malaria, particularly *P. falciparum*, in receptive areas from where it had been absent (Najera, 1999).

According to Connor *et al.* (1999) and Najera (1999), most malaria epidemics, nevertheless, tend to recur in particular epidemic prone areas, which lie in a broken irregular fringe or along the limits of distribution of malaria endemicity, whether the limiting factors are temperature (latitude or altitude) or relative humidity (deserts). Meteorological variables such as rainfall, temperature and humidity influence patterns of malaria

transmission. The catastrophic malaria epidemic in Ethiopia in 1958; for example, was associated with unusually high rainfall over an extended period as well as with elevated temperatures and relative humidity (Lindsay and Martens, 1998). Similarly, the 1940 outbreak in Nairobi, Kenya, resulted from heavy rains, which followed 2 years of low rainfall (Lindsay *et al.,* 1998).

1.3.3 Malaria vectors and behaviour

The primary vectors of malaria in Africa are mosquitoes of the *Anopheles gambiae* complex (Black 1V *et al.,* 1996). According to White (1974), *An. gambiae* group of species are present throughout tropical Africa and its offshore islands (White, 1974, Mnzava and Kilama, 1986). Meanwhile, in southern Africa four members of the *An. gambiae* group of species occur: *An. gambiae, An. arabiaensis*, *An. merus* and *An. quadriannulatus* (Shelley, 1973; Gillies, 1987; le Sueur and Sharp, 1988, Sharp *et al*., 1990; Sharp and le Sueur, 1991; Coetzee, 1993). These species are morphologically very similar but with different behaviours and can also be distinguished by means of Polymerase Chain Reaction (PCR) method (Scot *et al*., 1993).

In a recent study carried out in the Jimma area of Ethiopia with the objective of defining the relationship between a southern African population of *An. quadriannulatus* and that in Ethiopia revealed a different vector species designated *An. quadriannulatus* species B (Hunt, 1998).

White (1974), Service (1986, 1993) and many others reported that *An. gambiae sensu stricto (s.s)* (of the gambiae complex) was probably Africa's most efficient malaria vector. Meanwhile, Gilles and Coetzee (1987) summed it up that the overall vector situation in tropical Africa is dominated by *An. gambiae, An. arabiensis* and *An. funestus*. In addition, in areas where these species occur in large numbers, *An. melas, An. merus, An. bwambae, An. moucheti* and man-biting populations of *An. nili* are other vector species. In a study carried out by Touré *et al*., (1994) in West Africa, also found *An. gambiae* to be Africa's major malaria vector. In the study, the close association of *An. gambiae* to man was indicated not only by its specific man-biting and indoor resting behaviour, but also by its peculiar heliophilic larval breeding habits in bare-edged, temporary water pools which are generally man-made.

Among the six sibling species of the *An. gambiae* group, the nominal taxon (*An. gambiae)* is characterised by specific man-biting and indoor resting habits which account for the highest vector capacity for human malaria parasites (Touré, *et al*., 1994). Vector capacity is defined as the rate at which a particular infection is transmitted from one person to another (Diuk-Wasser *et al.*, 2005). These bionomics have been used to support the hypothesis of a man-dependent speciation process which involves, in West Africa, various incipient species chromosomally recognised by different combinations of paracentric inversions (Coluzzi, *et al.,* 1985). Within this process, the Mopti chromosomal form appears to be one of the most recent evolutionary steps in view of its association with dry season irrigation (Touré, *et al.,* 1983, 1987; Petrarca *et al.,* 1986; Robert *et al*., 1989; Akogbeto *et al.,* 1992). In the same study, along the Mali transect, *An.*

gambiae Mopti was sympatric with *An. arabiensis. Anopheles gambiae* posses both endophilic (rest inside houses during the time required for blood digestion and maturation of the ovaries) and anthropophilic (man-biting) behaviour pattern. In contrast, *An. arabiensis* is both an anthropophilic, zoophilic and endophilic (feeds on man, animals and rests indoors, respectively), and exophilic (rest outdoors) vector. Meanwhile, *An. quadriannulatus* feeds on cattle and is not known to transmit pathogens to man. These three species spend the aquatic stages of their life cycle in temporary pools of fresh water (White, 1974). These different behaviours are important in terms of the vectors' ability to transmit malaria and their subsequent control by various interventions (Diuk-Wasser *et al.*, 2005).

The situation in Namibia is presumably similar to the rest of southern Africa. However, there is limited literature to support this hypothesis. A survey by De Meillon (1950), before the introduction of vector control by DDT in 1965, showed that *An. gambiae* occurred widely throughout Namibia but was most common in the northern regions. However, none of these *An. gambiae s.l.* species were identified to species level, as accurate and reliable identification techniques were not available then. However, subsequent identifications by the enzyme electrophoresis (Miles, 1979) and by the chromosome analysis techniques (Hunt, 1973) have revealed the presence of *An. arabiensis* as the predominant member of the *An. gambiae* group followed by *An. gambiae* and *An. quadriannulatus* (Coetzee, 1989 and Green, 1992). *Anopheles arabiensis* is said to be predominant or is the exclusive species in dry and semi-arid areas (White, 1974; Mnzava and Kilama, 1986). This statement ties in well with the findings of La Grange (1988) who, using electrophoresis identified 629 *An. arabiensis*, 12 *An.*

gambiae and 3 *An. quadriannulatus* from a total number of 644 specimens from Namibia. *Anopheles arabiensis* breeds in a variety of habitats including rain puddles and iishana (large bodies of water that collect after the rains). De Meillon also found that *An. funestus*, another major group of malaria vectors in Africa (Coluzzi, 1984), was common near the Kavango River (De Meillon, 1951).

1.3.4 Geographical distribution of *Anopheles gambiae sensu lato (s.l.)* in Africa

Out of about 400 species of *Anopheles* mosquitoes throughout the world, about 60 species are known to be vectors of malaria under natural conditions (Bruce-Chwatt, 1985). Of these, some 30 species are of major public health importance.

Anopheles gambiae is widespread in nearly all African countries south of the Sahara and is probably the world's most efficient malaria vector (Gillies and De Meillon, 1968; White, 1974, Service, 1986, 1993). Larvae occur mainly in temporary habitats such as pools, puddles, hoof prints and borrow pits, but also in rice fields. *Anopheles arabiensis* is also widespread in most African countries but seems to prefer rather drier savanna areas (White, 1974; Service, 1986; Mnzava and Kilama, 1986; Coetzee *et al.*, 1993; Touré, 1994; Sharp, 1991; Coetzee *et al.*, 1993). The breeding habitats of Anopheles arabiensis are the same as those of *An. gambiae.* *Anopheles quadriannulatus* occurs in Ethiopia, Zanzibar and southern Africa (White, 1974; 1980; Sharp *et al.*, 1984; Gillies and Coetzee, 1987; Service 1986, 1993). However, Mnzava and Kilama (1986) found no specimen of *An.*

quadriannulatus in Zanzibar Island, despite using outdoor calf-baited traps. Hunt *et al.* (1998) found that the Ethiopian population is a different species and designated it as *An. quadriannulatus* B. *Anopheles quadriannulatus* feeds on cattle and is not known to transmit pathogens to man (Coetzee *et al.*, 1993). *Anopheles bwambae* is known from mineral springs in the Semliki forest of Uganda (Service, 1986; Gillies and Coetzee, 1987). It is a rare species and is not considered an important malaria vector, though it can transmit malaria within its very restricted range (Service, 1986, 1993). *Anopheles melas* is a salt-water breeding species and occurs along the coast of west Africa to Congo (White, 1974; Service, 1986; Gillies and Coetzee, 1987). It is common in lagoons and mangrove swamps and does not breed in fresh water. *Anopheles merus* is the east African equivalent of *An. melas*, it breeds in salt-water lagoons and swamps along the coast of east Africa, although some studies have shown that *merus* occurs inland (Mosha and Mutero, 1982). *Anopheles merus* has been implicated as a vector of malaria in Tanzania and Kenya (Bushrod, 1981; Mosha and Petrarca 1983). *Anopheles funestus* is widespread in Africa south of the Sahara (Service, 1986; Gillies and Coetzee, 1987). According to the literature, *An. funestus* is considered as the most important vector after *An. gambiae* and *An. arabiensis* (White, 1974; Service, 1993; Gillies and Coetzee, 1987), others feel that it's role may be underestimated (Coluzzi, 1984; Mnzava *et al.*, 1993). It prefers more or less permanent waters, especially with vegetation, such as swamps, marshes, edges of streams, rivers and ditches. It also prefers shaded habitats.

1.4 Malaria control efforts in Africa

Malaria is a complex disease that, even under the most optimistic scenario, will continue to be a major public health threat for decades in Africa and one of the major causes of ill health and death (Beales *et al.*, 1989; Stanley *et al.*, 1991; WHO, 1996; WHO, 2003; Malaney *et al.*, 2004). During the 1940s and early 1950s, malaria control activities in Africa were very limited in terms of health programmes and interventions, except for very specific settings, such as selected urban centres or sites of economic importance, primarily related to settlements of expatriate populations (Teklehaimanot *et al.*, 1999). During the malaria eradication period, Africa hosted several pilot pre-eradication projects, but was never included in the Global Malaria Eradication Programme, in spite of the burden of the disease on the continent. There were several reasons why this could not be implemented including the general underdevelopment of health services, communication and infrastructure. The limited participation of African countries in the eradication efforts had long-term negative impacts in terms of human resources and capacity building for malaria control in the continent (Gramiccia and Beales, 1988). Although, elimination of malaria is a formidable task in most parts of the sub-Sahara Africa, control of malaria is feasible.

The "WHO Inter-regional Conference on Malaria Control in countries where time-limited eradication is impractical at present", Brazzaville, (1972), reached the conclusion that insecticides remain "the most effective weapon in the hands of malariologists for controlling rural malaria" and recommended that intra-domiciliary insecticide spraying "should be

promoted in situations where this measure is considered by experts to be the method of choice" (WHO, 1972). These are situations where the vectors rest long enough on sprayed surfaces before and after taking a blood meal to pick up lethal doses of the insecticide (Curtis and Mnzava, 2000). Moreover, control programmes are able to organize, deploy and sustain the intervention (WHO, 2005). According to Kouznetsov (1977) and Beales *et al.* (1991), since the introduction of this method, considerable experience has been gained in tropical Africa during the last three decades. Between the two world wars, antilarval measures were the traditional, and almost the only malaria control measures applied on a relatively large scale in Africa. This agrees well with events in South Africa where focal larviciding remained an important tool despite its decrease in use during the early 1930s (le Sueur *et al.*, 1993). However, these operations were restricted to urban areas of political, strategic and economic importance. Some successful attempts in the control of malaria by house spraying with pyrethrum were already underway. This work is said to have been pioneered by Giemsa in 1911 at the military barracks of Dar es Salaam (Clyde, 1967) and that during the following two years, the method was successfully used by Balfour (1913) in cabins and holds of steamers on the Nile River. Systematic application of this method brought down the epidemics of malaria in South Africa (De Meillon, 1936; le Sueur *et al.*, 1993).

Despite the advent of residual insecticide spraying, malaria control in tropical Africa has not been that easy. Early attempts for malaria control started to a large extent, following the recommendations of the First African Malaria Conference, Kampala, 1950 (Molineaux and Gramiccia, 1980).

During this conference, it was recommended that "whatever the original degree of endemicity, malaria should be controlled by modern methods as soon as feasible and without awaiting the outcome of further experiments" (Beales *et al.*, 1991; Kouznetsov, 1977). A number of pilot projects using a variety of control methods, singly or in association, but including indoor spraying with residual insecticides, were undertaken in Africa (Macdonald, 1957; Pampana, 1963; Molineaux and Gramiccia, 1980; Bruce-chwatt, 1984). This included southern Africa where large-scale malaria control programmes using house spraying with DDT and Benzene hexachloride (BHC) were initiated in 1952-1953 in South Africa, Swaziland, Zimbabwe, Madagascar and Mauritius (Kouznetsov, 1977). The control operations yielded good results at the limits of tropical Africa where malaria is seasonal and of the unstable type (South Africa, Swaziland, Zimbabwe, high plateau and south coast of Madagascar). In addition, in the islands (Mauritius and Reunion), malaria transmission was interrupted in large territories and malaria incidence and prevalence were drastically reduced (Molineaux *et al*., 1980, Kouznetsov, 1977; Bruce-chwatt, 1993).

In Cameroon, two rounds of DDT spraying at six-month interval produced a rather moderate reduction of the parasite rate and no interruption of transmission. It was hinted that the resistance of *An. gambiae s.l.* and the *An. funestus* groups to deposits of DDT was responsible for this limited success, and an association of chemoprophylaxis with insecticidal spraying was therefore advocated (Pampana, 1963; Molineaux & Gramiccia, 1980).

In Nigeria, six monthly spraying for four years with DDT and dieldrin produced a significant reduction in indoor resting vectors (Molineaux and Gramiccia, 1980). In Burkina Faso, DDT and dieldrin were sprayed once or twice a year, but inhibition of malaria transmission was not observed (Molineaux *et al.*, 1980, Bruce Chwatt, 1993). According to White (1999), it is far more beneficial to reduce mosquito longevity than to reduce mosquito density, for the purpose of limiting transmission potential. Clearly, the more malaria vectors live longer the more chances of being infected and thus transmit malaria to humans. The first country to revise its malaria control strategy for these reasons was South Africa, where systematic house spraying with pyrethrum was introduced from 1931 onwards (le Sueur, 1993).

Reports of complete failure of residual spraying and drug administration to interrupt transmission of malaria were received from West Africa (Molineaux *et al*., 1980). The failure to interrupt malaria in tropical Africa led to a temporary exclusion of Africa from the Global Malaria Eradication Programme, which started in 1955 (Kouznetsov, 1977, Molineaux *et al*., 1980). However, the results of the first few years of malaria control operations in Africa south of the Sahara were deliberated at length at the Second African Malaria Conference held in Lagos at the end of 1955. It was recognized that in large areas of tropical Africa, malaria was little or not at all responsive to the measures applied. This lack of success was ascribed to the high intensity of transmission, the behaviour of *An. gambiae*, the development of resistance in *An. gambiae* to dieldrin, the characteristics of residual insecticides, high cost of residual insecticides and the effect of absorption on mud surfaces (Kouznetsov, 1977, Molineaux *et al.*, 1980).

Thus, this suggests that residual house spraying can contribute to a significant reduction in the transmission of malaria in areas of lower endemicity than those with stable transmission.

1.5 Current malaria control strategy in Africa

The most widely accepted strategy for malaria control is the Global Malaria Control Strategy (provision of early diagnosis and prompt treatment), which is promoted by WHO (WHO, 1993a; 1993b). A recent review of the implementation strategy was made by the 20th Malaria Expert Committee (WHO, 2000). Provision of early diagnosis and prompt treatment is recognised as the cornerstone of malaria control in sub-Saharan Africa (Bosman *et al.,* 1999). Implementation of vector control (mainly insecticide-treated nets and indoor residual spraying) varies widely in different regions (Curtis and Mnzava, 2000; Goodman *et al.,* 2001), some regions resort to selective vector control, epidemic risk monitoring, detection and forecasting in epidemic prone countries (WHO, 1996; Bosman *et al*., 1999; WHO, 2001). Meanwhile, some countries cannot afford indoor residual spraying and resort to insecticide treated nets as a means of malaria vector control.

With the Declaration of Alma Ata, in 1978, WHO promoted the idea of malaria control in the context of primary health care, but the Global Malaria Control Strategy was translated into strengthening malaria control efforts in Africa (Teklehaimanot *et al.,* 1999).

During the Ministerial Malaria Conference in Amsterdam, The Netherlands, in 1992, the Global Malaria Control Strategy was endorsed by over 92 Ministers of Health from endemic countries and the major international partners (WHO, 1993). It was subsequently endorsed by the World Health Assembly (WHA), the Economic and Social Council of the United Nations, the United Nations General Assembly and by the Organization of African Unity (Teklehaimanot *et al.*, 1999). The four technical elements of this global strategy are:

i. to provide early diagnosis and prompt treatment;
ii. to plan and implement selective and sustainable preventive measures, including vector control;
iii. to detect early, contain or prevent epidemics;
iv. to strengthen local capacities in basic and applied research, to permit and promote the regular assessment of a country's malaria situation, in particular the ecological, social and economic determinants of the disease.

There is no doubt that provision of early diagnosis and treatment can reduce malaria mortality and specific research is not needed to support this. According to Bosman *et al.*, (1999), experiences in malarious areas of sub-Saharan Africa have shown that the presence of a qualified clinician able to provide rapid diagnosis and effective anti-malarial treatment free of charge produces a dramatic reduction in malaria mortality.

In many developing countries, including Namibia, malaria control programmes aimed at reducing *Anopheles* vectors involve the use of indoor residual insecticide spraying. However, these vectors have shown resistance to numerous insecticides in Africa, including DDT, various organo-phosphates, and some carbamates (Kamwi, 1993). By 1986, the WHO reported that some important *Anopheles* species were resistant to one or more insecticides, 49 species had developed resistance to DDT and 10 species to pyrethroids. Organo-phosphate resistance has been reported in 24 species and carbamates resistance in 14 species (WHO, 1992). The WHO Expert Committee on Vector Biology and Control (WHO, 1992) reported that 10 species of *Anopheles* show some degree of pyrethroid resistance, although only one of these cases is documented in the literature (Malcolm, 1988).

In Botswana, Swaziland and Zimbabwe where malaria is limited to certain areas and where the disease is seasonal, unstable and subjected to epidemics, malaria control mainly consists of indoor residual insecticide spraying during the transmission season and the treatment of malaria cases through the general health services (Beausoleil, 1984). This is the current situation in Namibia (Kamwi, *personal experience*). In Namibia intra-domiciliary spraying strategy, using DDT (50% wettable powder) was only introduced in Oshakati in 1965 and Kavango and Caprivi in 1974 (Hansford, 1975). The current recommended dosage of DDT, however, is 75% wettable powder at 2 gm/m^2 (Najera and Zaim, 2002).

Research on development of new insecticidal compounds is expensive and increasingly difficult – especially that the market for public health is small

compared to that in agriculture. The possibility of having, in the immediate future, a vast array of valuable compounds is therefore not very likely. However, the available insecticides, if properly used, can still play a major role in the reduction of malaria transmission. Up to now, most vector control has been attempted through centrally organized insecticidal spray teams, and these have had their successes, for example, in the 1950s and 1960s, in greatly reducing malaria prevalence in almost all parts of Asia by DDT spraying in houses (Curtis, 1991). Whereas most of the efforts were directed to malaria vectors, indirect benefits have been accrued with the control of other vector-borne diseases such as leishmaniasis and others (Bryceson, 1996).

During the 1980s, the commercial availability of pyrethroids, which are highly insecticidal but with low mammalian toxicity, revived interest in bed-nets (Lu Bao Lin, 1991). Trials with bed-nets treated with these insecticides have since been reported from many countries (WHO, 1989). In malaria control programmes, emphasis has been placed on the use of bed-nets impregnated with pyrethroid insecticides (ITNs) for personal protection (WHO, 1983). The effectiveness of bed-nets impregnated with permethrin in the laboratory followed by testing on a village scale has been reported by Lines *et al.*, (1987) and Charlwood and Graves (1987). Similar results of a trial of pyrethroid impregnated bed-nets have been reported from the Gambia (Snow *et al.,* 1988) and in an area of Tanzania holoendemic for malaria (Njunwa *et al.*, 1991).

In recent years, evidence has been accumulated to show that when ITNs are used with high coverage rate under programme conditions, they

significantly reduce malaria-associated morbidity and mortality in children (Alonso *et al.,* 1991; Lengeler *et al.,* 1996; Lengeler, 2004). Based on these findings, the World Health Organization has been promoting the national scaling up of this intervention (Lengeler, 2004). The biggest challenge, however, is on the best strategy to scale up their use while at the same time ensuring equity and access (Stevens *et al.,* 2005). Whereas a combination of social marketing through the private sector and highly subsidized (free distribution) by the public sector is recommended, national efforts for scaling up has not been without problems. Recent experiences, however, have indicated that when the distribution of ITNs by the public sector is linked to other health services such as vaccinations (in Ghana, Togo and Zambia), high coverage and re-treatments rates are achieved (WHO, 2005). With the introduction of Long lasting insecticidal nets (LLINs) to the market, the historical problem of maintaining high re-treatment rates will most likely be taken care of (Guillet, 2004).

1.5.1 House spraying for malaria vector control in South Africa: the past and present

The use of intra-domicilliary spraying of pyrethrum for malaria vector control in southern Africa started in Natal province of South Africa in 1930 (le Sueur *et al.,* 1993). Pyrethroid extract from flowers was used in houses for spraying as a short-term knockdown insecticide (De Meillon, 1936) and was superseded by the more residual organochlorines (DDT and Dieldrin) after the Second World War. Chemical insecticides have been used against *Anopheles* mosquitoes in malaria control programmes for over 60 years, with varying success (Park Ross, 1936; Harrison, 1978). Additional control

measures included the use of focal larviciding, which remained an important tool despite its decreased use (le Sueur, 1993). Since 1946 the *Anopheline* mosquitoes involved in the transmission of malaria have been controlled by the intra-domiciliary application of DDT, which continues to be highly effective (Sharp *et al.,* 1988). The impact of indoor residual spraying was reported by park Ross in 1935 through the reduction in the number of cases annually.

In 1946 pyrethrum was replaced as the insecticide of choice by DDT, which was used for both indoor residual application and larviciding (Smit *et al.,* 1992, Sharp *et al.,* 1988). Complete coverage of indoor residual spraying was reported to have been achieved by 1958 (Sharp & le Sueur, 1996). After the introduction of house spraying in the affected areas, *An. gambiae* and *An. funestus* were eliminated from all the affected provinces of South Africa with the exception of *An. arabiensis* (Sharp *et al.,* 1996). The eliminated species were highly susceptible to indoor house spraying with a residual insecticide, due to their high preference to exclusively bite man and rest indoors before and after a blood meal.

Despite all the successes cited above, DDT did not go unchallenged. According to Bouwman *et al.* (1990), the use of DDT was found to be an emotive issue and its continued use had been the cause of severe criticism by the media and various conservation bodies who regularly expressed concern about the environment and the health of the population protected from malaria. Further, a study done in Kwazulu-Natal revealed that the mean intake of DDT via breast milk by infants may have a detrimental

effect on the development of breast-fed infants (Smit *et al.*, 1992; Bouwman and Schutte, 1993; Bouwman *et al.*, 1994).

On the social practice, Mnzava *et al.* (1998), reported a high frequency of re-plastering (over 48%), in the villages of Kwa-Zulu-Natal irrespective of the insecticide being used which questioned the rationale of the continued use of DDT for house spraying. These problems are not only unique to South Africa. Hansford (1975) reported the same for Caprivi and Kavango Regions of Namibia and (Kamwi, *personal experience*) observed re-plastering in these same regions to this end.

Despite these claims the WHO Study Group on Vector for Malaria and other mosquito-borne diseases was not convinced that these reasons justified the discontinued use of DDT for house spraying (WHO, 1997). Instead WHO (1993, 2001) and Curtis (1994) supported the continued anti-malaria use of DDT where *Anopheles* species remain susceptible. In addition, Onori *et al.* (1993) in his contribution on the rationale and technique of malaria control states that DDT toxicity to humans is very low and there is no evidence that the millions of people whose houses were treated with DDT are at any risk from exposure to it.

In 1996 the South African Malaria Control Programme shifted to pyrethroids, - insecticides regarded as more environmentally-acceptable, not being bioaccumulative, not staining walls and, in some instances, having less excito-repellancy effects than DDT (Hargreaves, *et al.*, 2000, Sharp, *et al*., 2000). In less than 4 years after these changes, *An. funestus* was reported resting indoors in sprayed houses in KwaZulu-Natal and also found to be resistant to synthetic pyrethroids (Hargreaves, *et al.*, 2000).

Similarly, pyrethroid resistance or tolerance in the malaria vector *An. gambiae sensu stricto* has been reported from both west and east Africa (Vulule *et al.,* 1994, 1996, 1999). In northern KwaZulu-Natal Province, malaria increased as a result of reduced efficacy to pyrethroids forcing the control programme to switch back to the use of DDT. Re-introduction of use of DDT in KwaZulu-Natal significantly reduced the incidence of malaria to low levels within one year (Sharp *et al.,* 2002).

1.5.2 A review of malaria control in Namibia

Namibia's land surface covers an area of approximately 824 116km^2 (Figure 1), it is basically a flat and dry country, and can be divided into three geographical areas: True desert – Namib, subtropical northern region and arid lands, which cover most of the country. Namibia is divided into 13 political regions. The population of Namibia currently stands at 1, 830, 330 (Census, 2001).

Namibia is generally not a humid country. Average relative humidity is higher than 80% in the most humid months in northern Namibia, compared with maximums of 50-60% in the south (Mendelsohn *et al.*, 2003).

Namibia stretches about 1,320 km between the northernmost and southernmost points and 350 km in breadth at its narrowest point in the south. It spans a distance of some 1,440 km where it is widest, between the mouth of the Kunene River and Impalila Island, far to the east where the borders of Namibia, Botswana, Zambia and Zimbabwe converge on a single point (Mendelsohn, *et al.,* 2003).

The rainy season is from the months of November to April, with peak rains in February-March. The rest of the year is virtually without rain. Annual precipitation is highest in the north-east Region (Kavango and Caprivi) (348-871mm) and diminishes towards the north-west (321-828mm) and the south (255-710 mm). Caprivi receives the highest average rainfall of more than 600 mm in contrast to the lowest totals of less than 50 mm in the south-west and along the coast (Mendelsohn *et al.*, 2003).

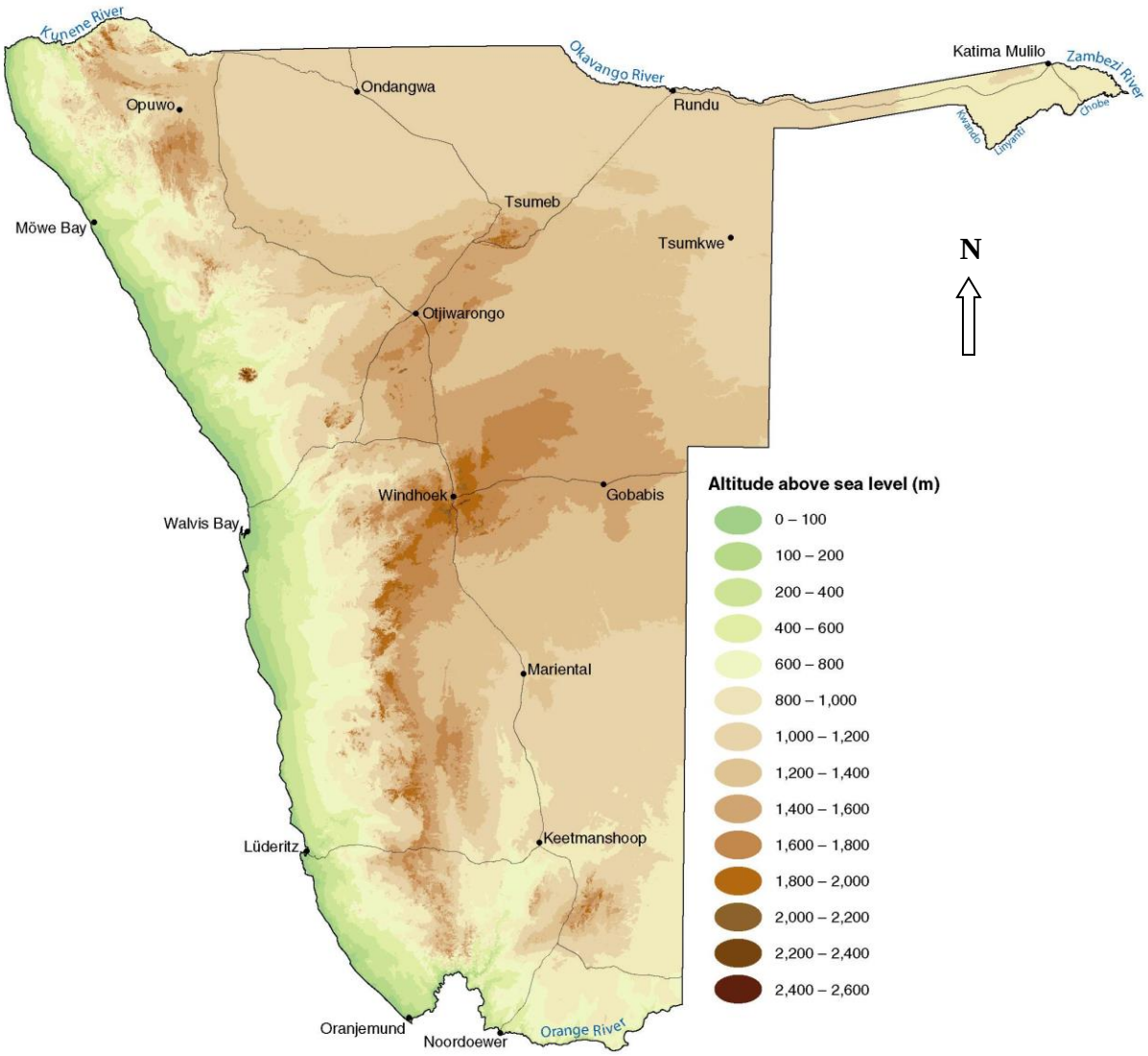


Figure 1: Physical map of Namibia showing main towns and altitude (m) above sea level. (*Source: Mendelsohn et al., 2003*)

The rainfall is extremely variable from year to year, and this has been shown to have a dramatic effect on the malaria epidemiology (Service, 1993). The only perennial rivers are on the country's borders, such as the Orange River in the South, and the Kunene, Kavango, Zambezi and Kwando in the north with Chobe River in the far north-eastern frontier. Over 60% of total population live in the malarious north, an area of some 140 000km^2 comprising of 16.98% of the total surface area of the country (Service, 1993).

Much of the north-eastern region (Caprivi) consists of flood-plains that are inaccessible for about 4-6 months of the year except by boat or helicopter. The Zambezi, Kuando-Linyanti, and Chobe Rivers form oxbow lakes (mulapos), which flood during the rainy season. Not surprisingly, these conditions create malaria control problems. In contrast, in the north-west, the land is very flat and water courses are imperceptible depressions with no well-defined river courses (shallow rain water pools) these are called iishana. Virtually all iishana are formed as a direct result of rainfall.

The distribution of malaria is strongly related to rainfall as is the case in north-western regions of Oshana, Ohangwena, Omusati and Oshikoto where Iishana form habitats for mosquito breeding. Thus, areas in which there is some risk of malaria usually receive more than 300mm of rain per year, while the most risky areas generally have more than 500mm as is the case with Caprivi and Kavango Regions (Mendelsohn *et al*., 2002). There is a clear relationship among the country's topography, the rainfall pattern and the distribution pattern of incidence of malaria. The rainfall data illustrated in (Figure 2 substantiate the malaria data illustrated in Figures 3 and 4).

Temperatures also affect the distribution of malaria, such that most risky areas in Namibia have average temperatures above 20º Celsius. Breeding of mosquitoes cannot take place in temperatures below 20ºC (Service, 1993).

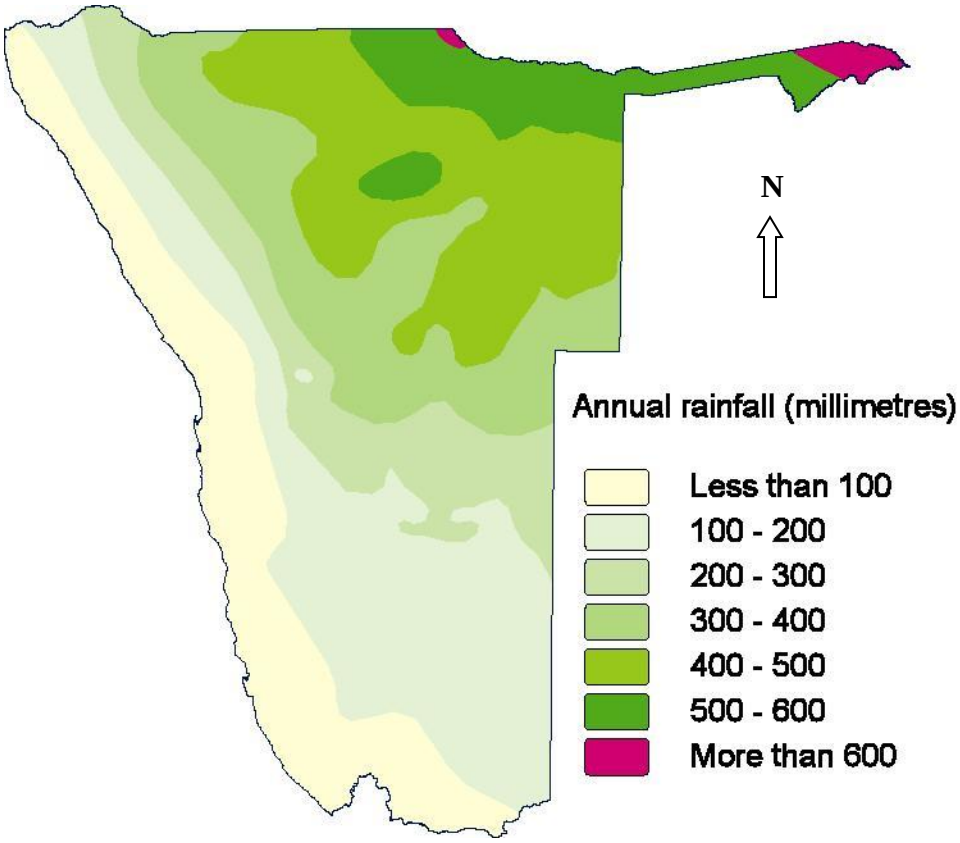


Figure 2: Annual rainfall (millimeters) in Namibia
(*Source:Obeid et al., 2001*)

Rates of Malaria infection peak about two to three months after much of the summer rain has fallen each year. The annual peaks are much clearer and predictable in Katima Mulilo and Rundu where the seasonal rains fall at much the same time every year (Renshaw, 1995; Obeid *et al*., 2001). In the south-central regions around Oshakati, in contrast, the timing of rainfall is

much more variable and seasonal outbreaks of malaria occur earlier in some years and later in others (Obeid *et al*., 2001).

Malaria is endemic in the northern regions of Namibia with an average of 400,000 outpatients, over 30,000 inpatient cases and more than 800 deaths registered annually due to malaria (Obeid *et al.*, 2001). Malaria cases vary from year to year. The highest number of malaria cases ever recorded in the country was 473 326 in 2001 with 1 320 deaths. The incidence of the disease varies from region to region, with a mean number of 255 cases per 1,000 population for the whole country in the years between 1995 and 2001. The distribution of malaria cases in Namibia during 2000 and 2003 is illustrated in Figure 3 and Figure 4 respectively.

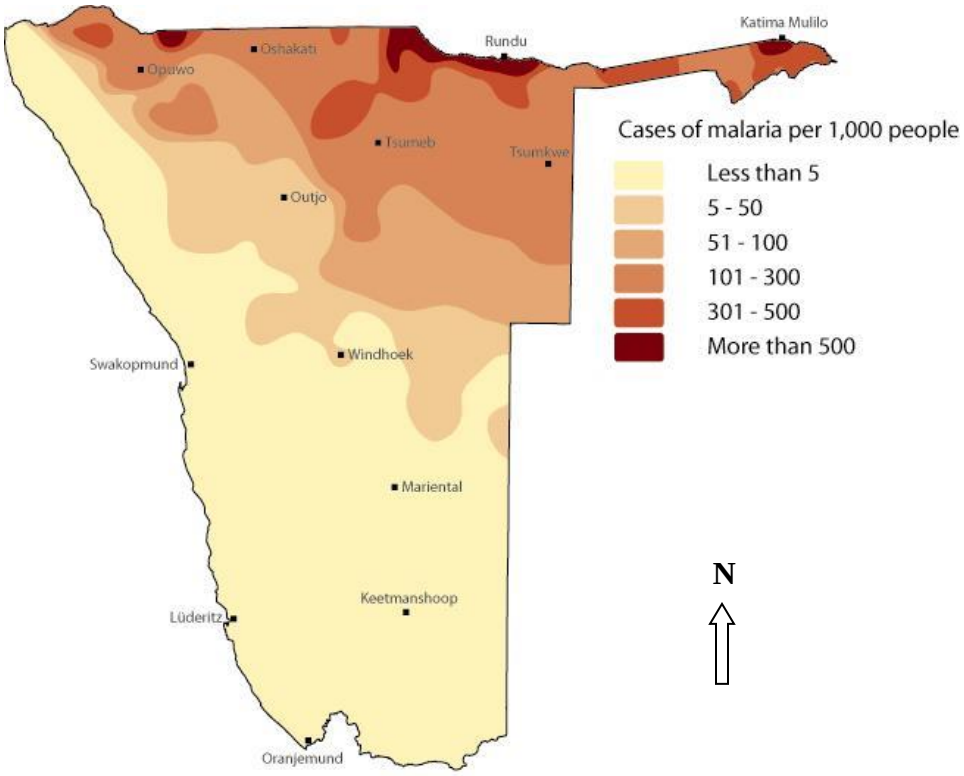


Figure 3: The incidence of malaria (number of new cases per 1,000 people) in Namibia during 2000 ***(Source: Health Information System, MoHSS, 2003, Namibia)***

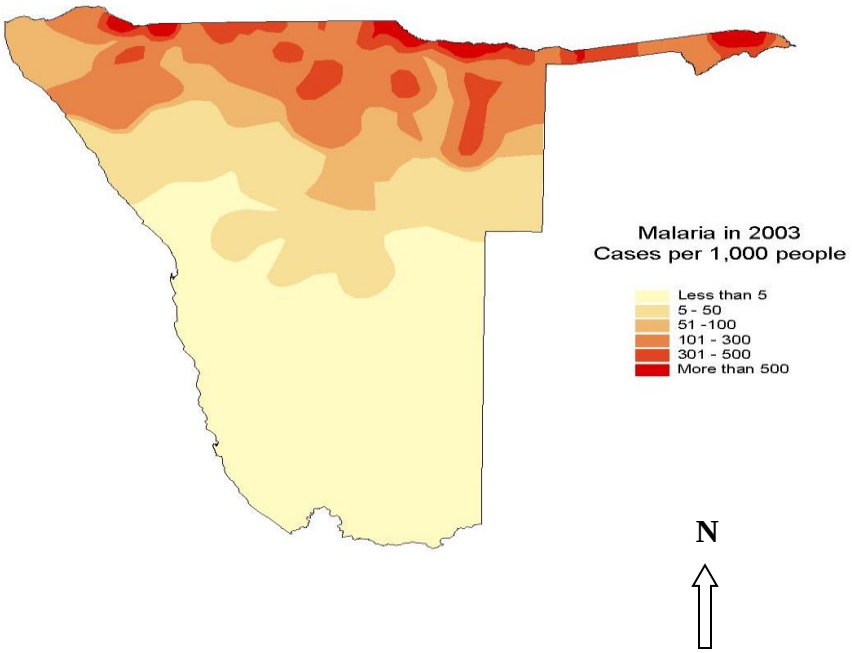


Figure 4: Cases of malaria per 1000 population in Namibia during 2003
(Source: Health Information System, MoHSS, 2003, Namibia)

The malaria mortality rate varied between 30/100,000 and 93/100,000 during the years 1996 and 2001. It is the most important cause of admission in northern regions of Caprivi, Kavango, Ohangwena, Omusati, Oshana, Oshikoto and Kunene (Obeid *et al*., 2001). About half of all new patients visiting health facilities in these regions during 1992-1995 (accounting for 46% of all diagnosis) were due to malaria (MoHSS Epidemiological Report, 1995). It is especially prevalent among children with almost one out of every four inpatients being hospitalised for this disease (Obeid *et al.*, 2001).

Thus, malaria is the leading cause of morbidity and mortality amongst the under five year olds, and the third most important cause of death among adults (MoHSS, 1995; MoHSS, 2002). In the northern regions of Namibia for example, malaria ranks among the ten top leading diseases in the country (Epidemiological Report, 1990, 1995, 1997; Obeid *et al*., 2001). Malaria accounted for 20% and 44% of all new cases under the age of 5years at the outpatient department (OPD) during 1992 and 1996 respectively, in north-eastern Namibia (MoHSS, Epidemiological Report, 1996).

It is possible that more than half the population is infected on average each year in the most risky areas of Katima Mulilo, along the Kavango River and Mahenene in Omusati region (Obeid *et al*., 2001). Rates of infection decline progressively to the south-west of these worst areas, so that there is almost no risk south of a line that runs roughly from Gobabis to Okahandja and then west to Khorixas (Obeid *et al*., 2001).

In the 1960s, house-spraying operations were carried out principally from May to November in Owambo on account of flooding during the rainy season. Difficulties were experienced in order to evenly spray roofs which were 1-2 metres from the ground and which had entrances 0.5-1 metre high. It was difficult to estimate the amount of insecticides to apply because of the type of thatching grass used (Hansford, 1975). Hence there has been a considerable variation in the amount of 75% DDT used per structure, varying from 0.054kg/m^2 in 1966 to 0.144 kg/m^2 in 1974 (WHO recommends 0.2kg/m^2). During 1990 the target spraying dosage of DDT decreased from 0.4-0.6kg/m^2 to 0.25-0.26kg/m^2. To date, no chemical

analysis has been made to determine the actual amount of DDT being sprayed. However, this is a problem this study was not intended to address. In Kavango and Caprivi, spraying was done twice annually from 1974 to 1990, in August-November and in January-March. However, WHO malariologists (Meek, 1991; Service, 1993; Kassasky, 1994; Renshaw, 1995; 1996) recommended that the most appropriate time to spray is just before the beginning of the rains, such as in October. This ties in well with recommendations made by Hansford (1975) that a main spray cycle before the summer rains should cover as many houses as possible. The World Health Organization, recommends at least 85% coverage of all house structures targeted for spraying based on appropriate epidemiological stratification (WHO, 2005). However, this has not been realised given numerous reasons including logistics.

The first malaria survey to investigate the distribution and malaria vector species in Namibia was conducted from February to June 1950 (De Meillon, 1950; WHO, 1951). Since then, there had not been any major study carried out covering the entire country. This is despite the fact that since the early 1960s there had been an ongoing indoor residual insecticidal spraying in the northern malarious areas. According to De Meillon (1950), the ideal malaria survey to investigate the malaria vectors should include all-the-year-round information, for in this way the true degree of malaria endemicity and the biology and behaviour of the vectors are best assessed. The survey of 1950 presumed that the malaria vector was *An. gambiae s.l.* (De Meillon, 1950). However, Kuschke (1968) found *An. gambiae* (pool-breeder) and *An. funestus* (river breeder) in Kavango and Caprivi Zipfel (now Caprivi region).

In March 1965, the then Directorate of Health for South West Africa commenced intradomiciliary spraying with 50% DDT and DDT mixed with kerosine on a small scale and gradually expanded to cover the whole of then Owamboland (Hansford, 1975; and Els, *personal communication*) from 1969. DDT mixed with kerosine were used for brick built houses while 50% DDT which was later replaced by 75% DDT was used for all other house structures.

According to a memorandum dated 16th May 1979 that was signed by Dr Hetzeroth, Director for Health Services in the then Administration for South West Africa, it was decided to restart the vector control programme in the Caprivi Region as from May to December 1979. In addition, between 1963 to 1989 Darachlor (chloroquine + pyrimethamine) tablets were distributed from January to June at a dosage of 2 tablets for every person 10 years old and above and 1 tablet for every child below 10 years. This was done once a week (Els, *personal communication*). This suggests that the existing vector control programme prior to 1979 may have collapsed in some areas of operation. The spraying operations were conducted from May to November on an annual basis, except for the Kavango Region where two spraying seasons were introduced from the late 70s (August to November and January to March). The reason for the changes was due to thatching which took place every two years from June to September. Secondly, most people were not at home during January to March due to ploughing and harvesting. Malaria control included the use of Darachlor for parasite control and residual insecticidal spraying (Hansford, 1975).

Although, there is no data available for the number of malaria cases around that time, the comparison illustrated above suggests that DDT residual spraying had some impact on malaria control at the time.

The evaluation of the malaria vector control programme was by means of mass blood smear surveys. The survey started in former Owamboland during 1966 and in Caprivi and Kavango during 1967. According to Els (*personal communication*), a Control Health Inspector who worked for the Malaria Control Programme from 1963-1989, the reduction in malaria prevalence had given an idea of the effectiveness of DDT on malaria transmission and in regular sprayed areas of the effectiveness of good spraying coverage. The results of the blood smear surveys were also used to target areas where spraying was essential and to identify areas where spraying could be terminated. The available data from these surveys conducted during April to May showed the prevalence of malaria to have fallen from 49% before the commencement of DDT spraying to 0.06% during 1974 for Owambo (De Meillon, 1950, Hansford, 1975). In areas where the spraying coverage was poor the prevalence of malaria would be higher (Hansford, 1975).

From the late 60s to 1991, staff members of the National Institute for Tropical Diseases, in Tzaneen, South Africa, undertook yearly parasitological surveys during the peak transmission season of April to May from the former Owamboland, Kavango and Caprivi. The objective of the surveys was to evaluate the quality and impact of the control operations. During each survey, between 8 000 to 16 000 blood smears were collected and sent to the Institute, for examination and analysis (Hansford, 1975,

Teklehaimanot *et al*., 1990). This was an expensive exercise carried out over a two-month period. Although the Programme got the results from the blood smears surveys, the data were not utilized in any way to evaluate the control programme and or re-orientate the control strategy where necessary.

Reports from these mass blood surveys in 1965, before the onset of DDT residual house spraying in 1965, revealed that the prevalence of malaria for the former Owamboland and Kavango was 47.6% and 64.8% respectively (Department of Health, 1988). In 1988, a survey was carried out to compare incidence of malaria in sixteen villages: eight on the Angolan side and eight on the Namibian side of the Kavango River, directly opposite each other (La Grange *et al.,* 1988). It was found that the overall slide positivity and spleen rates across all age groups in villages on the Angolan side were 25% and 54% respectively, whilst on the Namibian side they were 15% and 28%. Highest slide positivity rates from Angolan villages were in children in the one to four-year age group (53%: 35/66). Whereas those on the Namibian side were 37%: 17/46 in the 10-14 year age group. Hansford (1975) recommended that mass blood surveys in Owamboland be discontinued while recommending the continuation for Kavango and Caprivi. The reason for this is not known.

1.5.3 The problem of DDT residual spraying

DDT residual spraying has been the mainstay of malaria vector control from the 1960s in the northern regions of Namibia until recently. However, there had been some problems. For example, Caprivi Region is unique due to flooding in certain years. Heavy floods occurred in 1958, 1968, 1978,

1987, 1988, 1998 and 2003 in Caprivi (Namibia Weather Bureau report 1998). During these floods most houses in the most eastern Caprivi Region were flooded. Yet there has been use of DDT house spraying since 1973 to-date. In north-western Namibia, most people live in very small short houses. It is difficult to do indoor house spraying while standing. This makes it very difficult for spray men to apply the recommended dosage of $2g/m^2$. As a result high dosages of DDT are being applied at about $2.6g/m^2$. A similar situation is observed in Opuwo district where residents live in low-roofed "rondavels" made of sticks and cow dung. These people are nomadic and live in these "rondavels" for a few months and move elsewhere in search of green pasture. The same is true for Tsumkwe in the central region.

Some difficulties are also experienced by vector control programme in Namibia that may compromise the effectiveness of the spraying operation, for example, shortage of transport for spraying teams. The other examples include, grass-roofs that are replaced every 2-4 years from June (when grass is mature) until the commencement of the rains in November and significant numbers of the population are out attending crops in Kavango and Caprivi between January and March resulting in low coverage. This may compromise the effectiveness of insecticide residual spraying.

1.5.4 The problem of insecticide resistance in mosquitoes

By 1946 two species of *Anopheles* were reported to be resistant to DDT, but by 1980 a total of 51 *Anopheline* species showed a degree of resistance to one or more insecticides (Bruce-Chwatt, 1991). Forty-seven were resistant to Dieldrin and 34 to DDT, while resistance to organo-phosphates has been recorded in ten species and resistance to carbamates in four species (WHO, 1980).

Bruce-Chwatt (1991) reported that the physiological resistance of *Anopheles* to DDT is a serious problem and that the impact of such resistance on malaria control during the eradication era was obvious. The extension of resistance from DDT to other chlorinated hydrocarbons (HCH and Dieldrin) has led to the non-use of these compounds in many parts of the world.

According to Brown (1986) 56 *Anopheline* species have developed DDT resistance. It should be realised that DDT is still widely used as a residual adulticide for malaria control in some African countries including South Africa, Ethiopia, Swaziland and Namibia (*Kamwi, personal experience*).
Anopheles arabiensis in the Sudan is resistant to Malathion (WHO, 1980). Meanwhile, resistance to Permethrin has been reported in the laboratory in *An. stephensi* (Kamwi, 1993), *An. arabiensis* and *An. gambiae* (WHO, 1980). *Anopheles funestus* has been implicated as a major malaria vector in sub-Saharan Africa including Namibia where pyrethroid insecticides are widely used in agriculture and public health. Samples of this species from northern Kwazulu/Natal in South Africa and the Beluluane region of

southern Mozambique showed resistance to pyrethroid insecticides (Hargreaves *et al.*, 2000; Brooke *et al.*, 2001). Similarly, Brooke *et al.*, (2000) reported resistance to Dieldrin in *An. gambiae Giles sensu stricto* in Nigeria and Cote d'Ivoire.

1.6 Problem of drug resistance

One of the major obstacles in malaria control is the development of resistance by the malaria parasite (*P. falciparum*) to chloroquine. Chloroquine, the cheapest and most widely available antimalarial drug, has lost its clinical effectiveness in most parts of Africa (WHO, 2003). Despite the worldwide spread of chloroquine resistance, chloroquine remains the drug of choice for treatment of uncomplicated malaria in Namibia. Sulphadoxine Pyrimethamine (SP) is used as a second-line drug. Quinine is used for the treatment of severe and complicated malaria.

According to the World Health Organization (WHO, 2003) drug resistance is defined as the ability of a parasite strain to survive and/or to multiply despite the administration and absorption of a drug in doses equal to or higher than those recommended but within the limits of tolerance of the subject.

Chloroquine resistant *P. falciparum* was first noted in Africa during 1978 and in Namibia during 1984. In recent years clinicians working in the malarious areas of Namibia have reported increasing numbers of cases of chloroquine treatment failure. Since then a number of drug resistance studies have been carried out. An *in-vitro* resistance study carried out in

Kavango region in 1990 found that 60% of the patients responded successfully and 40% were indicative of RI resistance (Resistance, delayed recrudescence) to chloroquine, (MoHSS, 1995).

An *in-vivo* study carried out by MoHSS and WHO in Rundu in 1990 revealed that 82% responded successfully, 6% RI, 6% RII (Resistance, early recrudescence) and 6% RIII resistant. Another *in-vivo* study carried out in Ombalantu in 1993 showed that 57% responded successfully, 3% RI, 23% RII and 17% RIII resistant. A therapeutic efficacy study conducted during April 1996 in Katima Mulilo found the following: 6% of the patients were indicative of early treatment failure, 9% of late treatment failure and 85% of treatment success. The sample size of this study was very small with a high defaulter rate. This means it failed to meet the WHO specifications for any decision to be made to continue or change the drug policy.

An *in-vivo* drug resistance study was carried out during 1997 in three sites, Rundu, Outapi and Katima Mulilo. The results from these studies were not conclusive, as there was a high drop out rate. However the results are as follows; Rundu parasitological failure; 2% were indicative of RIII resistance, 10% RII, 10% RI, 8% RI/Sensitive and 70% were sensitive. Clinical response; 95.8% were indicative of treatment success and 4.2% of treatment failure. In the Katima Mulilo study the sample size was not large enough to reliably make a conclusion, but showed the following results, 19% RIII, 31% RII and 50% sensitive. Clinical response; 5.8% were indicative of early treatment failure and 94% of treatment success. Ombalantu, parasitolgical response; 3.6% were indicative of RIII, 21.4% of

RII, and 75% RI/Sensitive. Clinical response; 81.5% responded successfully whilst 18.5% were indicative of treatment failure.

Furthermore, a similar resistance study was conducted in March 1998 in Ombalantu and the following results were obtained; Parasitological response 70% RI, 5% RI/S, 21% RII and 2% RIII resistant. Clinical response 14.6% were indicative of early treatment failure, 2% late treatment failure and 83% of adequate treatment response. From these studies the indications are that treatment success based on Parasitological success ranged from 50 – 83% with the 28 days of follow up.

1.7 Economic impact of malaria in Namibia

Malaria continues to be a major obstacle to economic development of rural populations (Malaney *et al.,* 2004), as transmission is either perennial or peaks during the planting and harvesting periods when there is greatest need for agricultural activity. Apart from its impact on health in terms of mortality and morbidity, malaria has considerable impact on the economy of the country. According to WHO (1998) the three aspects of its economic impact are as follows:

(i) direct cost from the treatment and prevention of malaria incurred by the individual,
(ii) indirect cost resulting from economic losses, and
(iii) impact on the quality of life of individuals and families, which is difficult to quantify.

Since the inception of Roll Back Malaria in 1998, and particularly since the Abuja Summit in 2000, malaria prevention and control have once again become domestic and international priorities. International spending for malaria in Namibia has increased at least twofold since 1998 (WHO, 2003), and this does not even include the complementary financing of a range of primary health care services (such as reproductive health and Integrated Management Child Illnesses (IMCI) that also have an impact on malaria.

According to WHO (2000), the economic impact of malaria for Africa, south of the Sahara, was estimated in 1987 to be US $800 million and would have reached US $1700 million by the end of 1995. In 2002, approximately US$ 200 million was earmarked for malaria control worldwide, compared with an estimated US$ 60 million in 1998 (WHO, 2003). Meanwhile, Teklehaimanot & Bosman (1999) revealed that recent estimates on the direct and indirect costs of malaria exceeded US $2 billion in 1997 and that this figure is likely to increase every year. The World Health Organisation (WHO, 1998), estimated that by the year 2000 malaria would cost Africa's economy $3.6 billion a year, as a result of working hours lost and the cost of treatment (Chinnock, 1997). Namibia being a member state of WHO, has a share in this fund.

According to the WHO, Country Office in Namibia (*Kamwi, personal communication*), the WHO financial support to Namibia rose from a sum of U$617,000.00 in 1990 to US$1,151,000.00 in 2000 and by mid 2001 it rose to a sum of US$6,735,642. Of this figure, WHO financial support for the Malaria Programme for the period 1990-2000 was US$495,567.00. Meanwhile, during the year 2000 alone the Ministry of Health and Social

Services spent N$2 193 124.80 (US$ 274 140) on chemicals for residual spraying and N$4 060 692.00 (US$ 507 586) on drugs for malaria control respectively.

1.8 Statement of the problem

The distribution of malaria in Namibia is strongly related to rainfall, which contributes to malaria vector breeding. Caprivi Region receives the highest average rainfall of more than 600 mm per year, and diminishes towards the North western regions of Namibia which receive 321-828 mm (Obeid *et al*., 2001, Mendelsohn *et al*., 2003. In addition, the northern part of the country is characterized by perennial rivers (Kunene, Kavango, Cuando, Chobe and the Zambezi) which contribute favourably to mosquito breeding. According to De Meillon (1950) the malaria vectors in Namibia belong to the Anopheles *gambiae* group of species. De Meillon also found that *An. funestus* another major group of malaria vectors in Africa was common near the Kavango and Zambezi Rivers. Because of its high tendency to bite and rest indoors, *An. funestus* is easily controlled by insecticide residual house-spraying or with the use of ITNs (Mnzava *et al.*, 1993). The former Department of Health, South Africa introduced indoor house spraying in northern Namibia using 75% DDT wettable powder for malaria vector control way back in 1964 (Hansford, 1975). However, there had not been any evaluation of this programme which was inherited by the Ministry of Health and Social Services to suggest any conclusive evidence as to whether *An. funestus* had been eradicated in northern Namibia or not. Moreover, it was decided in the present study to investigate whether or not DDT is still effective for malaria vector control.

In KwaZulu-Natal, Sharp *et al., (*1993) observed that *An. arabiensis* had a tendency of biting indoors and resting outdoors. The biting and resting behaviour of the local malaria vectors in Namibia is largely not known. Clearly, this called for an investigation, as the behaviour of malaria vectors is crucial in the transmission and control of malaria.

In many parts of Namibia, health facilities report high numbers of clinical malaria cases even when cases are generally expected to be low. Microscopic examination of malaria is not done at clinics and in most health centres. This problem and a combination of measured temperature or reported fever and the presence of malaria parasites in the blood had to be investigated. The latter provided a precise geographical and seasonal distribution of malaria in Namibia. These data were used to establish the prevalence, density and episodes of parasites of malaria caused by *P. falciparum*.

In recent years unsubstantiated reports of decreased chloroquine efficacy in the treatment and management of malaria have been received from health facilities (MoHSS, 1995-2003). Studies carried out in various parts of Namibia have shown a rising level of chloroquine resistance (MoHSS, 1996-2004). In addition, studies from Botswana, which shares a border with Namibia, have reported unacceptably high chloroquine resistance levels in the Chobe sub-district with subsequent replacement of chloroquine with sulphadoxine pyrimethamine as the first line drug (Kamwi, personal experience). It was therefore necessary to establish the susceptibility status

of *P. falciparum* to chloroquine in order to justify any policy change in the use of antimalarial drugs.

The availability of accurate data is essential to make effective planning, implementation and evaluation of malaria control strategies. The present study reviewed and analysed the existing data on malaria control and made recommendations as deemed necessary.

1.8.1 Working hypothesis

i. The malaria vectors in Namibia are presumed to be *An. arabiensis* and that given the long use of DDT residual spraying which has been used for decades *An. funestus* may have been eradicated from Namibia.

ii. The vector behaviour and distribution of *An. arabiensis* may have changed significantly over the years as a result of the long term DDT spraying operations in the country.

iii. There is a significant over-diagnosis of malaria cases in clinics and health centres, especially during the low transmission seasons resulting in wastage of the scarce antimalarial drugs.

iv. The resistance of malarial parasites against chloroquine has been increasing steadily resulting in increased morbidity and mortality due to malaria infections.

1.8.2 General Objective

The general objective of the study was to determine the vector species in malaria transmission, seasonal abundance, behaviour of malaria vectors and efficiency of the diagnostic and treatment procedures and the overall malaria control scenario in Namibia.

1.8.3 Specific objectives

1. Determine the vector species responsible for malaria transmission in selected areas of northern Namibia.

2. Investigate the biting and resting behaviour of malaria vectors and their implications for malaria vector control.

3. Determine the relationship between seasonal variation of malaria vector density and prevalence of malaria.

4. Investigate the relationship between fevers and clinical malaria.

5. Verify the level of *P. falciparum* resistance to chloroquine.

6. Analyze retrospective malaria data for efficient planning and targeting of selective vector control operations.

CHAPTER TWO: MATERIALS AND METHODS

2.1 The Study Area

The study was carried out in Bukalo area about 45km south of Katima Mulilo and Kalimbeza village 24^0 37E, 17^0 48S, situated 25km east of Katima Mulilo district, Caprivi Region and Mahenene area about 50km from the north-western Outapi hospital, Omusati Region, northern Namibia (Figure 5).

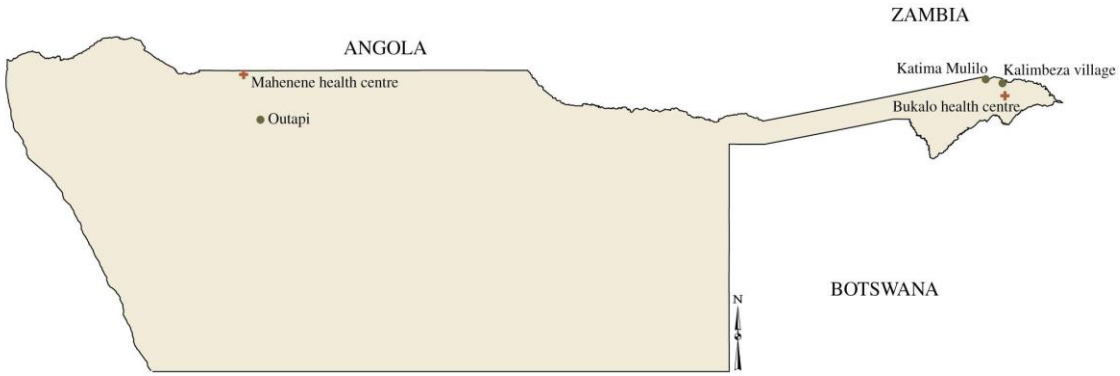


Figure 5: Map showing study sites in northern Namibia

The two sites of Mahenene and Bukalo were selected for this study based on the demographic, epidemiological and climatic conditions that are believed to represent the various malaria endemic areas in northern Namibia (Service, 1993b).

However, entomological studies were carried out primarily in Kalimbeza village, Katima Mulilo district from June 1999 to July 2000. It would have been appropriate for the study to cover the northern malarious regions but logistics and the insecurity posed by Unita bandits in Kavango and western Caprivi made it impossible to cover these areas. As a result, sampling of

mosquitoes in Divundu situated 200km east of Rundu, Kavango region and Caluque situated about 80km northwest of Outapi hospital and bordering southern Angola in Omusati region were collected for a month only.

2.1.1 Studies on malaria vector species and seasonal abundance in Katima Mulilo District, northern Namibia

2.1.2 Study sites

Kalimbeza village in Katima Mulilo district was selected for the sampling of mosquitoes on the basis of community distribution. In this village, fifteen sprayed and nine unsprayed houses were selected for mosquito collection. One exit window trap was placed in each of the 24 selected huts in order to collect mosquitoes.

2.1.3 Mosquito collections at Kalimbeza village

A field research assistant was identified and trained on how to handle mosquitoes, how to use an aspirator and how to transfer mosquitoes into press tubes containing isopropanol (see plate 1.0). The Principal Investigator worked along with the Field research assistant to gain experience for a month. The field research assistant who was a resident of Kalimbeza village collected mosquitoes between 06h00 to 08h00 from Monday to Friday for a year.

2.1.4 Mosquito abundance and biting behaviour

Mosquitoes would enter in the huts for blood meal from humans. They would enter huts by any means, through the eaves or through the door. After blood feeding, these mosquitoes would attempt to fly out through the exit window traps and in the process would get trapped.

All mosquitoes were then trapped by means of exit window traps of the Muirhead-Thomson design (Muirhead-Thomson, 1947). Mosquito collection was done by means of an aspirator from exit window traps into press cups (Plate 1.0). The exit window traps were inserted in the walls of the huts between July 1999 and June, 2000 in Kalimbeza village. It should however, be stated that the initial exercise was meant to collect mosquitoes from Outapi in Omusati and Andara in Kavango Regions respectively for a year. However, this was not realised as a result of the logistical problems experienced to conduct this exercise in Outapi. Similarly, Kavango Region was infiltrated by Unita bandits resulting in insecurity. Thus the research was only conducted in Kalimbeza Village, Katima Mulilo District.

Plate 1.0 *Field research assistant (Precious Rahele Matome) collecting mosquitoes by means of an aspirator from an exit window trap at Kalimbeza village, Katima Mulilo, Caprivi Region, July 1999 to June 2000.*

The mosquitoes that were collected for a month in Andara and Outapi were processed along with the collection from Kalimbeza village.

The number of mosquitoes (*Anopheles*) caught in each of the huts through an exit window trap were counted and recorded on data sheets. Other species such as *Culex quinquefasciatus* which were not malaria vectors were excluded. The number of fed and unfed mosquitoes was also recorded.

Mosquito data were recorded on a data sheet including the date of collection and code number of the hut. Mosquitoes were taken to Oshakati insectory for further processing and indentification.

2.1.5 Species identification

Mosquito specimens collected from Kalimbeza Village, and the mosquitoes caught for a month only (July, 1999) from Andara in Kavango and Mahenene in Omusati Regions respectively were identified morphologically using the key of Gillies and De Meillon (1968); and Gillies and Coetzee (1987).

The following data were recorded on a data sheet: dates of collection of mosquitoes, method of collection, sentinel sites, whether mosquitoes were blood fed or unfed. The mosquito specimens were then sent to the Medical Research Council (MRC), Durban, South Africa for molecular identification using the *An. gambiae* species specific Polymerase Chain Reaction (PCR).

2.1.6. Species identification of the *Anopheles gambiae* complex by means of Polymerase Chain Reaction (PCR)

The (PCR) method for species identification of members of the *An. gambiae* complex was carried out using the method of Scott *et al*. (1993). It is one of the most convenient methods and utilizes a cocktail of five 20-base oligonucleotide primers to amplify species-specific DNA products of members of the complex. *Anopheles gambiae* and *An. arabiensis* are unequivocally identified by unique fragment sizes; *An. merus* and *melas* share the same fragment size but are differentiated by locality. *Anopheles merus* is found in East and Southern Africa while *An. melas* is found in West Africa.

2.1.7 Polymerase Chain Reaction Identification procedure

One leg of a mosquito was placed in a 0.5 ml micro-centrifuge tube. A 12.5 µl PCR reaction mixture containing: 10x reaction buffer (100mM, 500 mM, KCL) (pH 8.3), 0.2 µM of each dNTP, 0.132 µM *An. quadriannulatus* primers, 0.264 µM primers (universal, *arabiensis, gambiae, merus*) and demonized water and 0.5 units Thermostable DNA polymerase (Taq) were added. Each individual tube with a mosquito leg and PCR reaction mix was centrifuged for two minutes at 14 000 revolutions per minutes (rpm) to release DNA from the tissue. A positive control for *An. gambiae*; *An. arabiensis*; *An. merus and An. quadriannulatus* was included by using the legs from laboratory colony material for amplification. A negative control was prepared from PCR reaction mixture without DNA. The tubes were placed in a Hybaid thermocycler PCR machine programmed for an initial

cycle of denaturing at 94º C for one minute followed by 30 cycles of denaturisation at 94º C, annealing at 50º C and extension at 72º C, each step running for 30 seconds. Agarose gel electrophoresis: Ten microlitres of amplified DNA was mixed with 3 µl Ficoll dye (5 g of 50% Sucrose, 1ml of 0.5 M EDTA pH. 7.0, 0.1 g/mg Bromphenol blue, 1 g of 10% Ficoll and 9 ml distilled H_2O) and electrophoresed on a 2.5 % agarose gel stained with 0.03mg/100ml Ethidium bromide. The gel was taken from the tray, viewed on the UV light trans-illuminator and bands were read visually and compared to the standard 1Kb-plus (Gibco-BRL) size marker and the species standards.

Table 1. Lengths (base pairs) of *Anopheles gambiae* complex diagnostic PCR fragments (Scott *et al.*, 1993; Hunt *et al.*, 1998).

Species	Fragment length (base pairs)	Locality	Region
An. quadriannulatus species A and species B	153	Kalimbeza	Caprivi
An. arabiensis	315	Kalimbeza	Caprivi
An. gambiae	390	Kalimbeza	Caprivi
An. merus and An. melas	464	Kalimbeza	Caprivi

Table 2. *Anopheles gambiae* complex ribosomal DNA (rDNA) intergenic spacer species -diagnostic primers and their melting temperatures (T_m) (Scot *et al.*, 1993).

Primer name	Primer sequence (5′ to 3′)	T_m (°C)	Reference
UN	GTG TGC CCC TTC CTC GAT GT	58. 3	Scot *et al.*, 1993
GA	CTG GTT TGG TCG GCA CGT TT	59. 3	Scot *et al.*, 1993
ME	TGA CCA ACC CAC TCC CTT GA	57. 2	Scot *et al.*, 1993
AR	AAG TGT CCT TCT CCA TCC TA	47. 4	Scot *et al.*, 1993
QD	CAG ACC AAG ATG GTT AGT AT	42. 7	Scot *et al.*, 1993

Note: The universal primer (UN) anneals to the same position of the rDNA of all the five species, GA anneals specifically to *An. gambiae*, ME anneals to both *An. merus* and *An. melas*, AR anneals to *An. arabiensis*, and QD anneals to *An. quadriannulatus* (Scott *et al.*, 1993).

2.2. Malaria prevalence surveys

2.2.1 The Relationship between fevers and clinical malaria

Bukalo and Mahenene health centres of Katima Mulilo and Outapi district were selected for screening suspected malaria patients in order to provide information on the true definition of a malaria case. Divundu and Kangongo clinics in Kavango region were further selected for the same reason. However, the security situation in Kavango and western Caprivi could not permit the completion of this exercise as stated above.

Two sets of questionnaires were administered to investigate the relationship between fevers and clinical malaria. One of the two questionnaires was carried out to assess malaria morbidity at Bukalo and Mahenene health centres respectively. Clinical thermometers were used to determine whether patients had fever or not. The questionnaire was also used to collect data on houses of these suspected malaria cases, if there were sprayed and if so when last sprayed. The suspected cases were further asked whether they had taken chloroquine in the last two weeks.

In addition, thick and thin blood smears were taken from each of the patients to check for the presence or absence of malaria parasites.

Thick and thin blood smears and body temperatures were collected from suspected malaria patients at the above mentioned health centres in order to determine malaria parasite prevalence and malaria episodes caused by *Plasmodium falciparum*. A total number of 462 and 714 thick and thin blood smears respectively were taken from suspected malaria patients at Bukalo and Mahenene health centres. Meanwhile 134 thick and thin blood smears were collected from Andara and the same number from Kangongo clinics.

The other questionnaire was administered cross-sectionally to determine malaria parasites from residents within the vicinity of the study sites. Thick and thin blood smears were carried out and examined to determine malaria parasite prevalence. The following information was included in both questionnaires date, name, age and sex, region, nationality, health facility, patient address, temperature and result of blood smear.

2.2.2 The relationship between seasonal variation of vector density and malaria prevalence

A total of 1, 969 thick and thin blood smears were taken from Bukalo, Silumbi, Lisikili and Isize combined schools from 18 villages surrounding these schools as cross-sectional survey. A total number of 2, 233 were collected from the 5 years old and above and 120 thick and thin blood smears were collected from the under 5 year old children from Outapi. Meanwhile a further total of 47 thin and 32 thick blood smears were respectively collected for the under five age group from Calueque during dry season.

The principal laboratory technologist from the National Vector-borne Disease Control Programme (NVDCP) trained nurses in these health facilities for two weeks to accurately take blood smears and body temperatures from sick people who reported at their facilities for the first time. The nurses took blood smears and body temperature recordings for 5 working days in a week for 12 months. A total of one thousand four hundred and forty-four (1444) blood samples were recorded.

The blood slides were each marked with a code number corresponding with a name during collection of blood smears. Blood smears collected were then transferred and parked in slide boxes and were brought to Oshakati and Katima Mulilo laboratories for examination of malaria parasites. Examination of blood smears was done with the assistance of the Medical technicians and technologists at Oshakati and Katima Mulilo respectively.

In order to investigate whether indoor residual spraying was effective, a cross-sectional survey of malaria was carried out from Outapi and Calueque, southern Angola where there is no insecticide spraying activity. Similarly, mosquito collections were carried out in Calueque using Pyrethrum spray catches. Permission to conduct the study from southern Angola was sought through the diplomatic channels. A total of 152 thick and thin blood smears were collected from residents of Calueque. Blood smears were collected from all age groups.

2.3 Microscopic examination of blood smears for malaria parasites

2.3.1 Collection of blood

A sterile blood lancet was used to prick a finger of each patient in order to get drops of blood to make thick and thin films.

2.3.2 Preparation of thick and thin blood films

Clean microscope slides were used and labelled with serial numbers including the name of the site.

A drop of blood was applied to each slide using a slide or a micro-capillary pipette. Blood was spread to form more or less a round smear. The blood films were then allowed to dry in open air.

2.3.3 Staining procedure

Dry blood films were stained with 3 % Giemsa solution.

For rapid staining, 10% Giemsa was used. The thin part of the slide was fixed in methanol for a while. The blood film was allowed to air dry. It was then stained in Giemsa for 30–45 minutes and allowed to air dry. The blood film was examined under 100 x oil objective for malaria parasites.

2.4 Therapeutic efficacy test of chloroquine in Katima Mulilo and Outapi districts (See Annex 4)

The therapeutic efficacy study was carried out over 28 days' follow-up period, in line with the WHO protocol (WHO, 1996; WHO, 2002). All patients with uncomplicated malaria that met the selection criteria as set out in the WHO protocol were followed up over 28 days' period. A total of 36 patients from Katima Mulilo and 29 from Outapi were enrolled for Chloroquine (CQ) efficacy test. Thick and thin blood smears were taken from these patients.

2.4.1 Community consent

The communities were sensitised about the study. The Principal Medical Officers for Outapi and Katima Mulilo hospitals acted as mediators between the study teams and the communities to which they were already known. Community leaders and the villagers were visited to obtain community consent. In addition, individual patients or their guardians (in the case of children) were only included in the study upon their consent, which is part of the Geneva Convention on Good Clinical Practice.

2.4.2 The Procedure for recruitment of candidates for the study

Patients of either sex, particularly outpatients were recruited for the study. Since the north-western and north-eastern parts of Namibia are regarded as unstable malarious areas, all age groups were included (i.e. both under and above five years). The patients had to be confirmed positive for *P. falciparum* malaria through laboratory microscopic examination of their blood films. Sixty patients were required for the final analysis of this study. The sample size was of such a size to cover for defaulters and those who were not suitable for the study. The defaulter rate did not exceed 10% of the study population. Patients came in for the first and the second day after their first visit for treatment (chloroquine treatment). Patients had to agree to be examined on day three, day seven, day fourteen, day twenty-one and day twenty-eight after their first visit. Patients were advised to visit the hospital if malaria worsened. As a result patients living in the vicinity of the study site were given priority for recruitment to the study in order to accommodate the follow-up. The body temperature (ºC) and weight (kg) of all patients were recorded by a registered nurse before they were examined by the medical officer. Chloroquine was then prescribed and first administered by a registered nurse. Patients were advised to come again on the next day for further administration of chloroquine. See Tables 3.16 and 3.17 for the distribution of study population by age group and gender in the study site.

2.5 Retrospective malaria data

2.5.1 Malaria data for efficient planning and targeting of selective vector control operations

The Ministry of Health and Social Services in Namibia has a well established Health Information System. One set of data being captured by the system is malaria cases from health facilities including clinics, health centres and hospitals. The malaria data from the outpatient department are captured by the computer system.

In order to plan for a cost effective malaria vector control, it was necessary to analyse the malaria data collected from health facilities as from 1995 to 2003. The inpatient and outpatient malaria data were captured, analysed and then plotted into the affected regions on a map.

2.5.2 Statistical data analysis

Several software packages were used to analyze the data. Statistical analysis was done using mainly SPSS version 10.1 for windows. A combination of statistical techniques was employed to fully analyze the data. A combination of frequencies, percentages and chi-square were employed with or without cross tabulation of variables where appropriate. Correlations were also used to establish relationship between important variables in the various studies. Frequencies and percentages were employed on the following data: vectors species in malaria transmission, physiological condition of the vectors species in sprayed and unsprayed

houses, malaria vectors in Namibia and southern Angola, seasonal abundance of vector species, the relationship between fevers and clinical maralia. In addition Chi-square tests and correlations were performed on the data on the relationship between fevers and clinical malaria, and seasonal abundance of vectors species in malaria transmission. Microsoft Excel was used for graphing and analysing data on retrospective malaria data and efficacy of chloroquine.

CHAPTER THREE: RESULTS

3.1 Vector species in malaria transmission and their seasonal abundance

Vector species in malaria transmission examined at the MRC, Durban for molecular identification using the *Anopheles gambiae* species specific PCR are given in Table 3. 1.

Table 3.1 *Anopheles arabiensis* as the most common (%) species in northern Namibia by study site and *An. funestus* group being common in southern Angola. Figures in brackets are column percentages while those without are the sample sizes.

Mosquito Species	**Kalimbeza**	**Kangongo**	**Andara**	**Outapi**	**Calueque, S. Angola**
An. Arabiensis	229 (89)	13 (68)	46 (79)	47 (76)	14 (30)
An. funestus	4 (1)	4 (21)	8 (14)	4 (6)	17 (36)
Other Species	25 (10)	2 (11)	4 (7)	11 (18)	16 (34)
Total (*n*)	**258**	**19**	**58**	**62**	**47**

The results show clearly that there are more *An. arabiensis* in northern Namibia than *An. funestus* as demonstrated by the collection in Kalimbeza, Kangongo, Andara and Outapi. The results further show that although the collection of mosquitoes in Calueque was done only for a day, the data at hand suggests that *An. funestus* is more abundant than *An. arabiensis*. These results, however, do not preclude seasonal variation of the two vector species.

Further more this table shows overall that in DDT sprayed Namibia, there are more *An. arabiensis* (89%) than the other species, while in unsprayed Angola there are more *An. funestus* than other species. It should be noted, however, that this is only the total number of mosquito species collected and does not take into account the different methods of collection. The results, therefore may have been influenced by the inherent behaviours of the different vector species in relation to the different methods of collection

Table 3.2: The mean number of *Anopheles arabiensis* collected by means of exit window traps and the feeding status in sprayed and unsprayed huts or dwelling units for 12 months in Kalimbeza village, Caprivi Region.

Spray Status	**Physiological condition**		**Number of huts** *(n)*
	Blood Fed	**Not Fed**	
Unsprayed	40±4.09	104±3.74	**5**
Sprayed	13±2.09	25±2.79	**15**

The chi-square value is 7.66 with 1 *degree of freedom* and a *p-value* of 0.006. This shows that the two classification variables are not independent i.e. the physiological condition (blood fed, not fed) depends on whether the house was sprayed or not. There was significant difference in the number of blood fed and not fed mosquitoes between sprayed and unsprayed houses. The mean of blood fed mosquitoes trapped was higher in unsprayed huts compared to the sprayed ones. Thus, the analysis reveales that the physiological conditions (blood fed, not fed) depends on whether the house was sprayed or not. It should be noted that the sample size for sprayed and unsprayed huts were not the same.

Table 3.3: Malaria vectors in northern Namibia and southern Angola

Mosquito species	Calueque Southern Angola	Namibia
An. arabiensis	14 (30%)	335 (84%)
An. funestus	17 (36%)	20 (5%)
Other species	17 (36%)	42(11%)
Total	**48**	**397**

The results in table 3.3 shows that over all in Namibia, *An. arabiensis* constituted 84% of mosquitoes, while in southern Angola, there are more *An. funestus* than any other species. It should be noted however that this is only the total number of mosquito species collected and does not take into account the different collection methods. The results therefore may have been influenced by the inherent behaviours of the different vector species in relation to the different methods of collection. It is worth noting that Namibia has a DDT intervention in place and this is not the case in southern Angola. This is the most likely factor responsible for the difference in these results.

3.2 The biting behaviour of *Anopheles arabiensis*

The biting behaviour of malaria vectors in Kalimbeza village, Katima Mulilo district is shown in Table 3.4.

Table 3.4: The physiological status of blood fed and or not fed (*Anopheles arabiensis*) in Kalimbeza village.

Physiological status of mosquito	Frequency	Percentage
Blood fed	398	31%
Not fed	896	69%
Total	**1294**	**100**

The results in Table 3.4. show that 69% of *An. arabiensis* captured in Kalimbeza village were not blood fed while only 31% were blood fed. This suggests that although mosquitoes could penetrate into the sprayed huts through the eaves, they came out of the huts before feeding on humans.

3.3 The seasonal abundance of vector species

Seasonal abundance of vectors in malarious areas in Andara and Kalimbeza villages is shown in Table 3.5.

Table 3.5: Seasonal abundance of vector species in transmission areas, northern Namibia

Season	Vector Species			Total
	An. arabiensis	*An. funestus*	Other	
Dry	52 (15%)	24 (65%)	27 (47%)	**103(23%)**
Wet	297 (85%)	13 (35%)	31(53%)	**341(77%)**
Total	349	37	58	**444**

Chi-Square statistic is 67.298 P=0.000 with 2 *degrees of freedom* (*n=444*). This is statistically significant indicating that the two classification variables (season and species) are not independent. Clearly there are more *An. arabiensis* during the wet season than the dry season. The opposite is true with *An. funestus* species. The distribution of the other species did not differ.

Table 3.6: Seasonal abundance of malaria vectors in Kalimbeza village, Katima Mulilo

Season	**Vector Species**			**Total**
	An. arabiensis	*An. funestus group*	Other species	
Dry	3 (1%)			**3 (1%)**
Wet	226 (99%)	4	25	**255 (99%)**
Total	229	4	25	**258**

The results show that there is more activities of *An. arabiensis* during the wet season, sample size (n=226) compared to the dry season (n=3). This is true for *An. funestus*, although the sample size is small but there was not a single mosquito collected during the dry season.

3.4 The relationship between fevers and clinical malaria

The following Tables 3.7, 3.8, 3.9 and 3.10 are showing the results of a study carried out on a sample of study subjects drawn from schools in Katima Mulilo and Outapi districts. None of the study subjects were having any clinical illness at the time blood samples were taken for testing the presence or absence of malaria parasites. Tables 3.11 and 3.12 are showing the results of a study carried out in a sample of patients who were seeking treatment at Bukalo and Mahenene health centres. While Table 3.13 is showing the result of a study of a sample population, i.e., from Calueque, southern Angola (*n=28*) and from Namibian (*n=1390*) who tested positive or negative for *P.falciparum.*

Table 3.7: The number of study subjects who tested positive or negative for *Plasmodium falciparum* during the cross-sectional survey, Katima Mulilo and Outapi districts.

Season	Blood smear results				Total	
	Positive		Negative			
	No.	%	No.	%	No.	%
Wet	104	5	2086	95	**2190**	100
Dry	113	5	2043	95	**2156**	100
Total	**217**		**4129**		**4346**	

The results of table 3.7 reveals that there was no significant difference between study subjects that had malaria and those that did not during the wet and dry season, (chi-square statistics is 0.555 on 1 *df*, p=0.456). This shows that malaria parasites are available at all times even in people who would look healthy in the northern malarious areas throughout the year.

Table 3.8: The number of study subjects who tested positive or negative for *Plasmodium falciparum* in Outapi, northern Namibia and Calueque, southern Angola.

Country	*Plasmodium falciparum* results				Total	
	Positive		Negative			
	No.	%	No.	%	No.	%
Outapi, Northern Namibia	97	4	2280	96	2377	100
Calueque, Southern Angola	16	11	136	89	152	100

The Chi-Square statistic is 13.9, which is very significant (P<0.000) i.e. there is a statistically significant difference between Angola and Namibia in terms of malaria positive prevalence. The prevalence of *P. falciparum* is higher in Angola (11%) than in Namibia (4%). The small sample size for Angola may not be representative thus results for Angola are not conclusive but may give an idea of the malaria situation there.

Table 3.9: The number of study subjects (by age group) who tested positive or negative for *Plasmodium falciparum* during the wet and dry season in Katima Mulilo and Outapi districts during 2000

Age category	Season	Blood smear results for malaria parasites				Totals	
		Positive		Negative			
		No.	%	No.	%	No.	%
Under Five	Wet	24	(6%)	386	(94%)	**410**	100
	Dry	15	(6%)	229	(94%)	**244**	100
Five and above	Wet	80	(5%)	1700)	(95%	**1780**	100
	Dry	98	(5%)	1814	(95%)	**1912**	100
Totals		**217**		**4129**		**4346**	

Results in tables 3.7, 3.9 and table 3.10 below show that malaria parasites are available in some individuals throughout the year even though they do not show any signs and symptoms of malaria, regardless of age.

Table 3.10: The number of study subjects who tested positive or negative for *P. falciparum* during wet and dry season in Katima Mulilo district during 2000.

District	Season	Blood smear results				Totals	
		Positive		Negative			
		No.	%	No.	%	No.	%
Katima Mulilo	**Wet**	58	6	924	94	**982**	100
	Dry	62	6	925	94	**987**	100
Outapi	**Wet**	46	4	1162	96	**1208**	100
	Dry	51	4	1118	96	**1169**	100
Totals		**217**		**4129**		**4346**	

The results show that the prevalence of *P. falciparum* is statistically higher in Katima Mulilo than in Outapi (chi-square statistic of 9.205 with a p-value of 0.002 and *df=3, n=4346*) in both the wet and dry seasons.

Table 3.11: The number of patients who tested positive or negative to *P. falciparum* for fever status at Bukalo and Mahenene health centres

Methods of diagnosis	Diagnosed as having malaria				Total	
	Positive		Negative			
	No.	%	No.	%	No.	%
Fever	1358	97.7	32	2.3	**1390**	100
Blood smear for malaria parasites	388	27.9	1002	72.1	**1390**	100

The correlation coefficient between having fever and having a positive blood smear result was r = 0.10 (p=0.71) indicating that the difference is not statistically significant. The Chi-square statistic for the same is 0.138 (*df*=1,

p = 0.710) which is clearly also not significant. This shows that there is no correlation between fever and clinical malaria. This suggests that there had been an over-diagnosis of patients as malaria resulting in wastage of both the drugs and financial resources. For example 72% of the people who had fever were in fact not having malaria parasites (Table 3.12). These results are important as the current trend is to move towards the more expensive combination therapies for the treatment of malaria (ACTs) due to CQ and SP failure in many countries including Namibia.

Table 3.12: The number of patients who tested positive or negative for *P. falciparum* per health facility in study sites.

Health facility	Frequency	Blood smear result	
		P.falciparum	**Negative**
Andara clinic	134	18%	82%
Bukalo health centre	462	23%	77%
Kangongo clinic	80	8%	92%
Mahenene health centre	714	35%	65%

The results in table 3.12 show that there is more malaria in Mahenene followed by Bukalo, Andara and Kangongo. But this may not show the true picture on the ground because of some discrepancies in sampling procedures as a result of the insecurity of the area at the time of data collection. This was during the Unita bandits' incursion into Kavango region.

Table 3.13: The number of the patients by nationality who tested positive or negative for *Plasmodium falciparum* in study sites

Nationality of patient	Blood smear result for malaria parasites				Total	
	Negative		Positive			
	No.	%	No.	%	No.	%
Calueque, S.Angolan	13	46	15	54	28	100
Namibian	1002	73	388	27	1390	100

The results show that even though the sample size for Angola is small, *(n=28)*, the prevalence of malaria is higher (54%), than that of Namibia (27%), which had a higher sample size (*n=1390*).

3.5 Retrospective malaria data

Figure 6 shows the analysis of the retrospective malaria data by region in Namibia between 1995-2003 for patients reporting to health facilities for care as outpatients.

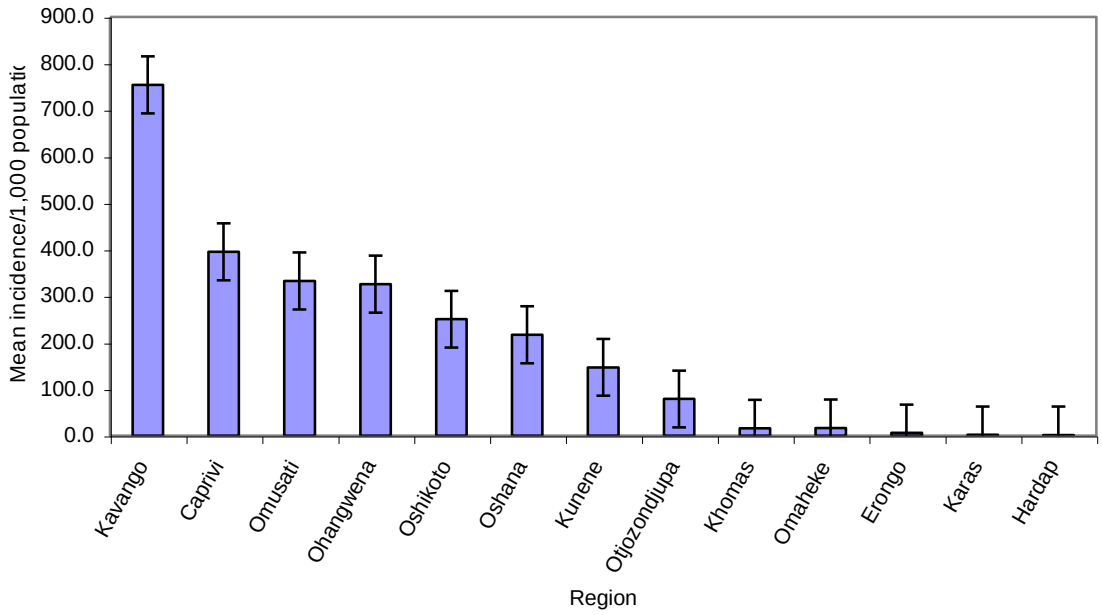


Figure 6: Mean malaria incidence per 1000 population by region, 1995-2003 in Namibia. Data was obtained from Outpatient Department. Bars represent standard error of mean. Source: MoHSS

Figure 6 demonstrates that malaria incidence is exceptionally higher in the Kavango region, followed by Caprivi, Omusati, Ohangwena, Oshikoto, Oshana, Kunene and Otjozondjupa regions.

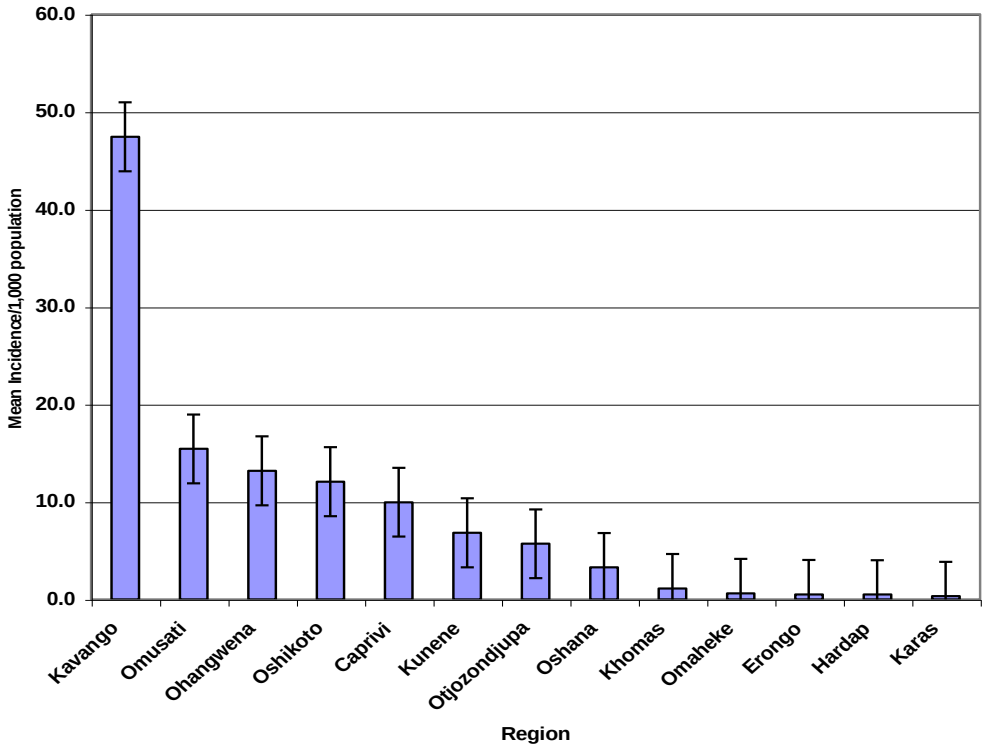


Figure 7 Malaria incidence per 1,000 population by region, 1995-2003 in Namibia. Data was obtained from patients admitted in health centers and hosptials in each region. Bars represent standard error of mean. Source: MoHSS

Figure 7 shows that the incidence of inpatient confirmed malaria cases were highest in Kavango region followed by Omusati, Ohangwena, Oshikoto, Caprivi, Kunene, Otjozondjupa and Oshana regions respectively.

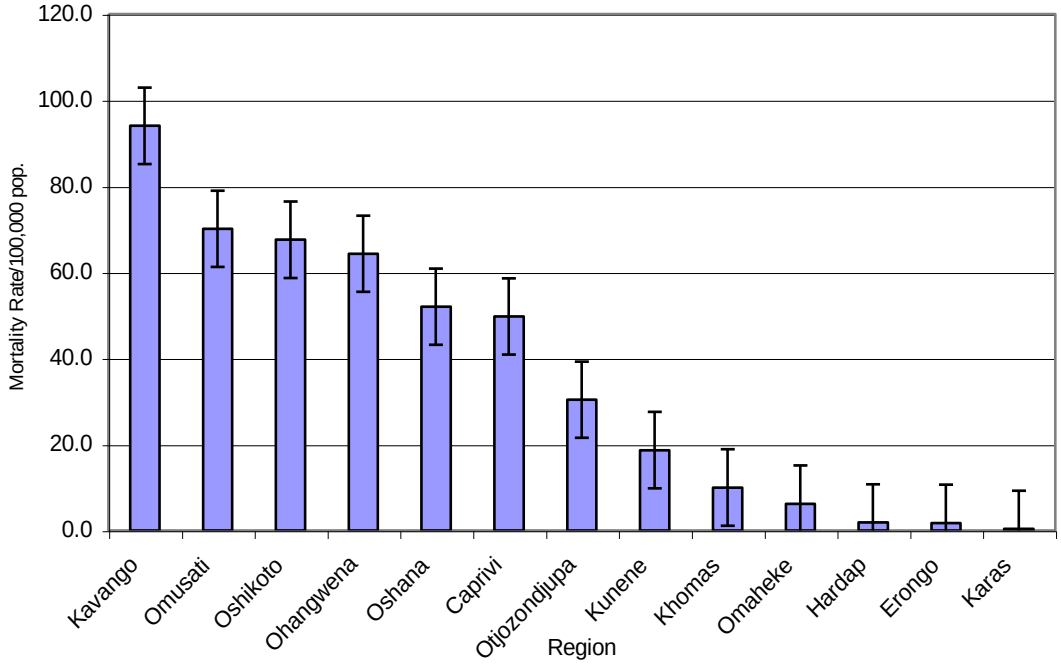


Figure 8: Mean annual mortality rate per 100,000 populations due to malaria by region in Namibia 1995-2003. Bars represent standard error of mean (pop.=population). Source: MoHSS

The mean hospital and health facility based mortality rate due to malaria was highest in Kavango region followed by Omusati, Oshikoto, Ohangwena, Oshana, Caprivi and Otjozondjupa regions had the highest mortality rate in descending order. As shown in the Figures 6 to 9, Kavango region has consistently shown the highest rates of mortality and morbidity due to malaria. The incidence of malaria in different regions of Namibia for outpatients of different ages is presented in Figure 9.

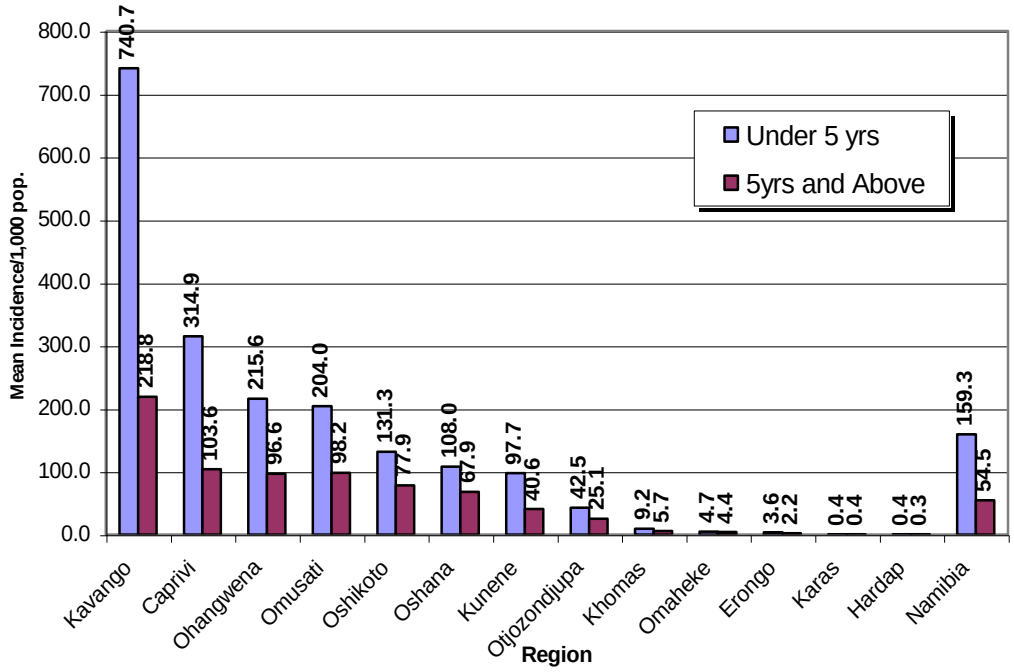


Figure 9 – Mean incidence of malaria per 1,000 population by age group, Namibia, 1995-2003 (outpatient department data). Source: MoHSS

Figure 9 shows the mean incidence of malaria by age group and by region. The incidence of malaria among under five-age group as compared to the five years and above age group was 3, 2.5, 2.4, 2.2, and 2.1 times higher in Kavango, Caprivi, Kunene, Ohangwena, and Omusati regions respectively. While the ratio of malaria incidence ranged between 1.7: to 1:1 in the remaining regions.

Figure 9 reveals that the under 5 years group is most affected with malaria in Kavango and Caprivi Regions, with a more than three fold burden of malaria among the young children. This is an indication that malaria is relatively more stable in Kavango and Caprivi regions as compared to other

parts of the country such as Kunene, Ohangwena, Oshikoto, Oshana and Omusati regions.

The contrast in malaria prevalence between the two northeast regions (Kavango and Caprivi) and the rest of the endemic regions is quite conspicuous.

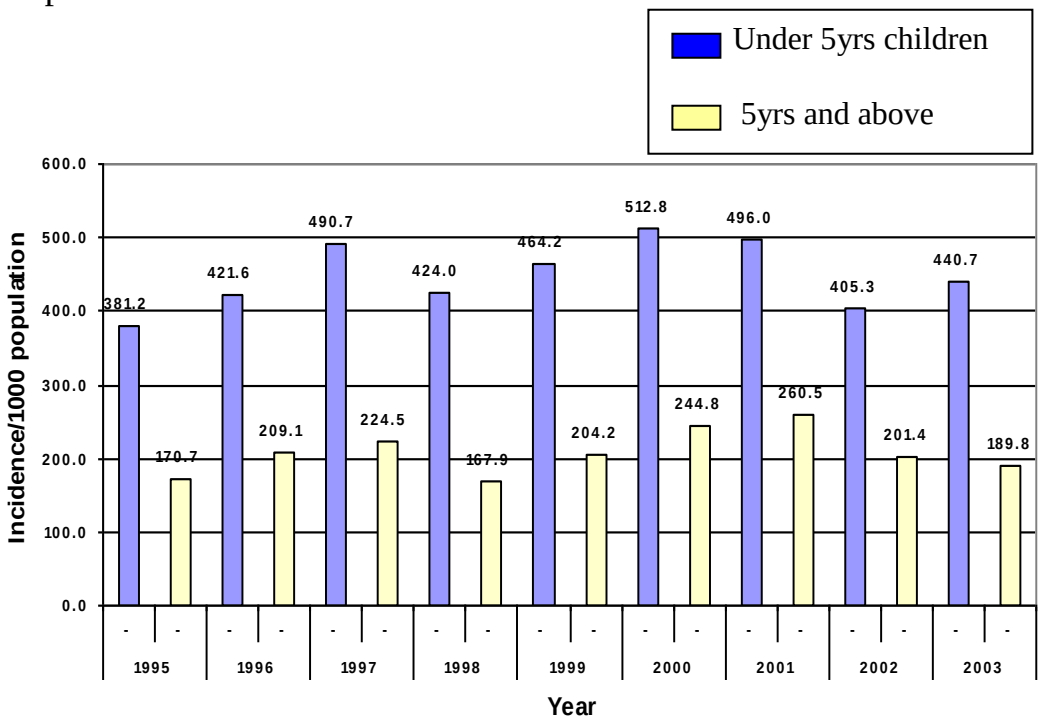


Figure 10: Incidence of malaria per 1,000 population by age group and year, Namibia, 1995-2003. Data was obtained from outpatient department. Source: MoHSS

Figure 10 shows the annual malaria incidence by age group and year at the national level. It shows that malaria on average, affects the under-five age group three times as much as the over five years age group.

3.6 Efficacy of Chloroquine (CQ) in the treatment of *Plasmodium falciparum* malaria

3.6.1 General characteristics of the study population

The chloroquine efficacy study includes 26.5% of the patients registered in all the study sites. The male to female ratio was 0.9:1. About 70.5% of the patients registered were from rural areas around the study sites.

Table 3.14: Distribution of study population by age group and study site, Namibia, 2003

Age	Katima		Outapi		Total (Outapi+Katima)	
	No.	%	No.	%	No.	%
U5	7	19.44	6	20.69	13	20.0
5+	29	80.56	23	79.31	52	80.0
Total	**36**	**100.00**	**29**	**100.00**	**65**	**100.00**

Table 3.15. Distribution of study population by gender and study site, Namibia, 2003

Sex	Katima		Outapi		Total	
	No.	%	No.	%	No.	%
Male	17	47.22	14	48.28	31	47.7
Female	19	52.78	15	51.72	34	52.3
Total	**36**	**100.00**	**29**	**100.00**	**65**	**100.00**

Only 20% of the subjects were children under the age of five years while those 5 years and above constituted 80% of the study population. The history of antimalarial drug use in the two weeks prior to recruitment into the study was reported by 7 of the 98. (7.1%) in the chloroquine study group.

3.6.2 Clinical findings

As shown in Figure 11, on Day 0 (first day of visit), over 90% of the patients reported fever in all the study groups, which declined significantly to 10%-15% by day 2 and to 1%-5% by day 3. Vomiting was reported by 14% on day 0, 36% on day 1 and 4% on Day 3 and 0% on Day 7. No history of convulsion was reported by any of the study subject.

On clinical assessment, on Day 0 (first day of visit) all patients had axillary temperature of $\geq 37.5^0$C. By Day 2, axillary temperature had dropped to less than 37.5^0C to 92.3% and 81.8% of patients in Katima Mulilo and Outapi study sites respectively.

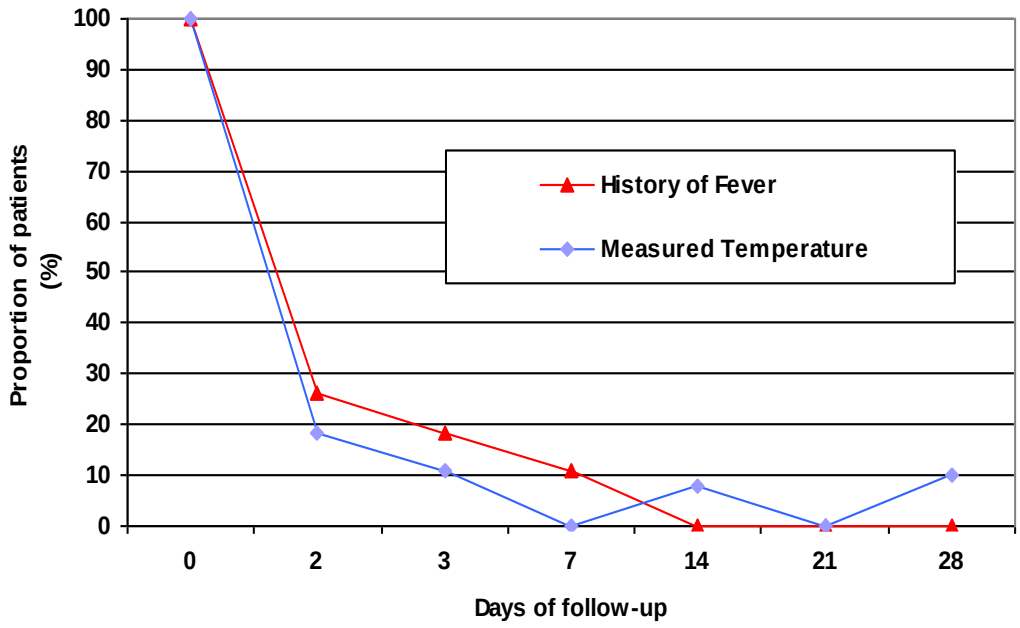


Figure 11: Proportion of study subjects with fever (body temperature as reported by patients and measured axillary temperature of $\geq 37.5^0$C) by follow-up days, Katima Mulilo study site, 2003

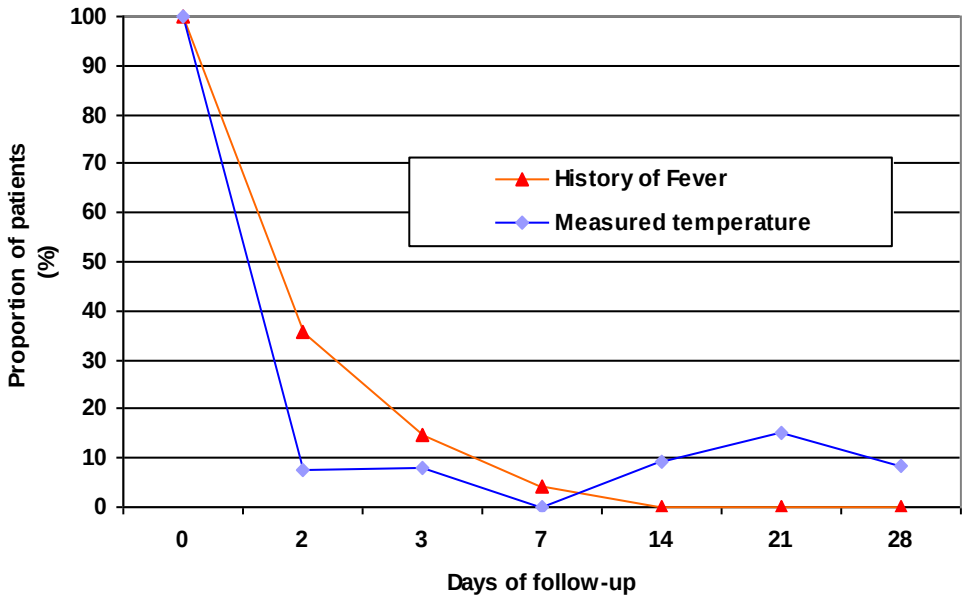


Figure12: Proportion of study subjects with fever (body temperature as reported by patients and measured axillary temperature of $\geq$ 37.5^{0}C) by follow-up days, Outapi study site, 2003.

In both study sites the parasite density, i.e. number of parasites per microliter of blood, on Day 0 ranged from 1,920 to 70,000/µl. This decreased to 0-25,600/µl by Day 3.

3.6.3 Treatment outcome

Table 3.16. Treatment outcomes in patients treated with chloroquine after 28 days of follow-up, in Outapi and Katima Mulilo study sites, 2003

Treatment Outcome	Katima Mulilo		Outapi	
	No.	**%**	**No.**	**%**
ETF[1]	12	38.7	5	18.5
LCF[2]	1	3.2	2	7.4
LPF[3]	8	25.8	8	29.6
APCR[4]	10	32.3	12	44.4
Total-Analyzed	**31**	**100**	**27**	**100**
Defaulters	4	11.1	2	6.7
Excluded[5]	1	2.8	1	3.7
Total Sample	**36**	**86.11**	**30**	**90**

There were nine (9) Treatment Failures (TFs) in the Katima Mulilo study in the first stage sample of 16 patients. Five of these were Early Treatment Failures (ETFs) and the remaining four were either Late Parasitological Failure (LPF) or Late Clinical Failures (LCF). Hence, the Treatment Failure rate for CQ was 67.7% (Table 3.16).

Similarly, in the Outapi study a total of 9 Treatment Failures (TFs) were recorded among the first 16 patients. Out of these TFs only 2 were Early Treatment Failures (Table 3.16).

This implies that in both study sites the TF rate is not significantly lower than 25% (WHO cut of point for acceptable treatment failure rate).

[1] ETF-Early Treatment Failure
[2] LCF-Late Clinical Failure
[3] LPF-Late Parasitological Failure
[4] APCR-Acceptable Parasitological and Clinical Response
[5] Excluded- patient exluded from the study due to clinical conditions that endanger the patient's life

CHAPTER FOUR: DISCUSSION

The recent upsurge of malaria in northern Namibia may be attributed to several factors, including good summer rainfall in recent years and the rising of *P. falciparum* resistance to chloroquine (Freese *et al*., 1991). Preliminary data presented during the National Malaria Policy Consensus Building Workshop, Ministry of Health and Social Services in 2004 also suggest a significantly reduced efficacy of sulphadoxine pyrimethamine.

4.1 Adult vector mosquito species in malaria transmission areas in northern Namibia

The distribution of adult mosquitoes in hut clusters from January to May in Kalimbeza village showed a population concentration around a stream of water out-flowing from Zambezi River where breeding took place, KM1-KM5 being an example (Table 3.1). The mosquito density in each hut cluster was higher in huts within the buffer zones of habitats, which had high larval densities. For instance, even though KM3 was sprayed, because it was close to the breeding site (stream) there were more mosquitoes collected in this exit window trap than was the case in KM12 an unsprayed hut (Annex 3) but far away from the stream, an occurrence also reported by le Sueur *et al*., (1988) and Sharp *et al.*, (1992). The trend of mosquito distribution reported here depicted that of rainfall pattern and floods in the Caprivi and Kavango Regions.

The mosquito population was generally low during the dry season although relatively high density was noted in huts close to the stream. The distance

from the point source of mosquito emergence had a weak correlation with mosquito density in hut cluster that were outside the stream (channel). For example, KM14, KM15 and KM17 which were furthest from any productive habitats recorded nil and 2 and 1, respectively during the entire study period.

The characteristics of individual huts could also have been responsible for the differences in mosquito population in the huts. For example, the number of people sleeping in each hut and their ages, the size and position of eave gaps and the availability of cattle, goats, sheep etc. were also considered as possible confounders that may have acted as bait to attract mosquitoes to individuals' huts. Mnzava and others reported similar findings in areas of predominantly *An*. *Arabiensis* in northern Tanzania and in Baringo, Kenya (Mnzava *et al.*, 1994; Mnzava *et al.*, 1995). This was observed in huts KM13-KM20 where there were less mosquitoes collected with an exception of KM16, which was an unsprayed hut close to cattle kraals. It is important to note that the data for mosquitoes collected was small, thus it would be advisable for researchers to pursue with this experiment.

4.2 *Anopheles gambiae* group of species identification, biting and resting behaviour

The species composition of a subsample (n=1289) of adult mosquitoes (Table 3.1) collected from Kalimbeza village by means of exit window traps identified by means of PCR technique shows that 89% were *An. arabiensis*, 5% *An. funestus* group and 11% were others which includes

Culex quinquefasciatus. The other species included in *An. funestus* group were *An. van eeden* and *An. rivulorum*, whereas in *An. gambiae* species there was only *An. arabiensis*. The low numbers of *An. funestus* group caught from exit window traps in Kalimbeza village could be related to the house-spraying activities with DDT. In fact, possibilities do exist that the *An. funestus* in this case could be from the Zambian side where there is no insecticide spraying. Namibia and Zambia share a common border and share Zambezi River on the north-eastern with an imaginary line on the northern side of the border. This study also revealed that *An. funestus* remains abundant (Table 3.3) in nearby Calueque an unsprayed area in southern Angola and yet no *An. funestus* were collected in Mahenene, on the Namibian side in a DDT sprayed area. This agrees with the findings of Sharp and le Sueur (1996) that *An. funestus* was eliminated from South Africa by DDT house-spraying in the 1950s.

Further, the results of adult composition of the *An. gambiae* group in this study were consistent with those reported by De Meillon (1950), Kuschke (1968), Coetzee (1987) and Meek (1991). Similarly, Green (1992) and Service (1993) also reported *An. arabiensis* to be the dominant species followed by *An. funestus*, a pool breeder, in Kavango and Caprivi. *Anopheles quadriannulatus* was equally identified in this study confirming the findings of Service (1993). This species is strongly zoophagic and is not a vector of malaria. Its presence, however, can create confusion because it is morphologically indistinguishable from *An. gambiae* and *An. arabiensis*, and control efforts may unnecessarily be directed against it (Service, 1993).

Unlike the findings of La Grange (La Grange, 1988), in which both *An. arabiensis* and *An. gambiae sensu stricto* were identified, in the present study, however, no *An. gambiae* species was found. This discrepancy could be explained by the fact that the species might have been genuinely missed in the present study, or due to the overlapping of the diagnostic electrophoretic markers in the Octanol Dehydrogenase – ODH loci (Mnzava and Kilama 1986), the few *An. gambiae* identified by La Grange (1988) could have been *An. arabiensis* as they were not confirmed by the cytogenetic method which was the golden standard then (Coluzzi *et al.*, 1979).

Resting places for anopheline mosquitoes are frequently inside houses. Female mosquitoes commonly enter a house/hut after dark, take a blood meal, and then, being heavily engorged with blood, fly to a nearby wall or ceiling where they normally rest until all blood is digested and they are gravid and thus ready to fly outside to lay eggs. According to Service (1993), female mosquitoes frequently prefer the lower portions of the interiors of huts/houses where temperatures are lower and the humidity is higher.

It is obvious that house-resting mosquitoes are of special importance in relation to control methods using residual insecticides. DDT insecticide indoor residual spraying and case management are the key strategies for malaria control in Namibia.

Although the sample size (*n=28*) for patients from Calueque, southern Angola who tested positive or negative for *P.falciparum* was smaller than

Namibian sample size (*n=1390*) results of this study shows that the prevalence of malaria is higher in unsprayed areas of southern Angola (Tables 3.13) than the sprayed northern Namibia. The other aspect which was demonstrated by this study is the fact that gametocytes can prevail in human blood both during dry and wet season of the year (Gilles, 1993).

It is interesting to note that these findings agree with studies carried out by the National Institute for Tropical Diseases in Tzaneen, South Africa which showed a much higher malaria prevalence and mosquito density in villages in Angola, where there was no spraying, than in neighbouring villages in Namibia, where sparying was done (Le Grange, 1988). This suggests that the malaria vectors in northern Namibia are susceptible to the current insecticidal residual spraying.

In May 2004, the Stockholm Convention on Persistent Organic Pollutants became operational. While enforcing strict measures to reduce environmental damage from persistent organic pollutants, the Convention stated that DDT is still needed in some countries for disease vector control. WHO recommends that countries select the insecticide for IRS based on local stuation analysis; DDT is one of the 12 insecticides that can be used for this purpose (WHO, 2005).

4.3. The mosquito vector density, seasonal variation and malaria prevalence

Of the 20 exit window traps, 15 were set in sprayed huts and 5 in unsprayed huts. The exit trap collections of adult mosquitoes showed that a substantial

number leave houses either as unfed, bloodfed or half-gravid with only a small proportion exiting as gravid. It is interesting to note that 36% of the total unfed adult mosquitoes were collected in unsprayed hut No KM1 compared to only 2.5% of the unfed adult mosquitoes collected in a sprayed hut No KM2 which was about 10 metres away from each other. This demonstrates that mosquitoes rested more in unsprayed huts as compared to sprayed huts. In addition, it was observed that of the 20 exit window traps, traps in sprayed huts KM14, KM15 and KM17 did not collect a single mosquito for the whole year. However, it is important to note that only 5 huts were unsprayed as compared to 15 sprayed huts. Thus these data do not give conclusive results. This is a challenge to the National Vector-borne Disease Control Programme and to the up-coming researchers.

The results in Table 3.1 call for further studies to compare with the findings reported by Sharp *et al.* (1990) in KwaZulu Natal, South Africa. In his study, Sharp and others caught more than 50% bloodfeds in exit traps in control and replastered huts and 32.6% in DDT sprayed huts as compared to only 17.9% reported in this study in sprayed huts. The results in Table 3.4 may suggest that some degree of indoor resting occurs after a blood meal in all huts in Kalimbeza village irrespective of the spray status. Although the data were small for unsprayed huts, they suggest that some vectors may have learnt to avoid sprayed surfaces indoors i.e. they do not rest (before and after blood meal) long enough to pick up a lethal dose (behavioural resistance).

The behaviour of a vector mosquito is a key factor in determining the outcome of a malaria control strategy based on house-spraying with residual insecticide spraying. *An. arabiensis*, which is the main malaria vector in northern malarious regions, exhibits behavioural polymorphism and has varied resting and feeding behaviours (Service, 1985; Sharp *et al*., 1990).

Although the numbers of mosquitoes reported in this study were relatively low as a representative sample size for the northern regions to warrant any conclusive observations to be made, nonetheless, these results may be indicative of changing the control measures and therefore require further studies to confirm. The most important findings are those of the gravid (Table 3.4) components which suggest there could be more indoor resting in northern Namibia than has been reported to date in KwaZulu/Natal, South Africa. However, this study was carried out in one village only (Kalimbeza) in Caprivi region as compared to that of Sharp *et al*. (1990), which comprised the whole of the Mamfene area of KwaZulu/Natal, South Africa. Thus, the results obtained in the current study need to be interpreted cautiously until supported by further research based in all the malarious regions of Namibia.

The seasonal abundance of mosquito vectors for Andara and Kalimbeza villages showed that 85% of female *An. gambiae* group were caught during the wet season compared to 15% caught during the dry season (Table 3.5.). At the onset of the cold season individuals of some species are killed off. They may then be found only at lower altitudes where temperatures are still suitable. According to Service (1993), in some species the males only die, while females seek shelter in protected sites from the cold (*hibernation)*.

Seasonal changes such as temperature, rainfall and humidity have an obvioius effect on anopheline populations and thus also on the incidence of malaria (Service, 1993). In most tropical countries breeding continues throughout the year, but in the dry season the population is very small due to paucity of breeding places; with the onset of rainy season numerous breeding places are created and the anopheline population increases explosively.

The distribution of the other species seems to follow the pattern of *An. arabiensis*. In Table 3.6 for example, it can be seen that 99% of the *An. arabiensis* were collected during the wet season as compared to 1% during the dry season. As expected, the abundance of *An*. *arabiensis* was rainfall dependent (White, 1974).

It is important to note that *C.quinquefasciatus*, a vector of lymphatic filariasis due to *Wuchereria bancrofti*, is the most abundant mosquito species in Namibia readily biting humans and is a common mosquito nuisance worldwide (Bushrod, 1981). This species is capable of breeding in polluted water. It is a mosquito associated with urbanization especially in towns with poor and inadequate drainage and sanitation (Service, 1996). Its population multiplies under these conditions. *C.quinquefasciatus* mosquitoes were collected in all the study sentinel sites. Since one of the requirements for the transmission of lymphatic filarias is high humidity (Rwegoshora *et al.*, 2005), its absence in Namibia is not therefore unusual.

4.4. The relationship between fevers and clinical malaria

This study addressed the relationship between fevers and clinical malaria at Bukalo and Mahenene health centres. It was clear from the results that the predominant human malaria species remains *P. falciparum* accounting for more than 97% of the blood smears examined. This is in agreement with the findings of Scrimgeour (1991) and Payne (1993), who reported *P. falciparum* to account for over 95% of all the recorded cases of malaria in Namibia. *P. malariae, P. ovale* and *P. vivax* make up for the remainder.

The study revealed that the correlation coefficient between having fever and having positive blood smear result was r = 0.10 (p = 0.71) which clearly is not significant. The Chi-square statistic for the same is 0.138 (*df* =1, p = 0.710) which is equally not significant. The current situation is that malaria diagnosis in clinics and in most of the health centres is based on clinical symptoms of reported fever. This therefore calls for the Ministry of Health and Social Services to consider introducing microscopes and well trained technicians to do the blood smear tests at least in health centres. The WHO currently restricts treatment based on clinical symptoms to children under the age of five years only - in the absence of microscopy and/or rapid diagnostic tests (RDTs) due to current treatment policies with expensive ACTs (International Artemisinin Study Group, 2004)

The results for cross-sectional surveys for patients who tested positive or negative for *P.falciparum* during wet and dry season did not show much difference. For example, Katima Mulilo had a total of 982 blood smears taken during the wet season. Of this, 924 (94%) were negative with 58 (6%)

positive. Similarly 987 blood smears were taken during the dry season. Of this, 925(94%) were negative and 62 (6%) were positive. The majority of the positive cases during the dry season were in fact gametocytes. This is an evolutionary strategy by the malaria parasites to ensure their survival and their pick-up by mosquitoes at the beginning of the transmission season (Bruce-Chwatt 1988).

4.5. Therapeutic efficacy of chloroquine

The National Policy and Strategy for Malaria Control (MOHSS, 1995), recommended chloroquine as the drug of choice for the treatment of uncomplicated *P. falciparum* malaria. The drug efficacy study was carried out at two sentinel sites, i.e., Outapi and Katima Mulilo Hospitals. The results revealed that there were 9 Treatment Failures (TFs) in the Katima Mulilo in the first stage sample of 16 patients. Five of these were EFs and the remaining four were either Late Parasitological Failures (LTF) or Late Clinical Failures (LCF). Unfortunately, it was not possible to continue recruiting the required 42 sample size to get a conclusive result from this study due to very few cases at the end of the malaria transmission season. Hence, the Treatment Failure rate for CQ was 67.7%. The findings of this study calls for more studies to be done by the National Vector-borne Disease Control Programme and researchers alike considering drug change. Similarly, in the Outapi study a total of 9 TFs were recorded among the first 16 patients. Out of these TFs only 2 were Early Treatment Failures (ETFs).

This implies that in both study sites the TF rate is not significantly lower than 25% (WHO cut of point for acceptable treatment failure rate).

According to Trape (Trape, 2001), between 1978 and 1988 *P. falciparum* resistance to chloroquine has been reported in most countries of tropical Africa. In fact hospital studies in various African countries have documented a 2 or 3 fold increase in malaria deaths and admissions for severe malaria, an increase temporarily related to the emergence of chloroquine resistance (WHO, 2004). This therefore calls for an urgent need to change treatment policies in Africa (WHO, 2005). Based on this, a number of countries in Africa have already changed their malaria treatment policies.

According to Verhoeff *et al*., (1997) and Roper *et al.,* (2003), the first African country to introduce SP for treatment of uncomplicated *P. falciparum* malaria and to abandon chloroquine was Malawi. However, the province of KwaZulu-Natal in South Africa replaced chloroquine with sulfadoxine-pyrimethamine (SP) as a first-line therapy in 1988. Thus, the population in KwaZulu-Natal was exposed to first-line SP selection early. In 1996, results of a study based at Mosvold hospital in the Ingwavuma district of KwaZulu-Natal showed that 20% of patients given SP did not clear the parasites within 14 days of treatment.

By 2000 this proportion had increased to 70%, whereon the province switched to using coartemether (Roper *et al*., 2003). The recent unpublished report by the Namibia's Ministry of Health and Social Services (MoHSS) on studies of the therapeutic efficacy of SP has shown some decline in efficacy in the districts of Outapi (APCR=73.3%) and Rundu (APCR=79.7%) study sites. This observation is above the limit of 25% as

set by WHO. However, this report found the efficacy of SP for Katima Mulilo to be high (APCR=90.9%). The Malaria Policy Review Committee has recently recommended to the MoHSS to consider changing from CQ to Artemether/lumefantrine (Coartem).

The most important issue is the implication that the above findings have for malaria control in Namibia and southern Africa in general. Countries in this sub-region have malaria control programmes based on vector control in combination with rapid case detection, as is the case with South Africa and effective treatment. Although all components of the programme are important for the control of the disease, overall success is dependent on the reduction of transmission brought about by the control of the 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. For many years, the insecticide of choice was DDT, and in some countries such as Namibia, South Africa, Swaziland and Ethiopia DDT is still preferred. In South Africa, since 1996, the malaria control programme had shifted to pyrethroids, regarded as more environmentally acceptable, not being bio-accumulative, not staining walls and in some instances, having less excito-repellancy effects than DDT (Hargreaves, *et al*., 2000). But this had a reverse effect with resurgence of *An. funestus* in KwaZulu-Natal (Hargreaves, *et al.*, 2000). To date South Africa has shifted back to DDT residual spraying (Mabaso *et al.,* 2004).

For anopheline mosquitoes, Malcolm (1988) concluded that physiological resistance to pyrethroids was not as widespread as had been suggested, and the risk of cross-resistance between pyrethroids and DDT may have been

over-emphasized. Thereafter, it was reported that a single mutation in the S6 transmembrane segment of domain II in the sodium channel sequence is associated with knockdown resistance (kdr) to both pyrethroids and DDT in the housefly, *Musca domestica* (Williams *et al.*, 1996). KDR is common in *An.gambiae* in West Africa with only a single report from East Africa (Kenya).

Having noted that, some difficulties are experienced by the vector control programme in Namibia, a number of key issues need to be taken into consideration. Firstly, where compliance with indoor residual house-spraying is low, communication strategies to highlight its importance needs to be incorporated into the national vector control activities. Secondly, the use of ITNs, which has taken a very low profile in Namibia need to be introduced carefully accompanied by well designed promotional efforts. This will not only take care of serious potential threat to vector resistance to pyrethroids in current use (Etang *et al.*, 2004) but more importantly address problems of compliance with indoor residual house-spraying, if they arise.

4.6. Retrospective malaria data analysis

Analysis of the retrospective malaria data in the country (Figures 6 to 9) demonstrated that malaria incidence was highest in Kavango region followed by Omusati, Ohangwena, Oshikoto, Caprivi, Kunene, Otjozondjupa and Oshana respectively. On the other hand, the analysis of mortality data revealed that Kavango, Omusati, Oshikoto, Ohangwena, Oshana and Caprivi regions experience the highest rates in descending order.

The data revealed that the under five-age group in Kavango and Caprivi were three times more frequently affected than the five years and above age groups (Fig 9). This is an indication that malaria is relatively more stable in Kavango and Caprivi regions as compared to other malaraia endemic regions. On the other hand, malaria remains unstable in the mainland in these two regions for reasons that the inhabitants do not have sufficient immunity. This ties well with the fact that the majority of the population lives in mainland far from the perennial rivers where transmission is limited between November and May. Nevertheless, when temperature is conducive breeding may take place along the rivers, resulting in short-lived transmission.

Malaria transmission in an area may be classified as stable and unstable (Cheesbrough, 1991). Stable malaria occurs in an area where malaria transmission occurs for at least 6 months in a year and is intense. In such areas, children suffer repeated attacks from the age of a few months. Those who do not die, have a substantial immunity by the age of five or six years. When immunity is established, patients may still suffer attacks of malaria but these are comparatively mild and last for only a few days. Older people are little affected. There is little variation in the incidence of malaria from year to year although there may be marked seasonal fluctuations (Cheesbrough, 1991; Gilles, 1993). The trend of malaria transmission in Kavango and Caprivi is such that the people living along the Kavango, Zambezi and Chobe Rivers experience stable malaria. In contrast, the people who live in mainland experience unstable malaria. Mosquito breeding takes place at all times along these rivers once temperatures peak

up above 20^{o}C (*Kamwi, personal experience*).

In unstable malaria the amount of transmission varies from year-to-year. The transmission season is short and infection of any one individual is comparatively infrequent so that immunity is unable to reach a high level. When an outbreak of malaria occurs, usually following explosive breeding of mosquitoes, it does so in the form of an epidemic with people of all ages being susceptible and often severely at risk (Gilles, 1993). This is the situation experienced in Oshana, Omusati, Oshikoto, Ohangwena, Otjozondjupa, Kunene, part of Omaheke and mainland of Kavango and Caprivi Regions.

As mentioned above, the data (Figures 9 and 10) suggest that malaria in Kavango region is more prominent in the age group below five years than those five years and above. The age group below five years do not have immunity even in areas of stable malaria. The acquision of immunity is slow. In areas of high malaria transmission, in the first few months of life, an infant is normaly protected by IgG antibodies from its mother and by the high concentration of haemoglobin F in its red cells (Cheesbrough, 1991). Following repeated infection, an infant normally acquires a natural immunity to malaria by about the age of 5 years. The contrast between the Kavango and the other endemic regions is quite striking. The majority of the residents in this region live along the Kavango River, which is perennial with relatively stable transmission resulting in some protective immunity in the adult population. The remainder live in mainland and are less affected by malaria. By contrast malaria affects under-five age group two to three times as much as adult population in the four regions of Omusati,

Ohangwena, Oshana and Oshikoto. This suggests that malaria transmission in these regions is less stable.

The southern part of Namibia has the lowest incidence, as most of the region is free of indigenous malaria transmission. Malaria transmission does not take place in southern Namibia including Karas, Erongo, Haradap and part of Omaheke Regions for reasons that the rainfall pattern and humidity are not conducive for malaria vector breeding (Obeid *et al.*, 2001). However, according to the Health Information Report (1999-2001), malaria has increased in central and southern parts of the country where it was rare before.

In Namibia, malaria epidemics were registered at increasingly frequent intervals than before i.e. in 1990, 1996, 1997, 2000 and 2001. These results compel the malaria control programme to strengthen selective vector control in the northern regions of Namibia. Similarly, there is a need to consider introducing treated nets to pregnant women and children under the age of five years who live in the malarious regions.

4.7. Conclusions and recommendations

Work carried out in this project has very important application to the control of malaria in Namibia and elsewhere, as follows:

1. According to this study, the most abundant species of malaria vector collected was identified as *An. arabiensis* (Table 3.1). This species appears to be the significant malaria vector within the *An. gambiae* group in northern Namibia. An insignificant number of *An. funestus* was also collected from Andara, Kangongo, Outapi and Kalimbeza sentinel sites. For this reason, there is a need to explore further the presence of *An. funestus* along the boarder of Kavango and Angola and that of Caprivi and Zambia. Further investigation is needed to establish whether or not this species is indigenous. The possibility of introduction from Angola and Zambia cannot be completely ruled out thus calling for closer malaria border coordination activities. The susceptibility status of *An. funestus* to insecticides needs also to be monitored.

2. The proportion of adult blood fed mosquitoes caught in exit window traps is worrying (Table 3.2 and 3.4). A high frequency of blood fed and survivorship indicate that a large proportion of indoor biting mosquitoes are no longer resting indoors and are therefore not coming into contact with the sprayed insecticide. This suggests that this fraction of mosquitoes is capable of transmitting malaria even in the presence of the insecticide. This could have some negative impact to the control programme. The challenge remains for the National

Vector-borne Control Progarmme to further monitor the proportion of vectors resting in-doors and out-doors. And based on their findings, they should respond appropriately.

3. The results of this study suggests that the current insecticides (DDT, deltamethrin and permethrin) used by the National Vector-borne Disease Control Programme are effective against the local malaria vectors - *An. arabiensis* and *An. funestus* group (Table 3.3). The continuation of the use of these insecticides for malaria vector control in Namibia is recommended as long as the vectors continue to be susceptible to these insecticides. To ascertain this, the vector control programme would need to strengthen its current capacity to monitor insecticide resistance in well chosen sentinel sites across the malaria endemic regions of the country.

4. Malaria remains highest in the northern regions affecting more of the under five-year age group with more than three-fold burden recorded in Caprivi and Kavango respectively (Figure 9). In contrast malaria affects the under five age group twice as much as adult population in the Ohangwena, Omusati, Oshana and Oshikoto regions (Figure 10). This suggests that malaria transmission in these regions is less stable for immunity to build up. It is therefore essential to undertake early diagnosis and treatment of cases with effective antimalarial drugs.

5. The predominant human malaria species remains *P. falciparum* (Table 3.11 & 3.12). The study has shown that the correlation coefficient between having fever and having positive blood smear

result was not significant. This means that having fever does not necessarily consititue the diagnosis of malaria. Therefore, this calls for the MoHSS to consider introducing diagnostic facilities at peripheral health facilities to minimize over-diagnosis and wastages of drugs – especially now that the current trend is to move towards the more expensive artememsinin based combination (ACTs) therapy as first line treatment for uncomplicated malaria.

6. The study has further demonstrated a much higher malaria prevalence and mosquito density in non-sprayed villages of southern Angola than in the neighbouring villages of Namibia where spraying was done (Table 3.1 & 3.13). This point to the importance of vector control interventions as one of the strategies for malaria prevention. Unlike Namibia, Zambia and Angola put a lot of emphasis on curative methods – neglecting malaria prevention by vector control.

7. The studies carried out in the two sentinel sites of Katima Mulilo and Omusati have indicated that the efficacy of chloroquine is below the acceptable limit (Table 3.16). The MoHSS recently held a national Consensus Workshop where Members of the Malaria Policy Review Committee recommended the replacement of CQ with Artemether-lumefantrine (Coartem) which is recognised as an ideal first line drug in all adults except for pregnant mothers (MoHSS, National Malaria Policy, 2005). Mefloquine replaces CQ/Proquanil combination as first line chemoprophylaxis in Namibia. In addition, the Ministry of Health and Social Services further launched a Malaria Epidemic Preparedness and Response Guidelines in April 2005 (MoHSS,

Malaria Epidemic Preparedness and Response Guidelines, 2005).

8. Given the current malaria situation in Namibia, and the fact that malaria vector control is the main prevention strategy, there is a need to strengthen the national capacity to be able to plan, implement, monitor and evaluate the vector control interventions in place. There is indeed a need for the Government of Namibia to continue to allocate adequate resources for capacity in entomology and vector control, resources for strengthening of physical infrastructures and for operations.

9. Currently, there is a general trend of most malaria endemic countries to decentralize their health services to the lowest administrative levels (Cueto, 2004). The advantages of this policy especially in the diagnosis and curative services are obvious. It should be pointed out, however that, most vector control interventions – especially insecticide residual spraying – (the main vector control method employed in Namibia), are usually of a specialized nature. Retaining these interventions as vertical as possible is the most cost-effective approach.

REFERENCES

Ameneshewa B., E. Awaseb, Haidula L. and A. Ekandjo (2000). *Report on a mission to two malarious regions of Namibia to support the regions in vector control and initiate Roll Back Malaria Initiative*; 15-27 October 2000, Windhoek Namibia.

Alonso P.L., S.W. Lindsay, J.R.M. Armstrong, M. Conteh, A.G. Hill, P.J. David, G. de Fegan, A. Francisco, A.J.Hall, F.C. Shenton, K. Cham, and B.M. Greenwood (1991). The effect of insecticide-treated bed nets on mortality of Gambian children. *Lancet*, **337:** 1499-1502.

Ayala F.J. A. A. Escalante and S. M. Rich (1998) Evolutionary relationships of human malaria parasites. In: *Malaria: parasite biology, pathogenesis, and protection* (Sherman IW,ed). ASM Press, Washington DC, USA, pp 285-300.

Balfaour A. (1913). A year's antimalarialwork at Khartoum, Trop.Med. Hyg., **1,** 1: In: *Malaria control by application of indoor spraying of residual insecticides in tropical Africa and its impact on community health.* (Kouznetsov R. L. ed). Division of Malaria and other Parasitic Diseases, WHO, Geneva.

Beales P.F., V.S. Orlov. and R.L. Kovzaetsov (1989). *Malaria and Planning for its Control in Tropical Africa*. Moscow: World Health Organization / United Nations Development Programme.

Bennett S. M.K. Aikins, M.C. Thompson, M.K. Cham, B.A. Cham, and B.M. Greenwood (1995). Mortality and morbidity from malaria in Gambian children after introduction of an impregnated bed net programme. *Lancet*, **345:** 479-483.

Black IV W.C. and L.E. Munstermann (1996). Molecular taxonomy and systematics of arthropod vectors. In: *The biology of disease vectors*.(eds) Beaty B.J. and Marquardt W.C. University Press of Colorado.

Bosman A., Y. Kassankogno and A.V. Kondrachine (1999). Implementation of malaria control. *Parassitologia* **41:** 391-393.

Bouwman H., A.J.Reinecke, R.M. Cooppan and P.J. Becker (1990). Factors affecting levels of DDT and metabolites in human breast milk from Kwazulu. *Journal of toxicology and environmental health,* **31:** 93-115.

Bouwman H., A. Coetzee and A. Schutte (1990). Environmental and health implications of DDT-contaminated fish from the Pongola Flood Plain. *J Afr Zool* **104:**275-286.

Bouwman H., R.M. Coopan, P.J. Bekker and S. Ngxongo (1991). Malaria control and levels of DDT in serum of two populations in KwaZulu. *J. Tox. Env. Hlth*., **33**: 141-155.

Bouwman H., P.J. Becker, R.M. Cooppan and A.J. Reinecker (1992). Transfer of DDT used in malaria control to infants via breast milk. *Bulletin World Health Organization.* **70:** 241-251.

Bouwman H., C. and H.J. Schutte (1993). Effect of sibship on DDT residue levels in human serum from a malaria endemic area in Northern KwaZulu. *Bull. Envron. Contam. Toxicol*. **50:**300-307.

Bouwman H., P.J. Becker and C.H.J. Schutte (1994). Malaria control and longitudinal changes in levels of DDT and its metabolites in human serum from KwaZulu. *Bulletin of the World Health Organization*, **72** (6) **:** 921-930.

Brooke B.D., R.H. Hunt and M. Coetzee (2000). Resistance to dieldrin + fipronil assorts with chromosome inversion 2la in the malaria vector *Anopheles gambiae*. *Medical and Veterinary Entomology* **14,** 190-194.

Brooke B.D., G. Kloke, R.H. Hunt, L.L. Koekemoer, E.A. Temu, M.E. Taylor, G. Small, J. Hemingway and M. Coetzee (2001). Bioassay and biochemical analyses of insecticide resistance in southern African *Anopheles funestus* (Diptera: Culicidae). *Bulletin of Entomological Research* **91,** 265-272.

Brooke, B.D., R.H. Hunt and M. Coetzee (2000). Resistance to dieldrin + fipronil assorts with chromosome inversion 2La in the malaria vector Anopheles gambiae. *Medical and Veterinary Entomology* **14**, 190-194

Brown, A.W.A. (1986). Insecticide resistance in mosquitoes: A pragmatic review. *Journal of the American Mosquito Control Association,* **2,** 123-140.

Bruce-Chwatt LJ. (1988). History of malaria from prehistory to eradication. In Werndorfer W H and McGregor I (eds) *Malaria: Principles and Practice of Malariology*. Edinburgh: Churchill Livingstone, 1988: 1-59.

Bruce–Chwatt, L. J. (1991). *Essential Malariology*. 3rd edition, William Heinemann, London.

Brutland G.H. (1998). In *Parassitologia* **41**:1-3, 1999; Roma.

Bushrod F.M. (1981). The *Anopheles gambiae* Giles complex and Bancroftin filariasis transmision in a Tanzanian coastal village. *Ann.Trop. Med. Parasit.* **75:** 93-100.

Brysceson, A.D.M. 1996. Leshmanisasis. Mansons Tropical Diseases, Gordon Cook 20th Eidtion, Great Britian.

Charlwood, J.D. and P.M. Graves (1987). The effect of permethrin-impregnated bednets on a population of *An.farauti* in coastal Paua New Guinea. *Medical and Veterinary Entomology*, **1,** 319-327.

Cheesbrough M. (1991). *Medical laboratory Manual for tropical countries*, 2nd edition, **Vol**. **1:** 2-605; Butterworth-Heinemann, Cambridge.

Chinnock, P. (1997). Malaria Action at Last? *Africa Health* **19** (2): 17-24.

Clyde D. F. (1967). Malaria in Tanzania. London: Oxford University Press.

Coetzee M. (1989). Comparative morphology and multivariate analysis for the discrimination of four members of the *Anopheles gambiae* group in southern Africa. *Mosq. Syst*., **21**: 100-106.

Coetzee M., R.H. Hunt, L.E.O. Braack, and G. Davidson (1993). Distribution of mosquitoes belonging to the *An. gambiae* complex, including malaria vectors, south of latitude 15^0S. *S.Afri.J.Sci*. **89:** 227-231.

Coluzzi M., Sabatini A., Petrarca V. and M.A. Di Deco (1979). Chromosomal differentiation and adaptation to human environments in the *Anopheles gambiae* complex. *Trans. R. Soc. Trop. Med. Hyg.* **73:** 483-497.

Coluzzi M. (1984). Heterogeneities of the malaria vectorial system in tropical Africa and their significance in malaria epidemiology and control. *Bull. Wld. Hlth. Org*. **62**: 107-113.

Connor S.J., M.C. Thomson and D.H. Molyneux (1999). Forecasting and prevention of epidemic malaria: new perspectives on an old problem. *Parassitologia* **41:** 439-448.

Cook G. (1996). Manson's Tropical Diseases, Twentieth Edition, London, UK.

Cueto M., (2004). The origins of Primary Health Care and Selective Primary Health Care. *Am. J. Publ. Hlth*, **94 (11):** 1864 - 1874

Cullen, J.R., U. Chitpraprop, E.B. Doberstyn, and K. Sombatwattanangkul (1984). An epidemiological early warning system for malaria control in northern Thailand. *Bull Wld Hlth Orgion,* **62:** 107-114.

Curtis C. F. (1994). Should DDT continue to be recommended for malaria vector control? *Medical and Veterinary Entomology* **8:** 107-112.

Curtis C.F., C.A. Maxwell, R.J. Finch, and K.J.Njunwa (1998). A comparison of use of a pyrethroid either for house spraying or for bednet treatment against malaria vectors. *Tropical Medicine and International Health* **3 (**8) pp 619-631.

Curtis C.F and Mnzava A.E.P (2000). Comparison of house spraying and insecticide-treated nets for malaria control. *Bull-World-Health-Organ*. **78** (12): 1389-400.

D'Alessandro U., B.O. Olaleye, W. McGuire, M.C. Thompson, P. Langerock, S. Bennett and B.M. Greenwood (1995b). A comparison of the efficacy of insecticide-treated bed nets in preventing malaria in Gambian children. *Trans. Roy. Soc. Trop. Med.Hyg*. **89:** 596-598.

D'Alessandro U., P. Langerock, S. Bennett, N. Francis, K. Cham and B.M. Greenwood (1996). The impact of a national impregnated bed net programme on the outcome of pregnancy in primigravidae in the Gambia. *Trans. Roy. Soc. Trop. Med. Hyg*. **90:** 487-492.

Demas F.A. (1993). MSc Thesis. The effectiveness of DDT in controlling *Anopheles arabiensis Patton* in rural huts of North-western Namibia.

De Meillon B. (1936). The control of malaria in South Africa by measures directed against the adult mosquitoes in habitations, Quarterly *Bulletin of the Health Organization of the League of Nations*, **5:** 134-137.

De Meillon B. (1951) Malaria survey of South-West Africa. *Bull. Wld Hlth Org*. **4**: 333-417.

Diuk-Wasser M.A., Toure M.B., Dolo G., Bagayoko M., Sogoba N., Traore S.F., Manoukis N. and C. Taylor (2005). Vector abundance and malaria transmission in rice-growing villages in Mali. *Am. J. Trop. Med. Hyg.* **72 (6):** 725 – 731.

Etang J., Chandre F., Guillet P. and L. Manga (2004). Reduced bio-efficacy of permethrin EC-impregnated bednets against an *Anopheles gambiae* strain with oxidase-based pyrethroid tolerance. *Malaria Journal;* **3 (1)**: 46.

Freese J.A., M.B. Marcus and J. Golenser. (1991) *In vitro* sensitivity of southern African reference isolates of *P. falciparum* to chloroquine and pyrimethamine. *Bull. Wld.Hlth Org*., **69:** 707-712.

Freese J.A., B.L. Sharp, S.M. Ngxongo and M.B. Markus (1988). *In vitro* confirmation of chloroquine-resistant *P.falciparum* malaria in KwaZulu. *S.Afr.Med.J*. **74**: 576-578.

Gillies M.T. and B. De Meillon (1968). *The Anophelinae of Africa South of the Sahara* (Ethiopian Zoogeograhical Region). South African Institute for Medical Research No. 54, Johannesburg.

Gillies M.T. and M. Coetzee. (1987). A supplement to the *Anophelinae* of Africa south of the Sahara. Johannesburg, South Africa. Publ. *S. Afr. Inst. Med*. Res. No. 55.

Gilles H.M. and D.A.Warrell (1993). *Bruce-Chwatt's Essential Malariology*, 3rd edition, London, UK.

Goodman C.A, A.E. Mnzava, S.S. Dlamini, B.L. Sharp, D.J. Mthembu, J.K. Gumede (2001). Comparison of the cost and cost-effectiveness of insecticide-treated bednets and residual house-spraying in KwaZulu-Natal, South Africa. *Trop-Med-Int-Health*. 2001 Apr; **6(4)**: 280-95

Gramiccia G., P.F. Beales (1988). *The recent history of malaria control and eradication*. In: Malaria, principles and practice of malariology (WernsdorferW.H. & McGregor I, eds). Churchill Livingstone, London, UK, pp 1335-1378.

Green C.A. (1992). *Identification of malaria vectors in Namibia*. Appendix II. Unpublished consultancy report of the World Health Organization.

Greenwood B.M. (1990). Populations at risk. *Parassitologia* **6:**188.

Greenwood B.M. (1997). The epidemiology of malaria. *Ann.Trop.Med & Para*. **91** (7): 763-769.

Greenwood B.M. (1997). What is new in malaria control? *Ann. Trop.Med & Para*. **91** (5): 523-531.

Guillet P. (2004). Fifth update on long-lasting insecticidal nets: current status and programmatic issues. Geneva, World Health Organization. (*http://mosquito.who.int/docs/updateLLN_5.htm*, accessed 1st August 2005).

Habluetzel A, F. Esposito and S. Lombardi (1989). Immunotechniques for epidemiology of malaria: appropriate tools for integration of primary health care with malaria research and control. *Trans R Soc Trop Med Hyg* **83** Suppl: 15-19.

Hansford C.F. (1975) *Malaria: Report on visit to South West Africa*: 17-28 February 1975. Department of Health, Welfare and Pensions, Tzaneen, RSA.

Hansford C.F. (1987). *Malaria: Report on a visit to South West Africa*. Department of Health, Welfare and Pensions, Tzaneen, RSA.

Hargreaves K., L.L. Koekemoer, B.D. Brooke, R.H. Hunt, J. Mthembu and M. Coetzee (2000). *Anopheles funestus* resistant to pyrethroid insecticides in South Africa. *Medical and veterinary Entomology* (2000) **14:** 181-189.

Harrison G. (1978). *Mosquitoes, Malaria and Man*: A history of the hostilities since 1880. John Murray, London.

Hunt R.H. (1973). A cytological technique for the study of the *Anopheles gambiae complex. Parassitologia* **15**: 137-139.

Hunt R.H., M. Coetzee, and M. Fettene, (1998). The *Anopheles gambiae* complex: a new species from Ethiopia. *Trans.Roy.Soc.Trop.Med.Hyg*. **92:** 231-235.

International Artemisinin Study Group (2004). Artesunate combinations for treatment of malaria: meta-analysis. *The Lancet*, **363:** 9-17.

Kamwi R.N. (1993). MSc Thesis. Bioassay of mortality, feeding success and irritability of Dub – Apr and Iraq-s strains of *Anopheles stephensi* exposed to permethrin. University of Liverpool, UK.

Kassasky A. (1994). Evaluation of malaria control in Namibia. A World Health Organization Consultancy Report.

Knell A. J. (1991). *Malaria A publication of the tropical programme of the Wellcome Trust*, Oxford University Press.

Kouznetsov R.L. (1977). Malaria control by application of indoor residual insecticides in tropical Africa and its impact on community health. *Trop Doc* **7:** 81-91.

Kuschke W.H.G. (1968). *The Okavango and its endemic diseases*, Windhoek.

La Grange J.P. (1988). *Malaria situation in the Okavango/Angola border region*. Unpublished report of the Department of National Health and Population Development, National Institute for Tropical Diseases, Tzaneen, Republic of South Africa.

le Sueur D. and B.L. Sharp (1988). The breeding requirements of three members of the *An. gambiae* Giles complex (Diptera: Culicidae) in the endemic malaria area of Natal, South Africa. *Bull. Entomol. Res*. **78**:549-560.

le Sueur D. B.L. Sharp, C. C. Appleton (1993). Historical perspective of the malaria problem in Natal with emphasis on the period 1928-1932. *S Afr J Sci* **89**: 232-239.

Lengeler C, D. de Savigny and J. Cattani (1996). From research to implementation. In: *Net Gain: anew method of preventing malaria deaths*, (Lengeler C; J. Cattani and D. de Savigny; J.D.R.C., Ed) Ottawa, pp. 1-15.

Lengeler C. (2004). Insecticide-treated bed nets and curtains for preventing malaria. *Cochrane Database of Systematic Reviews*, **2004(3):** CD000363.

Li Z. and Lu B. (1989). Field trials and use of pyrethroid-treated mosquito nets for malaria control in China. In: *Appropriate Technology for Vector Control, (Curtis C.F. CRC, Boca Raton, Ed) Florida. pp. 33-39.*

Lines, J.D., Myamba, J. and C.F. Curtis, (1987). Experimental hut trials of permethrin-impregnated mosquito nets and eave curtains against malaria vectors in Tanzania. *Medical and Veterinary Entomology*, **1,** 37-51.

Lindsay S.W. and W.J. M. Martens (1998). Malaria in the African highlands: past, present and future. *Bull. Wld. Hlth. Org*., **76** (1): 33-45.

Lu Bao Lin (1991). Bednets treated with pyrethroids for malaria control. In: Target, G.A.T. (ed.). *Malaria, Waiting for the Vaccine*. John Wiley & Sons Ltd, England.

Mabaso L.H., Sharp B.L. and C. Lengeler (2004). Historical review of malaria control in southern Africa with emphasis on the use of indoor residual house-spraying. *Trop. Med. Inter. Hlth* **9 (8):** 846-856

Magesa S.M., T.J. Wilkes, A.E.P. Mnzava (1991). Trial of pyrethroid impregnated bed nets in an area of Tanzania holoendemic for malaria. Part 2 Effects on the malaria vector population. *Acta Tropica* **49:** 97-108.

Malcolm, C.A. (1988). Current status of pyrethroid resistance in Anophelines. *Parasitology Today*, **4,** 513-515.

Malaney P.I.A, Spielman A., J. Sachs (2004). The malaria gap. *Am. J. Trop. Med. Hyg.* **71 (Suppl 2):** 141 – 146.

Marsh K. and R.W.Snow (1999). Malaria transmission and morbidity. *Parassitologia* **41:** 241-246.

Meek S. (1991). *Entomological aspects of malaria control in Namibia*. World Health Organization Consultancy Report.

Mendelsohn J., A. Jarvis, C. Roberts and T. Robertson (2002). *Atlas of Namibia. A Portrait of the land and its People*. First published, Cape Town, South Africa.

Mendelsohn J., A.Jarvis, C.Roberts and T.Robertson (2003). *Atlas of Namibia. A Portrait of the land and its People*. Second impression, Tien Wah Press, Singapore.

Ministry of Health and Social Services (1995). *Annual report of the Ministry of Health and Social Services*, Windhoek, Namibia.

Ministry of Health and Social Services (1995). *National Policy and Strategy for Malaria* Control, November 1995, Windhoek, Republic of Namibia.

Ministry of Health and Social Services (1995). *Epidemiology Report*, Windhoek, Namibia.

Ministry of Health and Social Services (1996). *Health Information System Report*, MOHSS, Windhoek, Namibia.

Ministry of Health and Social Services (1996). *Epidemiology Report*, Windhoek, Namibia

Ministry of Health and Social Services (1997). *Epidemiology Report*, Windhoek, Namibia.

Ministry of Health and Social Services (20001).*Health Information Report* 1999-2001.

Ministry of Health and Social Services, (1992). *Demographic and Health Survey*, Windhoek, Namibia.

Ministry of Health, Botswana (1997). *Report on malaria conference* 27-29 August 1997; Grand Palm Hotel, Gaborone.

Ministry of Health and Social Services. Namibian Country Co-ordination Mechanism for HIV/AIDS, Tuberculosis and Malaria, Scaling up the Fight against HIV/AIDS, Tuberculosis and Malaria in Namibia; September 2002.

Ministry of Health and Social Services. National Malaria Policy, April 2005.

Ministry of Health and Social Services. Malaria Epidemic Preparedness and Response Guidelines, April, 2005.

Mnzava A.E.P. and W.L.Kilama, (1986). Observation on the distribution of the *Anopheles.gambiae* complex in Tanzania. *Acta Tropica* **43**. 277-282

Mnzava A.E. (1991). PhD Thesis, University of Basel, Switzerland.

Mnzava A.E.P., Rwegoshora R.T., Tanner M., Msuya F.H., Curtis C.F. and S.G. Irare (1993). The effects of house spraying with DDT or lambda-cyhalothrin against *Anopheles arabiensis* on measures of malarial morbidity in children in Tanzania. *Acta Tropica* **54:** 141-151.

Mnzava A.E.P., Mutinga M.J. and C. Staak (1994). Host blood meals and chromosomal inversion polymorphism in *Anopheles arabiensis* in the Baringo district of Kenya. *J. Am. Mosq. Contr. Assoc.,* **10 (4)**: 507-510.

Mnzava A.E.P**.**, Rwegoshora R.T., Wilkes T.J., Tanner M. and Curtis C.F. (1995). *Anopheles arabiensis* and *An. gambiae* chromosomal inversion polymorphism, feeding and resting behaviour in relation to insecticide house spraying in Tanzania. *Med. Vet. Entomol*., **9**: 316-324.

Mnzava A.E., D.le Sueur, B.L.Sharp, S.Ngxongo, D.J.Mthembu and M.V. Ntuli (1997). Malaria in South Africa: Are District Health Systems and Current Malaria Control Strategies Compatible? *S. Afr. Med. J*., 87 (5): 585-587.

Mnzava A.E., B.L.Sharp, N.Cuamba, A.Barreto (1997). Malaria vector studies in Maputo, Mozambique – implications for control. *S.Afr.Med.J.* **87** (**11**) 1611-1614.

Mnzava AEP, B.L.Sharp, D.Mthembu, D. le Sueur, S.S.Dlamini, J.K.Gumede and I. Kleinschmidt (2001). Malaria control – two years' use of insecticide-treated bednets compared with insecticide house spraying in KwaZulu-Natal. *S.Afr. Med. J*. **91** (**11**): 976-983

Molineaux L. and G.Gramiccia (1980). *The Garki Project. Research on epidemiology and control of malaria in the Sudan Savanna of West Africa. Geneva*: World Health Organization.

Molineaux L. (1997) Malaria and mortality: some epidemiological considerations. *Ann.Trop.Med.Par*., **91** (**7**), 811-825.

Molineaux, L. (1988). The epidemiology of human malaria as an explanation of its distribution, including some implications for its control. In: *Malaria, Principles and Practice of Malariology*, **2:** 913-999, **(**eds), Wernsdorfer & Mcgregor.

Mosha F.W. and V. Petrarca (1983). Ecological studies on *Anopheles gambiae* complex sibling species on the Kenya coast. *Trans. R. Soc. Trop. Med. Hyg;* **77:** 344-345.

Mouchet J.; D.Fontenille, J. Julvez, P.Carnevale, A.W.Onapa, O. Faye, S. Laventure, J. Sicoulon, S.Manguin (1998). Evolution of malaria in Africa for the past 40 years: impact of climatic and human factors. In: *J. Am. Mosq. Control Assoc.* 1998 June **14** (**2**):121-30.

Msuya F H M. and C F. Curtis (1991). Trial of pyrethroid impregnated bed nets in an area of Tanzania holoendemic for malaria part 4 Effects on incidence of malaria infection. *Acta Tropica* **49:** 165-171.

Muirhead-Thomson R. C. (1947). The effects of house spraying with pyrethrum and DDT on *Anopheles gambiae* and *Anopheles melas* in West Africa. *Bull. Ento. Res*. **38**: 449-464.

Najera J.A. (1999). Prevention and control of malaria epidemics. *Parassitologia* **41:** 339-347.

Najera J.A. and M. Zaim (2002). Global insecticide use for vector-borne diseases control. Geneva, World Health Organization; *WHO/CDS/WHOPES/2002.5.*

Nobre FF, A.LBraga, R.S.Pinheiro and J.A.Lopes (1997). GISEpi: a simple geographical information system to support public health surveillance and epidemiological investigations. *Comput Methods Programmes Biomed;* **53 (1):** 33-45.

Nordberg E and H. Oranga (1996). Health information for district level planning: a cross-sectional household survey in rural Kenya. East *Afr. Med. J.,* **73 (6):**364-369.

Njunwa, K.J., J.D.Lines, S.M.Magesa, A.E.P.Mnzava, J.J.Wilkes, M.Alilo, K.Kirumbi, and C.F.Curtis (1991). Trial of pyrethroid impregnated bednets in an area of Tanzania holoendemic for malaria. *Acta,* **49,** 87-96.

Obeid el S, J.Mendelsohn, M.Lejars, N.Forster, G. Brule (2001). *Health in Namibia, Progress and Challenges*; Windhoek Namibia.

O'Dempsey, T.J.D., T.F.McArdle, B.E.Laurence, A.C.Lamont, J.E.Todd, B.M.Greenwood (1997). Overlap in the clinical features of Pnemonia and malaria in African children. *Lancet,* **347:** 223-227.

Onori E, P.F.Beals and H.M.Gilles (1993). Rationale and technique of malaria control In: *Bruce-Chwatt's essential malariology* eds Gilles H.M. and Warrell D.A.

Organization of African Unity Summit (1997). Harare Declaration on Malaria Prevention and Control in the Context of African Economic Recovery and Development. In: *Organization of African Unity, Assembly of Heads of States and Governments*, 33rd Ordinary Session.

Pampana E.J. (1963). *A text book of Malaria Eradication.* 2nd edn. Oxford University Press, London.

Parker B.R and A.D Kaluzny (1982). Structuring information systems for more effective disease control programmes. *J. Med. Syst.,* **6:** 613-631.

Park Ross G.A. (1936). Insecticide as a major measure in the control of malaria, being an account of the methods and organizations put into force in Natal and Zululand during the past six years. Quarterly, *Bulletin of the Health Organization of the League of Nations*, **5:** 114-133.

Phillips R.S. (1997). A multi-pronged attack on malaria. *Parasitology Today*, **13:** 324-327

Payne D (1987). Spread of Chloroquine resistance in *Plasmodium falciparum. Parassitology* **3**: 241 - 246.

Pyne D. (1993). The planning and organization of *in vivo* and *in vitro* sensitivity studies in the malarious areas of Namibia (Ovambo) and the training of Namibian health workers in these activities. Unpublished consultancy report to the World Health Organization

Ravaonjanahary C (1997). *The role of vector control in malaria control programmes in Africa.* Intercountry seminar/workshop on control of vectors of malaria and plague. Antananarivo.

Republic of Namibia. *2001 Population and Housing Census,* March 2002. Windhoek.

Roper C., Pearce R., Bredenkamp B., Gumede J., Drakeley C., Mosha F., Chandramohan D., and Sharp B. (2003) Antifolate Antimalarial resistance in southeast Africa: a population-based analysis. *The Lancet* **561:** 1174-1181.

Ross R. On some peculiar pigmented cells found in two mosquitoes fed on malaria blood. *B.M.J.* 1897, **2:** 1786-1788.

Rwegoshora, R.T., Pedersen E.M., Mukoko D.A., Meyrowitsch D.W., Masese N., Malecela-Lazaro M.N., Ouma J.H., Michael E. and P.E. Simonsen (2005). Bancroftian filariasis: patterns of vector abundance and transmission in two East African communities with different levels of endemicity. *Ann. Trop. Med. Parasitol.,* **99** (**3**): 253-265.

Salako L.A. (1999). Malaria control priorities and constraints. *Parasitologia* **41:**495-496.

Schellenberg J.A. J.N. Newell, R.W. Snow, V. Mungala, K. Marsh, P.G.Smith, and R.J.Hayes, *(*1998). An analysis of the geographical distribution of severe malaria in children in Kilifi District, Kenya. *Int.J.Epid*., **27**: 323-329.

Scott J., W.Brogdon and F.Collins (1993). Identification of single specimens of the *An.gambiae* complex by Polymerase Chain Reaction. *American Journal of Tropical Medicine and Hygiene,* **49:** 520-529.

Scrimgeour D. (1991). *In vivo* resistance to chloroquine and fansidar in *Plasmodium falciparum* malaria in Kavango, Namibia. Unpublished consultancy report to the World Health Organization.

Schapira A. (1990). *The resistance of falciparum malaria in Africa to 4-aminoquinolines, and antifolates*. Kaleidoscope Ltd., Copenhagen.

Service W.M. (1985). *Anopheles gambiae* : Africa,s principal malaria vector, 1902-1984. *bulletin of the entomological society of America*, **31:** 8-12.

Service M.W. (1986). *Lecture notes on medical entomology*. Blackwell scientific publications, London, UK.

Service M.W., A.Voller and D.E.Bidwell (1987). The enzyme linked immunosorbant assay (ELISA) tests for identification of blood meals of haemotophagous insect. *Bulletin of entomological Research,* **76:** 321-330.

Service M.W. (1993a). *The* Anopheles *vector in Bruce-Chwatt's essential malariology*. Third edition, London, UK.

Service M.W. (1993b). *Evaluation of malaria control in Namibia and need for entomological training*. World Health Organization Consultancy Report.

Service M.W. (1996). Medical Entomology for students, 56-58. Blackwell Scientific Publication, London, UK.

Sharp B.L., F.C. Quicke and E.J.Jansen (1984). Aspects of the behaviour of five *Anopheline* species in the endemic malaria area of Natal. *J. ent. Soc. S. Afr*. **47:** 251-258.

Sharp B.L., S.Ngxongo, M.J.Botha, F.Ridl, D.le Sueur (1988). An analysis of ten years of retrospective malaria datafrom the Kwa-Zulu areas of Natal. *S. A. J. Sc.,* **84:** 102-106.

Sharp B.L. and J.A. Freese (1990). Chloroquine-resistant *Plasmodium falciparum* malaria in the Kavango region of Namibia. *S. Afr. Med. J*., **78**, 322-323

Sharp B.L., D. le Sueur and P. Bekker (1990). Effect of DDT on survival and blood feeding success of *Anopheles arabiensis* in northern KwaZulu, Republic of South Africa. *Journal of the American Mosquito Control Association.* **6**: 197-201.

Sharp B.L. (1990). Aspects of the epidemiology of malaria in Natal Province South Africa. Ph.D. thesis. University of Natal.

Sharp B.L. and D. le Sueur (1991). Behavioural variation of *Anopheles arabiensis* (Diptera: Culicidae) populations in Natal, South Africa. *Bull. Ento. Res.,* **81**: 107-110.

Sharp B.L., le Sueuer, D., Ngxongo, S.M., Bredenkamp, B. and Wilken, G.B. (1992). Transmission of malaria and vector control in the Mamfene area, Ubombo District, Natal Province (1986-1992).

Sharp B.L., D. D. le Sueur, G.B. Wilken B.L.F. Bredenkamp, S.M. Ngxongo and E. Gouws (1993). Assessment of the residual efficacy of Lambda-cyhalothrin. A comparison with DDT for the intradomiciliary control of *Anopheles arabiensis in South Africa*. *J. Am. Mosq. Contr. Ass.* **9**: 414-420.

Sharp B.L., D. le Sueur (1996). Malaria in South Africa – the past, the present and selected implications for the future. *S. Afr. Med. J.;* **86: (1)** 83-9.

Sharp B. L., D. le Sueur. (1997). Need for regional co-operation in malaria research and control *S. Afr. Med.J*., **87:**11.

Sharp B. L., M.Craig, S. Ngxongo, C. Martin, J. Tsoka, D. le Sueur (1998). The contribution and future potential of Geographical Information Systems to health delivery in South Africa. MRC, Durban.

Sharp B.L., M.H.Craig, B.Curtis, A.Mnzava, R.Maharaj, I.Kleinschmidt (2000). *Review of malaria in South Africa*.

Sharp B., Wyk P., Sikasote JB., Banda P. and I. Kleinschmidt. (2002). Malaria control by residual insecticide spraying in Chingola and Chililabombwe, Copperbelt Province, Zambia. *Trop. Med. Intern. Hlth*, 7 (**9**): 732-736.

Shelley A.J. (1973). Observations on the behaviour of An. gambiae sp. B in Kambole village in the Zambezi valley, Zambia. Ann. *Trop. Med. Parassitologia,* **67**: 237-248.

Smit H.E, Bouwman, H. Le Sueur (1992). DDT and community health. **3** (3) and (4) July-November 1992.

Smith D.C. and Sanford L.B. (1988). Laveran's germ: the reception and use of a medical discovery. *Am. J. Trop. Med. Hyg.;* 1988; **34**: 2-20.

Spencer H. C., D. M. Kariuki, and D.K. Koech (1983). Chloroquine resistance in *Plasmodium falciparum* from Kenyan infants. *Am.Trop. Med. Hyg*. **32:** 922-925.

Snow R.W, I. Bastos de Azevedo, B.S.Lowe, E.W.Kabiru, C.J.Nevill, S.Mwankusye, G.Kassiga, K.Marsh and T.Teuscher (1994). Severe childhood malaria in two areas of markedly different *falciparum* transmission in East Africa. *Acta Tropica;* **57** 289-300.

Soni P.N., B.L. Sharp, S. Ngxongo, and V. Gathiram (1993). Morbidity from *Falciparum* malaria in Natal/Kwazulu. S.Afr.Med.J., **83:** 110-112.

Stanley C., Jr. Oaks, S. Violaine, M. W. P. Greg, C. Charles and J.Carpenter (1991*). Malaria obstacles and opportunities.*

Stevens W., Wiseman V., Ortiz J. and D. Chavasse (2005). The costs and effects of a nationwide insecticide-treated net programme: the case of Malawi. *Malaria Journal*, 4:22;
http: //www.malariajournal.com/content/4/1/22

Teklehaimanot A., V.I.Cano and A.E.C. Rietveld (1990). *The malaria control programme of Namibia.* WHO malaria (CTD/MAL) mission to Namibia.

Teklehaimanot A., A.Bosman (1999). Opportunities, problems and perspectives for malaria control in sub-Saharan Africa. *Parassitologia;* **41:** 335-338.

Touré Y.T., V.Petrarca, F.Traoré, A. Coulibaly, H.M.Maiga, O.Sankaré, M.Snow, M.A.Di Deco and M.Coluzzi (1994). Ecological genetic studies in the chromosomal form Mopti of *Anopheles gambiae s.s.* in Mali. *Genetica,* **94:** 213-223.

Touré Y.T., V. Petrarca and M. Coluzzi (1983) Nuove entita del complesso *anopheles gambiae* in Mali. *Parassitologia*, **25:** 367-370

Touré Y. T., V.Petrarca and M.Coluzzi (1987). Patterns of geographical and seasonal distributions of the 'Mopti' karyotypes of *Anopheles gambiae* in Mali. Proceedings of the 3rd International Conference on Malaria and Babesiosis, Annecy, France, 172.

Trigg P.I.& Kondrachine A.V. (1998). Commentary: Malaria control in the 1990s. *Bull. Wld. Hlth. Org.*, **76:** (1) 11-16.

Unicef (1991). A situation analysis of children and women in Namibia. Windhoek: Namib Graphics.

United Nations Environmental Programme (2004). Stockholm Convention on Persistent Organic Pollutants, 2004. (*http://www.pops.int/documents*, accessed August 2005

Vulule J.M., R.F.Beach, F.K.Atieli, J.M.Roberts, D.L.Mount, and R.W.Mwangi (1994). Reduced susceptibility of *Anopheles gambiae* to permethrin associated with the use of permethrin-impregnated bednets and curtains in Kenya. *Medical and Veterinary Entomology*, **8,** 71-75.

Vulule J.M., R.F.Beach, F.K.Atieli, D.L.Mount and R.W.Mwangi (1996). Long-term use of permethrin-impregnated nets does not increase *Anopheles gambiae* permethrin tolerance. *Medical and Veterinary Entomology*, **10,** 71-79.

Vulule J.M., R.F.Beach, F.K.Atieli, J.C.McAllister, W.G.Brogdon, J.M.Roberts, R.W.Mwangi and W.A.Hawley (1999). Elevated oxidase and esterase levels associated with permethrin tolerance in *Anopheles gambiae* from Kenyan villages using permethrin-imprenated nets. *Medical and Veterinary Entomology*, **13,** 239-244.

Wernsdorfer W.H. (1991). The development and spread of drug resistant malaria. *Parasitology Today*, 7**:** 297 – 303.

White G. B. (1974). *Aopheles gambiae* complex and disease transmission in Africa. *Trans. Roy Soc. Trop. Med. Hyg*. **68:** (4) 278-301.

Williams, M.S., D. Martinez-Torres, C.A.Hick and A.L.Devonshire (1996). Identification of mutations in the housefly para-type sodium channel gene associated with knockdown resistance (kdr) to pyrethroid insecticides. *Molecular and General Geneses*, **253:** 51-60.

World Health Organization (1980). Resistance of vectors of disease to pesticides. 5th Report of the Expert Committee on Vector Biology and Control. *WHO Technical Report Series,* No.655.

World Health Organization (1989). The use of impregnated bednets and other materials for vector-borne disease control. *Document WHO/VBC/89.981, 45pp.*

World Health Organisation Regional Office for Africa, May 1998. African Initiative for Malaria Control in the 21st Century.

World Health Organization (1993). Use of DDT in vector control. Conclusions of the WHO Study Group on Vector Control for malaria and other mosquito-borne diseases. WHO/MAL/95.1071 WHO/CTD/VBC/95.997, Geneva.

World Health Organization (1993). A global strategy for malaria control. WHO, Geneva.

World Health Organization (1994). Antimalarial drug policies: data requirements, treatment of uncomplicated malaria and management of malaria in pregnancy. WHO/MAL/94.1070. Geneva: WHO, Division of Control of Tropical Diseases.

World Health Organization (1995). World Health Organization, Expert Committee on Vector Control, Technical report series 857, Geneva, Switzerland.

World Health Organization (1996). WHO/AFRO workshop on prevention and control of malaria epidemics, Windhoek, Namibia.

World Health Organization Regional Office for Africa, Brazaville (1996). Malaria Control in Africa. A framework for the implementation of the Regional malaria control strategy 1996-2001

World Health Organization (1997). Use of DDT in vector control. Conclusions of the WHO Study Group on Vector Control for Malaria and other Mosquito-borne Diseases, Geneva.

World Health Organization (1997). Guidelines on the use of Insecticide-treated mosquito nets for the prevention and control of malaria in Africa. CTD/MAL/AFRO/97.4.

World Health Organization (1998). Brundtland, G.H, Director-General Elect. Speech to the Fifty-first World Health Assembly, pp8, Geneva, Switzerland, A51/DIV/6, 13 May 1998.

World Health Organization Regional Office for Africa, Brazaville (1998). African Initiative for Malaria Control in the 21st Century, pp18.

World Health Organization (2000). WHO Expert Committee on Malaria. Twentieth Report, WHO, Geneva

World Health Organization/UNICEF (2003). The Africa Malaria Report 2003; WHO/CDS/MAL/2003.1093.

World Health Organization (2005). World Malaria Report 2004. Roll Back Malaria, Geneva, Switzerland; WHO/HTM/MAL/2005.1102.

APPENDIX

Appendix table 1: Assessment of Malaria Morbidity at Bukalo and Mahenene Health Centers

Date:……

Name:……..

Age:……Sex:……..

Nationality:…….

District:……

Region:…….

Health Facility Name:……

Hospital ☐ Health Centre ☐ Clinic ☐ Health Post ☐

Outpatient:……(Y/N)

Patient's Residential Address:…….

…….

…….

Laboratory Ref. Number:……..

How long have you had fever?:…….

Have you taken any antimalarial drug in the last two weeks?:….(Y/N)

Have you spent a night/nights outside your place of residence in the last two weeks:?…

……..(Y/N)

If yes, where?……

When was your house last sprayed?

Temperature:…….^{0}C

Blood smear number:…….

Number of parasites per 200 white blood cells (wbc):…..

Blood smear results:

Plasmodium: *falciparum* ☐ *malariae* ☐ *ovale* ☐ *vivax* ☐ *Mixed* ☐

Negative ☐

Appendix table 2: Cross-sectional survey of Malaria morbidity in study sites

District…….Health

Centre……..…….

Date	Code No	Name	Age	Sex	Address	Results

Appendix table 3: The **number of *An.arabiensis* collected by means of exit window traps and the feeding status in sprayed and unsprayed huts or dwelling units for 12 months in Kalimbeza village**

Dwelling Unit	**Status of Dwelling Unit**	**Feeding status Freqeuency**		**TOTAL**
		Blood fed	**Not Fed**	
KM1	unsprayed	117	352	469
KM2	sprayed	6	33	39
KM3	sprayed	73	107	180
KM4	unsprayed	39	88	127
KM5	sprayed	32	130	162
KM6	sprayed	2	9	11
KM7	sprayed	14	6	30
KM8	sprayed	7	15	22
KM9	sprayed	6	22	28
KM10	sprayed	27	18	45
KM11	unsprayed	13	64	77
KM12	unsprayed	28	15	43
KM13	sprayed	1	7	8
KM14	sprayed	0	0	0
KM15	sprayed	0	2	2
KM16	unsprayed	1	1	2
KM17	sprayed	1	0	1
KM18	sprayed	12	12	11
KM19	sprayed	7	9	16
KM20	sprayed	12	6	18
Total		**329**	**961**	**1289**

Appendix table 4 The total number of *An.arabiensis* collected by means of exit window traps and the feeding status in sprayed and unsprayed huts or dwelling units for 12 months in Kalimbeza village, Caprivi Region.

Spray Status	Entomological Condition[6]		Total
	Blood Fed (%)	Not Fed (%)	
Unsprayed	198 (28)	520 (72)	**718**
Sprayed	200 (35)	376 (65)	**576**
Total	**398**	**896**	**1294**

Appendix table 4: Therapeutic Efficacy Study Methodology

STUDY SITES

The therapeutic efficacy study was conducted in two sites. These are Outapi and Katima Mulilo representing the various epidemiological situations in the malaria endemic areas in northern Namibia. Two teams from Outapi and Katima Mulilo were trained at Rundu Hospital. The mean annual incidence of malaria in the two regions between 1995 and 1999 was 1868, 690 and 348/1000 population for Katima Mulilo and Outapi districts respectively. This shows that Katima Mulilo has highest and Outapi the lowest transmission. In addition, Katima Mulilo town have a higher cross border population movement while Outapi has the lowest. Availability of the required staff for the study and laboratory services was also taken into account in selecting these sentinel sites.

THE TEST

Patients of either sex were recruited for the study among patients attending the outpatient clinics. Since the northwestern and northeastern parts of Namibia are regarded as unstable malarious areas, all age groups were included (i.e. both under and above five years). After initial clinical assessment patients that met all the criteria were screened for the presence of *P.falciparum* malaria.

The actual sample size required for the study was 50. However, to compensate for possible dropouts, each site was required to recruit sixty patients per study site. The defaulter rate was kept below 10% in most of the study groups.

[6] Figures in brackets are row totals while those without are the sample sizes

All patients recruited gave a written consent following adequate explanation about study and their voluntarily participation in the study. All patients were required to come for follow-up on Day 1 (the second day of visit to the health facility) and Day 2 (the third day of visit) after their first visit (Day 0) for treatment. Thereafter, patients came for follow-up on Days 3, 7, 14, 21 and 28. Information was given to all patients that they can visit the hospital if their condition worsens, their illness shows no improvement or for any reason that could be a cause of worry to them. As a result patients living in the close vicinity of the study sites were given priority for recruitment to reduce the defaulter rate and facilitate follow-up.

The procedures followed on each patient contact day are described below under the heading "Daily Activity".

A. INCLUSION CRITERIA

The inclusion criteria were as follows:

1. Age above 6 months
2. *Plasmodium falciparum* mono-infection with parasite counts = 1,000- 100,000/µl
3. Axillary temperature $\geq 37.5^0$C
4. Absence of the following signs and symptoms of severe malaria:
    - Severe anemia <PCV 15%)
    - Persistent vomiting
    - Inability to sit or stand
    - Deteriorating level of consciousness
    - Coma
    - Hypoglycaemia
    - Renal failure
    - Severe pneumonia
    - Bleeding disorders

4. Ability to come for 28 Days of follow-up
5. Patient/parental consent

B. EXCLUSION CRITERIA

- Age younger than six months
- Inability to come for stipulated follow-up
- Development of danger signs
- Lack of (parental) consent
- History of recent antimalarial treatment
- Mixed infections
- Pregnancy

C. COMPOSITION OF STUDY TEAMS

- Local Health workers for tracing study subjects
- A medical Doctor
- A Laboratory technologist
- A nurse
- Driver

D. DAILY ACTIVITIES

Day 0 Activities

Screen patients by obtaining a positive history of fever

⇩

Measure axillary temperature and if temperature is ≥37.5^{0}C then weigh

⇩

Do thick and thin films in people who qualify ask to wait for the results

⇩

If parasite count is ≥1,000/µl, get parental consent and enroll

⇩

Complete Case Record Format

⇩

Give 10mg/kg of chloroquine

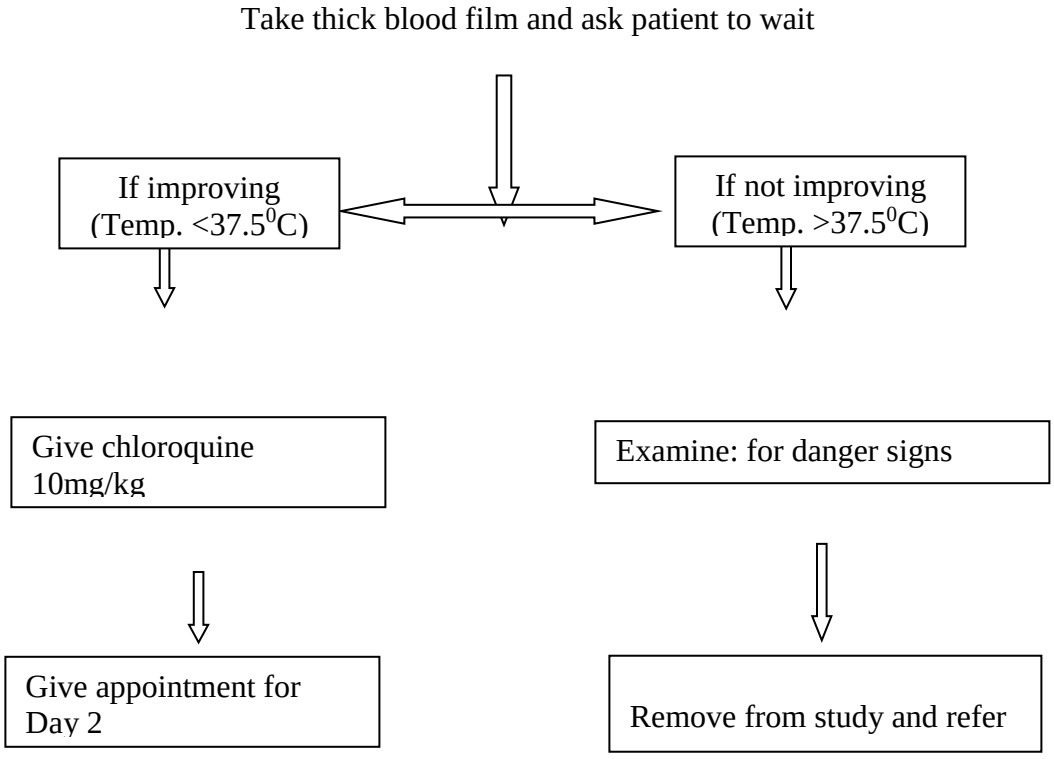
⇩

Give appointment for Day1

Give instructions to parents that if patient deteriorates or if any of the danger signs are observed then the patient should be brought back to the hospital/clinic immediately.

Day 1 Activities

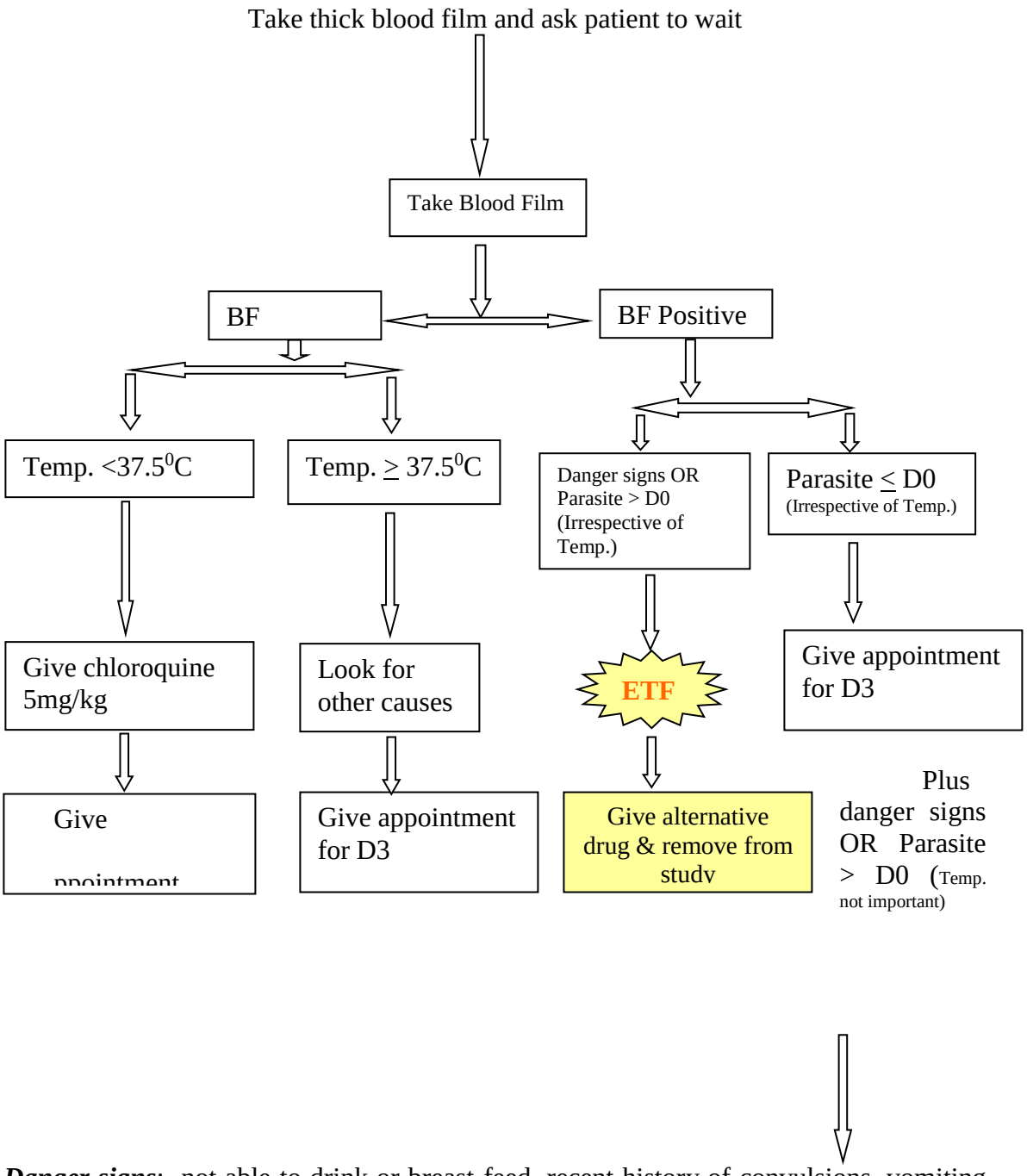
Ask about fever, vomiting, convulsions, coma, and inability to stand or eat.



Danger signs: not able to drink or breast-feed, recent history of convulsions, vomiting everything, unable to sit or stand, lethargy or unconsciousness

Day 2 Activities

Ask about fever, vomiting, convulsions, coma, inability to stand or eat

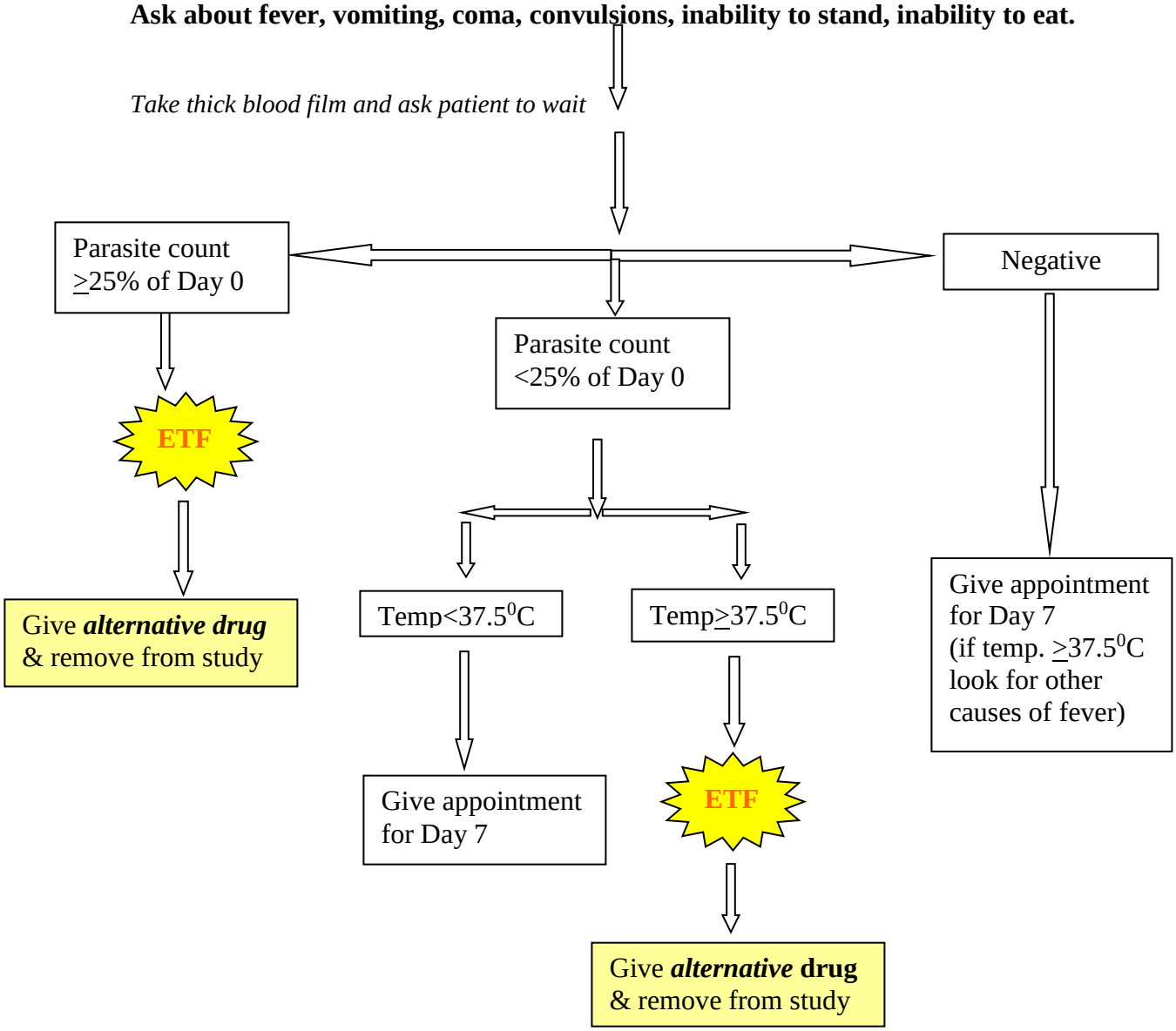


Danger signs: not able to drink or breast-feed, recent history of convulsions, vomiting everything, unable to sit or stand, lethargy or unconsciousness

Day 3 Activities

Ask about fever, vomiting, coma, convulsions, inability to stand, inability to eat.

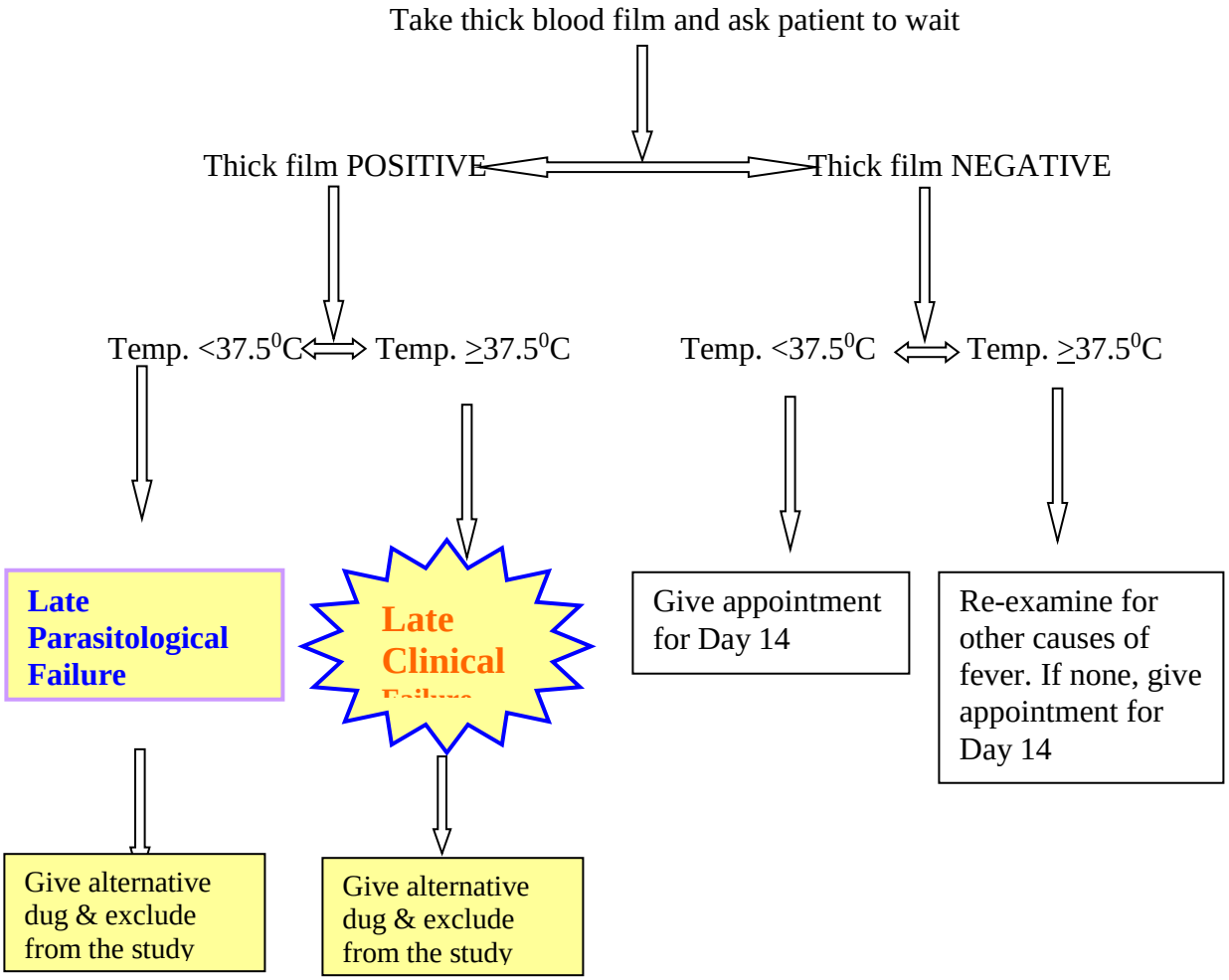
Take thick blood film and ask patient to wait



Danger signs: not able to drink or breast-feed, recent history of convulsions, vomiting everything, unable to sit or stand, lethargy or unconsciousness

Day 7 Activities

Ask about Fever, vomiting, convulsions, inability to stand, coma, inability to eat.

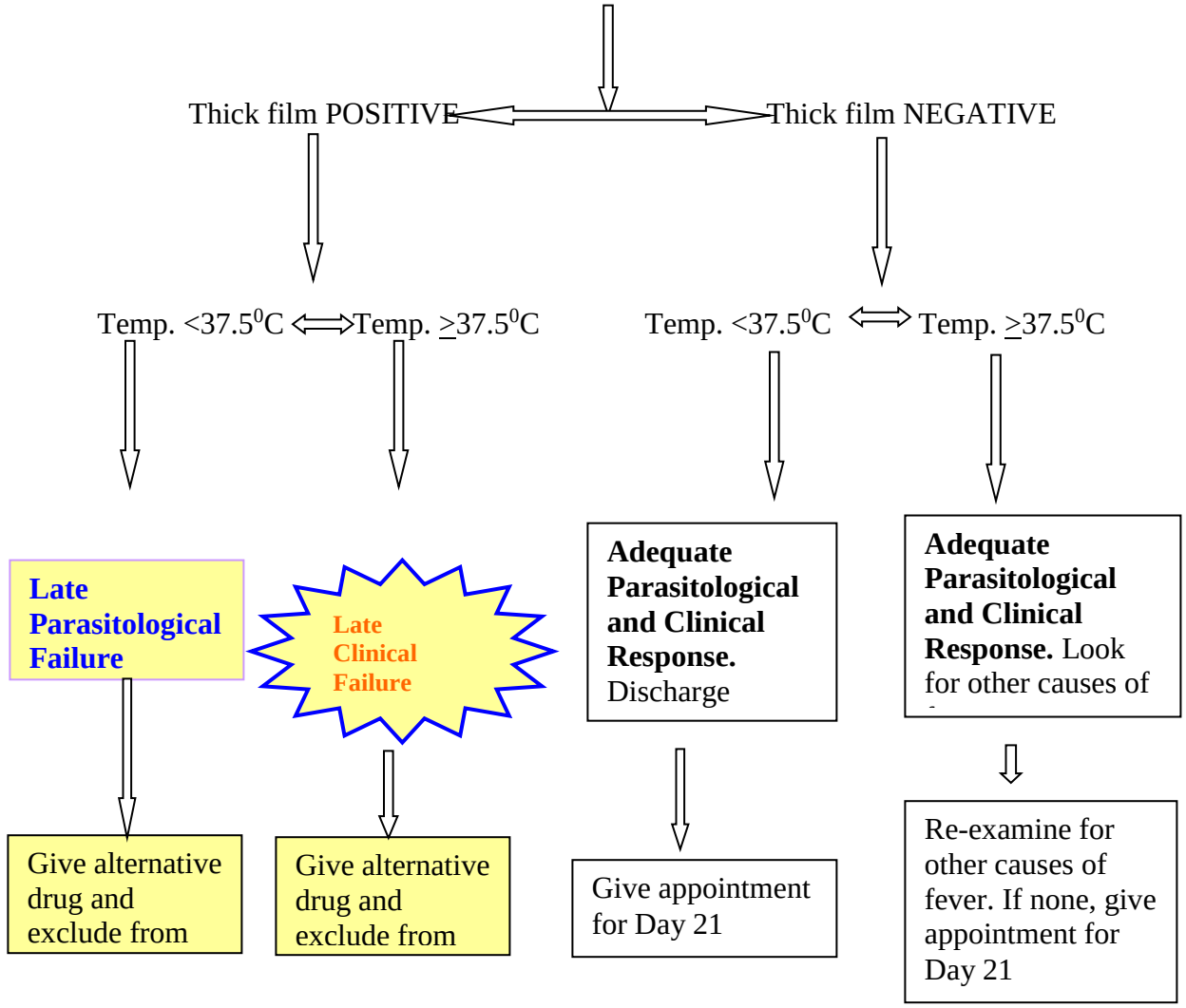


Danger signs: not able to drink or breast-feed, recent history of convulsions, vomiting everything, unable to sit or stand, lethargy or unconsciousness

Day 14 Activities

Ask about Fever, vomiting, convulsions, inability to stand, coma, inability to eat.

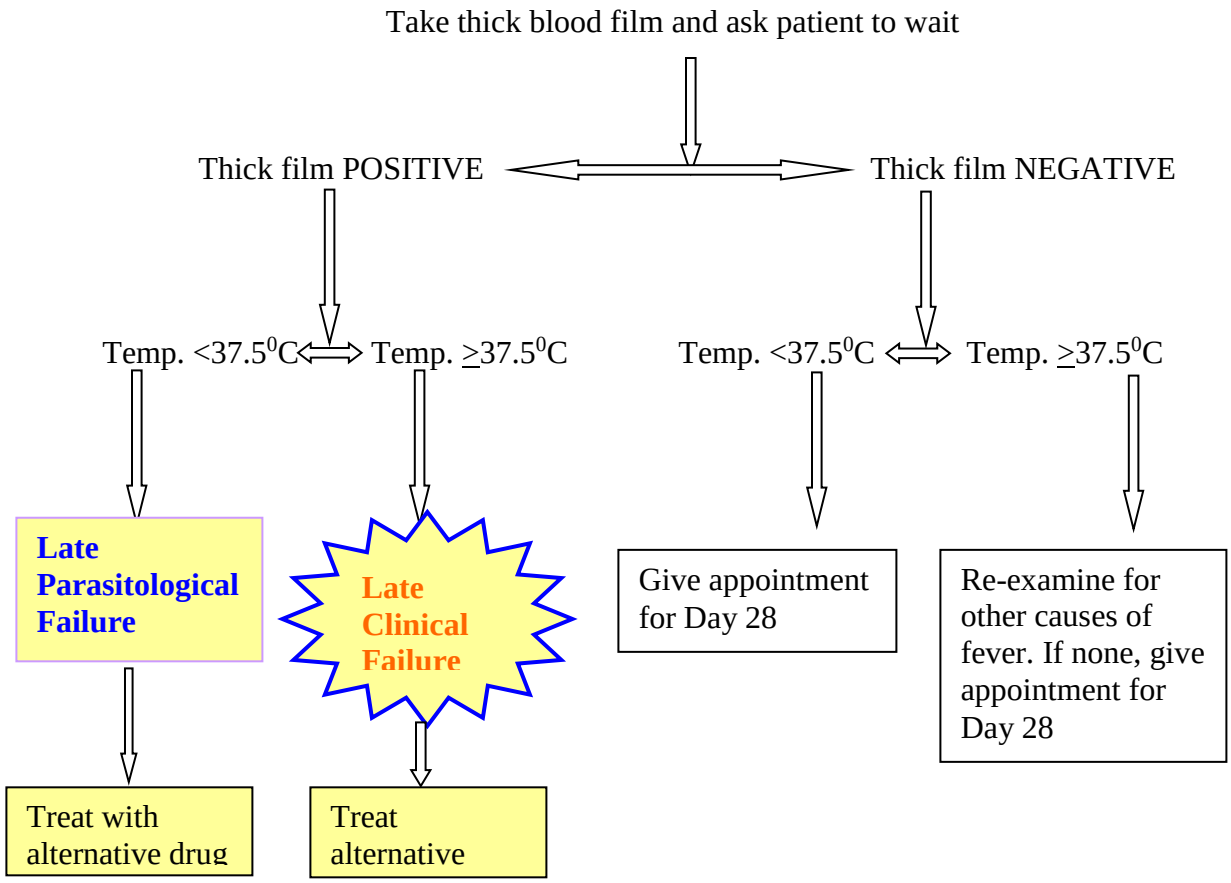
Take thick blood film and ask patient to wait



Danger signs: not able to drink or breast-feed, recent history of convulsions, vomiting everything, unable to sit or stand, lethargy or unconsciousness

Day 21 Activities

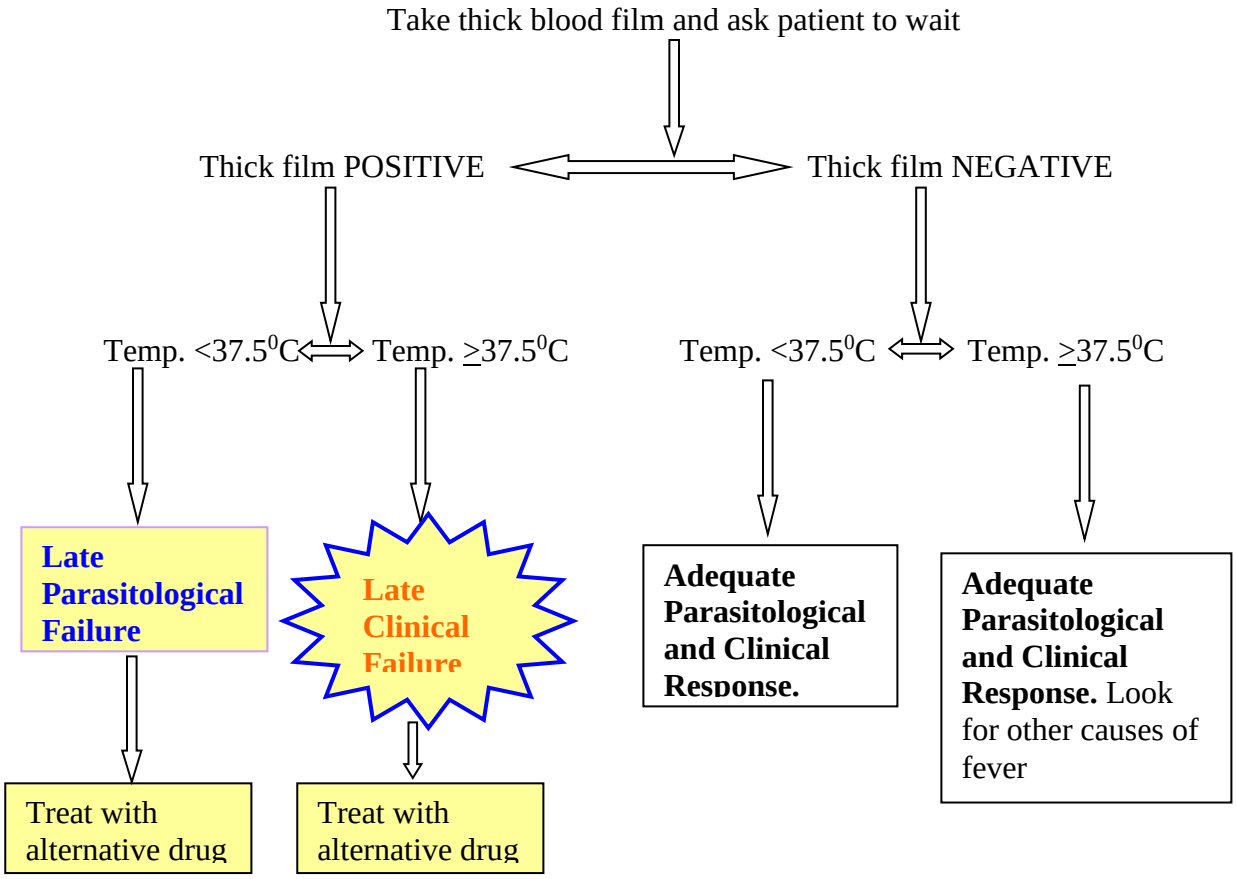
Ask about Fever, vomiting, convulsions, inability to stand, coma, inability to eat.



Danger signs: not able to drink or breast-feed, recent history of convulsions, vomiting everything, unable to sit or stand, lethargy or unconsciousness

Day 28 Activities

Ask about Fever, vomiting, convulsions, inability to stand, coma, inability to eat.



Danger signs: not able to drink or breast-feed, recent history of convulsions, vomiting everything, unable to sit or stand, lethargy or unconsciousness

F. MICROSCOPIC EXAMINATION

Collection of blood

A finger prick, using sterile blood lancet, was used to collect the blood film from each patient on all the follow-up days. Gloves were worn at all times. For each of the visiting days, ideally, two films were taken. On the same slide, both thick and thin films were taken on patient contact days except on Day 1. The thin film was used primarily for identification of parasite species in case it was impossible to identify the species in the thick film.

Preparation of thick blood films

- Clean microscope slides were used.
- The slides were labelled; and labels included the site, the study number, the day of visit and the date.
- Two blood slides were prepared for each patient on each patient contact day.
- A drop of blood was applied to each slide.
- Using a slide or a micro-capillary pipette spread the blood to form more or less a round smear.
- Slides were allowed to dry in open air.
- After preparation the slides were stained with 3 % Giemsa solution. For rapid staining, 10% Giemsa was used.

Preparation of 3 % Giemsa stain

- Take 1.5 ml of Giemsa stock solution
- Add 48.5 ml of buffer
- Allow to settle for one hour

Staining procedure

- Allow blood film to dry
- Fix the thin part in methanol
- Air dry
- Stain in Giemsa for 30–45 minutes
- Allow to air dry or heat dry - use minimal heat
- Examine under 100 x oil objective

Rapid or emergency method

- Prepare the thick smear
- Allow to dry
- Prepare 10% stain: 5ml Giemsa stock solution
- Add 45ml buffer
- Stain for 10 minutes
- Gently rinse excess stain off with tap water
- Dry with hair dryer (minimal heat) or air dry

Counting of parasites

Two tally counters were used, one for parasites and the other one for the white blood cells. All the parasites that occur within 200+ WBC were counted. All parasites and WBC within a field should be counted. If 500 parasites have been counted before reaching 200 WBC, counting is stopped after completing the count on the last field. If 200+ WBC are reached before 500 parasites, counting is stopped. Negative result is reported ONLY after 100 High Power Fields are read and no parasites are found.

Calculation of parasite density

The following formula was used to calculate the parasite density.

$$\text{Number of WBC} = \frac{\text{Number of parasites x 8000/µl}}{\text{WBC Counted}}$$

STORAGE

Slides were carefully stored in slide boxes after staining and during transportation. After using oil on the slides, they should carefully be placed face down on a clean piece of toilet paper or any other absorbent paper, in order to clean off the oil. All slides of study patients were kept for crosschecking at the end of the study. Where there are no boxes available, slides were carefully wrapped in tissue paper.

DATA COLLECTION

A logbook, containing all the patient data for the study was kept. These data include all those who were screened for the study, and all those who fulfil the enrolment criteria. This is an important part for the malariometric data for the environment, and is always useful.

FOLLOW -UP

It is critical that the drop out rate should be kept below 10% for the data to remain valid for the test. The address of each patient was carefully recorded. In addition the village, nearest clinic, the name of the mother/parent/guardian and a landmark to identify house or households was always recorded to aid in tracing patients that may not appear for on follow-up days. Transport was made available to pick those who do not come on follow-up days and drop patients after they have been seen at the hospital. This has greatly helped to reduce the defaulter rate. However, transport was not always available in some of the study sites resulting in higher than expected drop out of patients.

DOSAGE OF DRUGS USED

CHLOROQUINE

For patients with a body weight of greater than 25 kg oral dosage of chloroquine must be given as follows:

Day 0	chloroquine 10mg base/kg
Day 1	chloroquine 10mg/kg
Day 2	chloroquine 5mg/kg

All medications should be given under supervision, i.e., in the presence of a medical staff. If there is vomiting the dose should be repeated almost immediately. Experience has shown that children are unlikely to vomit if dose is repeated immediately, rather than waiting for half an hour as is usually recommended.

DATA ENTRY AND ANALYSIS OF RESULTS

The data was entered in into EPI-INFO (WHO/CDC) software. The patient case record form was installed on the computer on which the data can be entered in EPI-INFO. The data entry was done at the end of the study. Analysis was done using Microsoft Excel application.

The objective is to assess the number of treatment failures, i.e. early and late treatment failures, using the DLQAS (Double Lot Quality Assurance Sampling) system. Hypothesis: The proportion of treatment failures (TFs) is significantly above 25% in the study areas, and therefore, decision to change the drug policy is necessary. A high proportion of early treatment failures to the first line drug was

seen as an important indicator for the need of changing the first line treatment.

The treatment failure rate of 25% was taken as maximum acceptable limit with 95% confidence limit and 80% power. Accordingly, a first stage sample of 16 patients were analysed and the number of treatment failures was counted. If the TFs were found to be 5 or less then TFs were counted until the 6^{th} TF is noted or a second stage sample of 42 patients was reached. If 6 or more TFs were registered at any stage of the study, this was considered as an indication that the TF rate is not significantly lower than 25%, which means there is a need for changing antimalarial medicine policy.

INTERPRETATION OF FINDINGS

Early Treatment Failure (ETF)

- Development of danger signs or severe malaria on Day 1, Day 2 or Day 3, in the presence of parasitemia;
- Parasitaemia on Day 2 higher than Day 0 count irrespective of axillary temperature;
- Parasitemia on Day 3 with axillary temperature $\geq$ 37.5 °C;
- Parasitemia on Day 3 $\geq$ 25 % of count on Day 0.

Late Clinical Failure (LCF)

- Development of danger signs or severe malaria after Day 3 in the presence of parasitemia, without previously meeting any of the criteria of early treatment failure
- Presence of parasitemia and axillary temperature $\geq$ 37.5 °C (or history of fever) on any day from Day 4 to Day 28, without previously meeting any of the criteria of early treatment failure.

Late Parasitological Failure (LPF)

- Presence of parasitemia on any day from Day 7 to Day 28 and axillary temperature < 37.5 °C, without previously meeting any of the criteria of early treatment failure or late clinical failure.

Acceptable Clinical and Parasitological Response (ACPR)

- Absence of parasitemia on Day 28 irrespective of axillary temperature without previously meeting any of the criteria of early treatment failure or late clinical failure or late parasitological failure.

Acronyms

ACPR	Acceptable clinical and Parasitological Response
DLQAS	Double Lot Quality Assurance Sampling
ETF	Early Treatment Failure
LCF	Late Clinical Failure
TF	Treatment Failure
LPF	Late Parasitological Failure