

ASPECTS OF THE EPIDEMIOLOGY OF MALARIA IN NATAL PROVINCE,
REPUBLIC OF SOUTH AFRICA.

by

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ABSTRACT

This study investigated aspects of the epidemiology of malaria in the Natal province of the Republic of South Africa. In this study the Collins English dictionary definition of epidemiology is used where it is defined as the branch of medical science concerned with the occurrence, transmission and control of an epidemic disease.

Malaria has been a notifiable disease in the Republic of South Africa since 1958. Retrospective malaria case data from the Natal province as a whole was analyzed and the data from the KwaZulu and Natal areas of the province compared. Malaria cases were reported from 35 of the 65 magisterial districts in Natal province during the study period. In the Natal areas 91.5% of the cases were reported from eight districts and in the KwaZulu areas 96.4% of the cases came from three districts or as imports from Mozambique. The overall attack rate for both the Natal and KwaZulu areas using the total population figures for each area were very similar for the period 1986-1988 at 0.71 and 0.70 per 1000 head of population for the respective areas. The disease showed a distinct seasonal pattern in the KwaZulu areas with 86.9% of the cases being classified as indigenous and only 13.1% as imported. In the Natal areas, however, the seasonal pattern was not as marked and only 12.1% of the cases were recorded as indigenous and in excess of 82% as imported.

Three species of the *Anopheles gambiae* complex were found to occur sympatrically in Natal province, namely: *An.*

arabiensis, *An. quadriannulatus* and *An. merus*. Of these species *An. arabiensis* was found to occur at five localities during or after the notification of indigenous malaria cases from these areas. Due to the sympatric distribution of these species particular emphasis was placed on species identification and in particular the biting behaviour and control of *An. arabiensis* was investigated. The study found both morphological and behavioural differences between populations of *An. arabiensis* from those areas of the province with an intra-domiciliary residual insecticide vector control programme and those from the unsprayed areas. In the unsprayed areas the majority of the indoor resting *An. arabiensis* had fed on man whereas in the sprayed areas the majority of the indoor resting *An. arabiensis* were bovine fed. In the sprayed areas, however, the majority of the *An. arabiensis* caught leaving huts had fed on man.

The percentage survival of bloodfed *An. arabiensis* caught leaving huts in the DDT sprayed area was in excess of 72%. The data strongly suggest that optimal control of *An. arabiensis* will not be achieved using the current control strategy of the annual application of intra-domiciliary DDT.

PREFACE

This study represents original work by the author and has not been submitted in any form to another University. Where use was made of the work of others it has been duly acknowledged in the text.

The research described in this thesis was carried out at the Research Institute for Diseases in a Tropical Environment of the South African Medical Research Council, under the supervision of Professor J. van den Ende (Department of Medical Microbiology, University of Natal).

The convention used in this thesis in respect to decimal points is a full stop, in keeping with international scientific journals.

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CHAPTER 1

INTRODUCTION

1.1 INTRODUCTION

Mosquito borne diseases, foremost amongst them malaria, remain among the leading causes of morbidity and mortality in the tropical developing world. The yearly incidence of malaria cases is numbered in the millions. To the cost of human suffering or death from malaria must be added the cost of treatment of the sick, programmes for the control of the vector mosquitoes and the impact on economic development programmes (Gratz 1985).

The effective control of malaria on a large scale really started with the advent of residual insecticides e.g. DDT, BHC and dieldrin, and their intra-domiciliary application for the control of mosquito vector species. This was coupled with the availability of new, potent, inexpensive and relatively well tolerated drugs (Wernsdorfer 1980, Davidson 1982). By 1948, Venezuela, Cyprus, Italy, the United States of America, and Mauritius had embarked on the venture of eradication, a goal which was eventually achieved by four of these countries. The enthusiasm and confidence generated was such that in 1955 and 1956 the Eighth and Ninth World Health assemblies adopted a programme aimed at the eradication of the disease based on spraying houses with residual

insecticides (World Health Organization 1955a, 1956). Outstanding successes were achieved in the 1960s, but mainly at the northern and southern extents of the distribution of the disease. Tropical Africa with the highest incidence and the most stable type of malaria, was not included, on the grounds that it did not have suitable basic health services on which to build an eradication programme of this kind (Wernsdorfer 1980, Davidson 1982). The eradication programme met with various degrees of success: In more than 30 countries or territories, malaria was eliminated and reintroduction effectively prevented. In many others, the mortality and morbidity caused by malaria and the prevalence of the disease were reduced to a low level. In a significant number of countries, however, success was not obtained in the short or long term. The reason for these failures were many. These included; political instability, financial and administrative shortcomings, inflationary cost increases in respect of equipment, insecticides and other operational necessities (Bruce-Chwatt 1980, Wernsdorfer 1980, Fontaine 1983). The concept of eradication was abandoned in 1969 by the World Health Assembly of the United Nations as it was considered practically unattainable (World Health Organization 1969). The ensuing policy was to urge countries to contain the disease at levels with which their own general health services could cope, rudimentary though many of them were (Davidson 1982). The net result has been a marked resurgence of the disease in many parts of the world. A resurgence that has been exacerbated by the spread of insecticide resistance in the mosquito vectors (Bruce-

Chwatt 1980) and of drug resistance in the parasite (World Health Organisation 1984).

In 1986 at a conference held in Washington, D.C., U.S.A. on behalf of the U. S. Agency for International Development, Wurapa & Beausoleil (1986) presented a paper on the malaria situation in Africa, and made the following statements; "Malaria is still the most important parasitic disease in Africa, with an estimated incidence of 20 million cases per year. In Africa, south of the Sahara, some 200 million people are chronically infected, and, of these, about one third suffer acute manifestations of the disease in the course of a year". The magnitude of the problem is staggering and the evidence suggests there has been little change in the hyperendemic to holoendemic situation over most of tropical Africa. It is generally quoted that malaria in Africa is responsible for the death of one million infants and young children each year. This mortality figure is an extrapolation from the work done by Bruce-Chwatt (1952) in southern Nigeria. A recent study by Greenwood *et al.* (1987) in a rural area of the Gambia, found the overall mortality rate from malaria in children under the age of 5 years, to be 10 per 1000 per year. A figure remarkably similar to that obtained by Bruce-Chwatt (1952), 35 years earlier.

It is a generally expressed view, that to effectively control malaria in Africa, new control tools which only continued research can provide, coupled with the improved use of classical measures, would need to be applied on a

localised and long term basis.

1.2 MALARIA AND IT'S CONTROL IN NATAL, A HISTORICAL PERSPECTIVE.

Situated wholly within the sub-tropics (29° 32° E; 27° 31° S), Natal is bounded to the east by the Indian Ocean and to the north, west and south by Mozambique, Swaziland, Lesotho, Transkei and the provinces of the Transvaal and the Orange Free State (Figure 1). Natal is historically afflicted by endemic malaria, a condition that had to be taken into account by early European explorers and the Nguni people when considering settlement and military excursions. The endemic malaria area is in the north-eastern part of the province. This area consists of a coastal plain, bordered by the sea in the east, Mozambique in the north, Lake St. Lucia in the south and the 400-m isohyet in the west (Figure 2) and encompasses the districts of Ingwavuma, Ubombo and Hlabisa. In the past malaria epidemics occurred seasonally to the south of this area, and in the interior, severe, but localized, epidemics lasting over several seasons broke out from time to time (Park-Ross 1936). Severe outbreaks were recorded as far south as Durban in 1905 (Hill & Haydon 1905) and Umzinto in 1930 (Paterson, personal communication). During the late 1920s and 1930s, the disease had a marked effect on the agricultural development of the coastal belt. In 1928-29 a large number of employees in the sugar mills and plantations were afflicted. Of an estimated population of 6 000 Europeans at risk, 7 died; of 20 000 Asians, 151

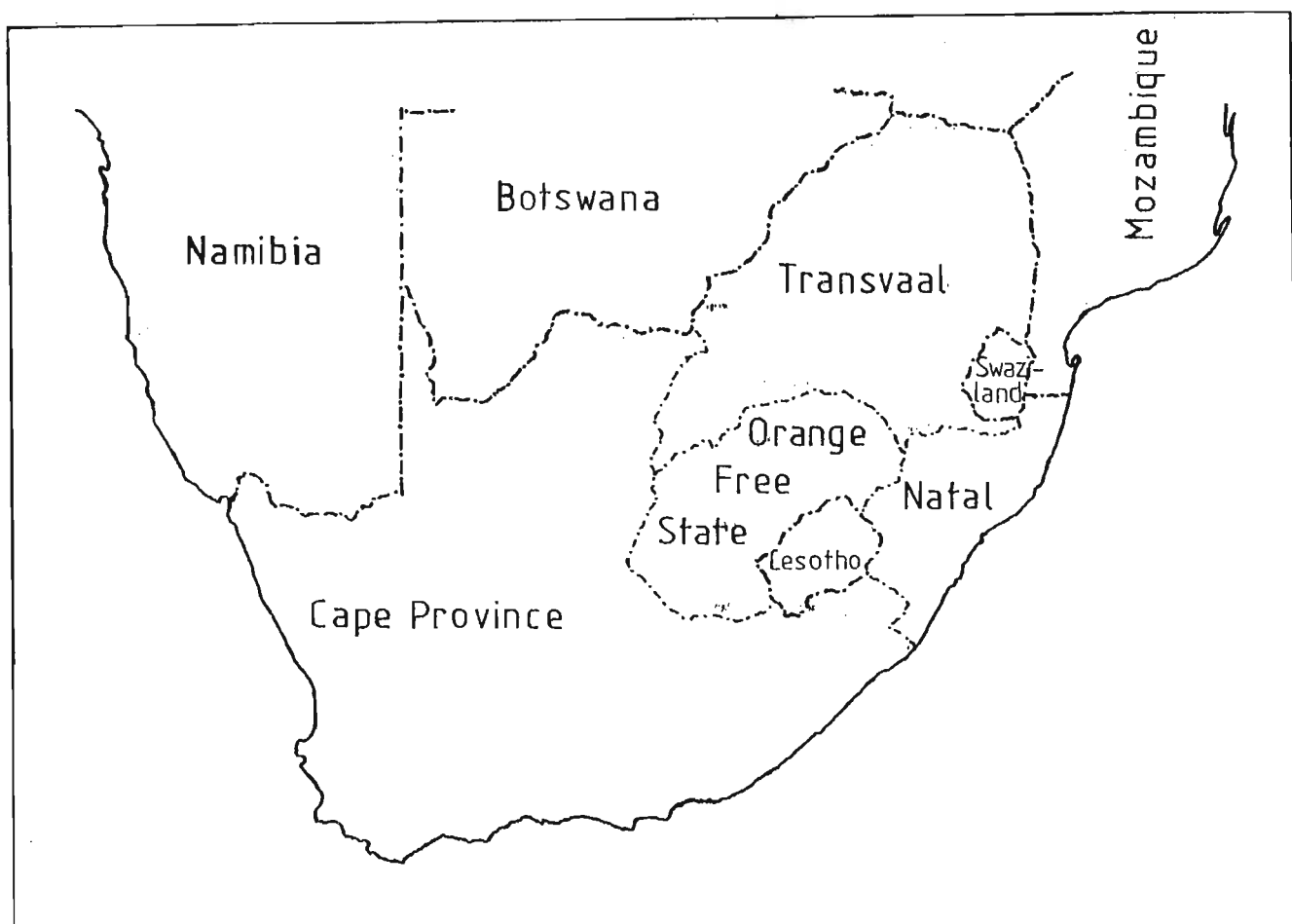


FIGURE 1. Map of the Republic of South Africa, showing the neighbouring states and it's four provinces.

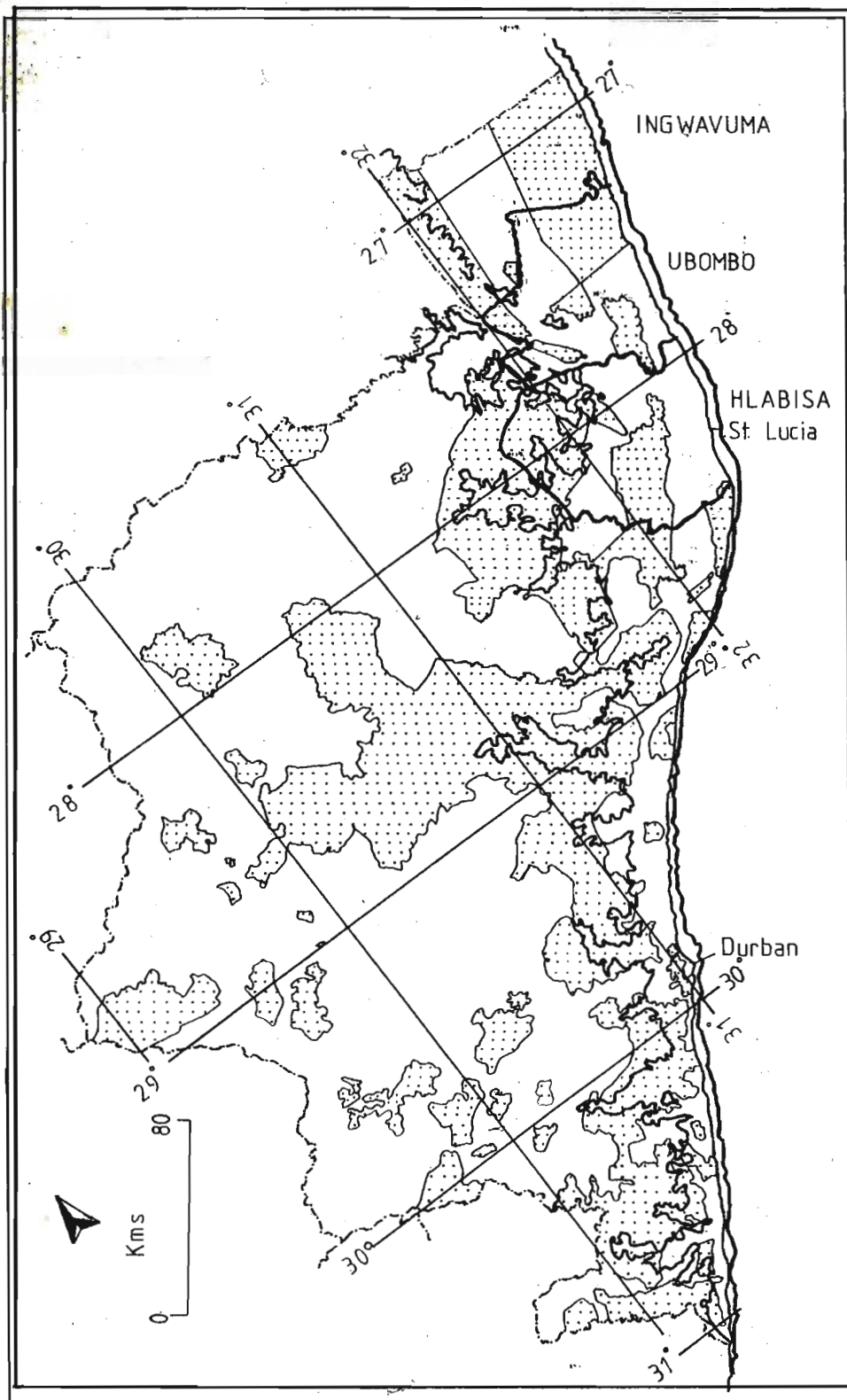


FIGURE 2. The province of Natal, showing that portion classified as KwaZulu (shaded area) and including the districts of Ingwavuma, Ubombo and Hlabisa. The overlay shows the 400 metre isohyet.

and of 215 000 blacks, 2 600 died (Nethercott 1974).

The need for malaria control was paramount, and at the invitation of the government, Professor Swellengrebel of the University of Amsterdam visited Natal in 1930 to take part in an investigation of the malaria situation in the Union of South Africa as a whole (Swellengrebel 1931). In his report the principles of species sanitation, which have been followed ever since, were laid down. As a result of this report, intensive anti-malaria measures were implemented. These actions were extended to include all the malaria areas of the province with the exception of the three northern districts, Ingwavuma, Ubombo and Hlabisa. These were excluded on the recommendation of Swellengrebel (1931), as they were highly endemic areas and the fear was expressed that the natural immunity of the population would diminish if a control policy was introduced.

Anti-larval measures using oil and Paris green (copper acetoarsenite) were introduced in 1932 and continued to be the main means of control until 1946. In 1934, pyrethrum was introduced as an intra-domiciliary knock-down insecticide; spraying was repeated weekly during the main transmission season and significant results were obtained (De Meillon 1936). Experimental work by De Meillon (1936) showed that, apart from being more effective, the control of adult mosquitoes cost only about a third of that of larval control. This work laid the foundation for the later worldwide use of intra-domiciliary residual insecticides against adult mosquitoes. In 1946 the use of pyrethrum was

discontinued and was replaced by DDT, both for house spraying and larviciding. This continued until 1953, when these measures were gradually extended to include the three northern districts, beginning with the spraying of all habitations within a three mile radius of mission stations and police camps. Anti-larval measures were abandoned in 1956. In the same year, malaria became a notifiable disease in the Union of South Africa. Total coverage with a residual insecticide in the northern districts of Natal was achieved for the first time in October 1958.

1.3 AIMS OF THE PRESENT STUDY AND THEIR MOTIVATION

"The *science* of malaria control, developed slowly and painfully from the beginning of the century to a relatively high state of sophistication, was almost overnight converted to the rather simplistic *technology* of malaria eradication. This basically required that one knows how to deliver 2 grams of something to every square metre of a sometimes elusive interior wall. The promise of malaria eradication in 5, 6, 7 or even 10 years had wide-ranging effects. One could not expect much success in efforts to recruit some of the better minds to careers in malariology when articles were appearing in the major journals on the epidemiology of a disappearing disease. One would be a little suspicious of an individual who today decided that specialization on the epidemiology of smallpox would make a good career. There is little doubt that we have lost a generation of malariologists and a generation of malaria research, losses

which will be difficult to recoup" (reprinted from the presidential address by G. M. Jeffery to the American Society of Tropical Medicine and Hygiene 1976).

South Africans were obviously not immune to the wide-ranging effects of the malaria eradication policy, as highlighted by the malaria research record for the Republic of South Africa (Figure 3). Appendix one contains a brief summary of the published scientific studies completed on malaria in the Republic of South Africa and utilised in compiling Figure 3. The first scientific publication on malaria was in 1905 on the malaria outbreak of this year in Durban, this was followed by one publication per decade for the next 20 years. There was a peak in malaria research during the period 1930 to 1940, prior to the widespread acceptance of vertical malaria control programmes. With the acceptance of these programmes research in malaria suffered tremendously, not only globally but also locally. This is evidenced in South Africa by the low scientific output in respect to malaria for the 40 year period 1940-1980. Research output, however, increased dramatically again during the 1980's. This increase in knowledge comes at a time when the malaria problem is again entering into the world spotlight as the effects of parasite resistance to chemoprophylaxis and treatment are being felt in developed countries.

The much needed change in attitude from the eradicationist policy as evidenced by this scientific output is in large the result of foresite on the part of Professor H.E.H. Paterson. Fifteen of the 23 scientific publications on

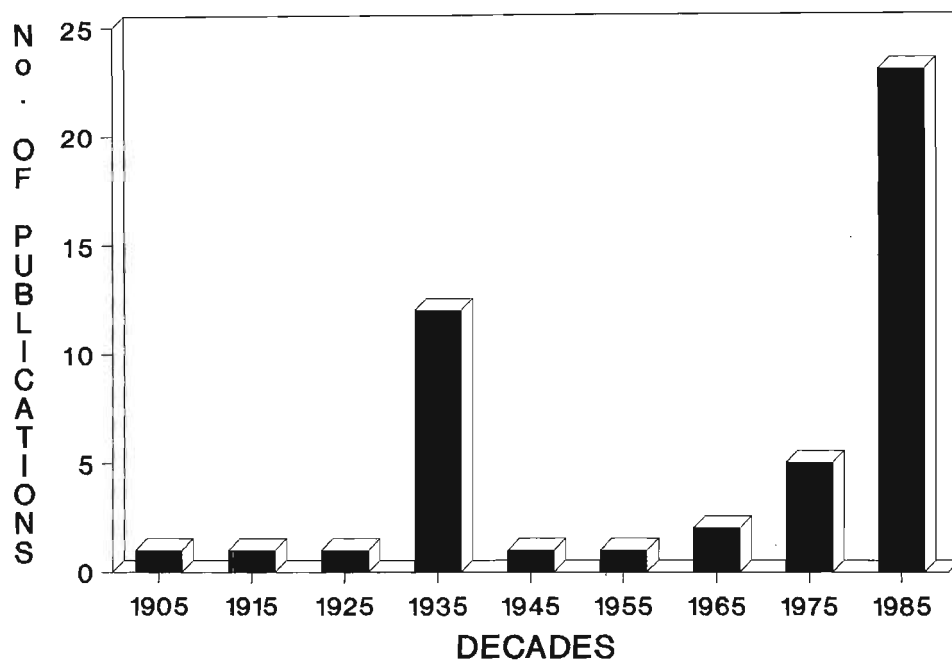


FIGURE 3. South African scientific publications on malaria, expressed as 10 year totals from 1900 to 1989.

malaria in the eighties include at least one author who studied for a postgraduate degree in science under the tutelage of Prof. Paterson.

Since the early studies of the 1930's; *An. gambiae* and *An. funestus*, the two major vectors of malaria in Africa, have been shown to be species complexes; the endemic malaria area of Natal has been subjected to a vertical malaria control programme where all homes in the high risk areas are subject to the annual intra-domiciliary application of DDT. Studies elsewhere in Africa have shown that house spraying with a residual insecticide differentially effects species and can change species composition in an area (Paterson 1963). This factor and the elucidation of the *An. gambiae s.l.* and the *An. funestus s.l.* as complexes of species, to a large extent, undermines the knowledge accumulated prior to the 1960s in regard to local circumstances relating to malaria transmission and it's control.

A knowledge of the extent of the disease, the identity of the vector, and it's biology and behaviour are essential to the planning and implementation of cost-effective vector control. Bearing this in mind and considering the lack of current knowledge of the dynamics of transmission and control of the disease in this area, it was considered essential that a broad-based study on the epidemiology of malaria in Natal be initiated.

The first consideration in any broad-based study of disease is to evaluate the extent of the problem. Malaria is a

notifiable disease in the Republic of South Africa and the first aim of this project was to analyse the malaria case data from Natal province. This analysis would outline the current prevalence and distribution of the disease. Specific studies on the mosquito vector would then be undertaken in the high risk areas. These studies would concentrate on the behaviour of the potential vector species in relation to disease transmission and control, placing special emphasis on specific species identification.

Due to the broad based nature of this study, it was of necessity that each section (chapter) include it's own introduction and motivation and closely follow the format of a manuscript prepared for publication. The final chapter (i.e. Chapter 9) of the thesis is a broad based summary that considers the results obtained in this study in relation to the epidemiology of malaria in Natal province.

CHAPTER 2

AN ANALYSIS OF RETROSPECTIVE MALARIA CASE DATA FROM NATAL PROVINCE

2.1 INTRODUCTION

Malaria has always been a major health problem for a large proportion of the population of Natal (Hill & Haydon 1905, Swellengrebel *et al.* 1931, Sharp *et al.* 1988). The control of the disease is considered essential for the continued social and economic development of the province. The control strategies in this province have been largely based on the malaria eradication programmes formulated in the 1950's and 1960's which approached malaria as an infection. This approach aimed at the eradication of the disease, required the total elimination of the parasite in a set time. The time frame placed on these programmes was a consequence of the high costs involved and the need to eliminate the parasite before the development of vector insecticide resistance.

"Today countries are faced with the difficult task of trying to control malaria in a situation where there is resistance not only of malaria vectors to insecticides but also of parasites to drugs. Thus control programmes, not limited in time, have had to be developed, brought in line with the other public health priorities and implemented with the resources available, taking into account the local

epidemiological situation as well as the socioeconomic development plans of the country. In such circumstances malaria must be considered in terms of a disease problem and not an infection. Such an approach means the continuing presence of infection in the community, maintained by mosquito transmission, but at the same time keeping it to a minimum level by the most effective utilisation possible of the available resources and technology. To achieve this, careful, pragmatic planning and replanning are essential, using an approach which may vary depending upon whether an anti-malaria programme is being implemented for the first time or whether an eradication programme is being reoriented to malaria control" (Beales *et al.* 1988).

As outlined by Beales *et al.* (1988) the analysis of the malaria case data and its geographical distribution is essential to the initiation of and the reorientation of existing control programmes to meet changing concepts in control strategy. The analysis of these data are also necessary for the ongoing assessment of the efficacy of existing control strategies and to enable pragmatic management decisions to be made. Epidemiology is most commonly defined as the study of distributions and the determination of health states within a population. Only when such an evaluation of a specific disease has been made can its importance be assessed and decisions made concerning containment. Further work relating to containment will obviously be based on this epidemiological foundation. This pattern of logic briefly outlines the motivation for

this study which analysed the malaria case data from Natal Province.

2.1.1 Natal Province in the context of the Republic of South Africa

The province of Natal, includes those areas classified as the KwaZulu areas and those classified as the Natal areas (Figure 2, Chapter 1). This province covers an area of 90,327 km². The KwaZulu areas cover approximately 33,150 km² or 36.7% of this area and malaria control in these areas falls under the jurisdiction of the KwaZulu Government Services. The remaining 63.3% of the province is classified as the Natal area and the Natal regional authorities of the Department of National Health and Population Development are responsible for malaria control in these areas. Over the 13 year period 1976-1988 Natal province accounted for 37.4% of the total number of malaria cases reported in the Republic of South Africa (Figure 4).

2.2 MATERIALS AND METHODS

2.2.1 Detection and classification of malaria cases

All cases of malaria are classified broadly according to the method of detection, that is, active or passive, and according to the origin of the infection, that is, indigenous to the area where it was discovered, or imported from another area of Natal/KwaZulu, or another country. Passively detected cases are those persons, usually with

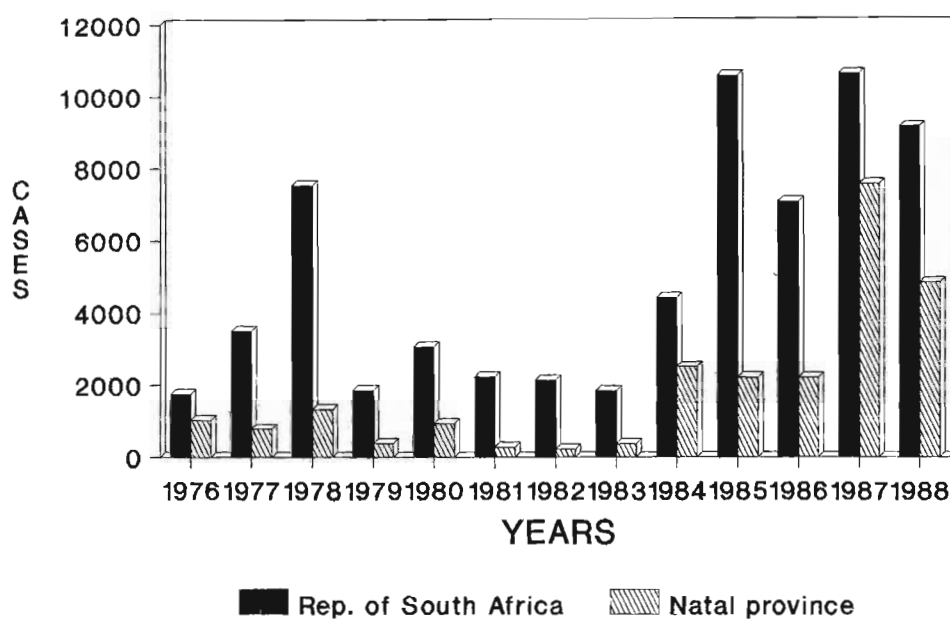


FIGURE 4. Comparative annual malaria case totals for the Republic of South Africa and Natal province for the years 1976 - 1988.

symptoms of malaria, who present themselves to a clinic or hospital. The active detection of cases is carried out in one of three ways: 1) Routine active surveillance is practised in areas of high endemicity. A surveillance agent has a section of territory that he covers once approximately every six weeks, and takes approximately 15 blood smears per week from the residents. 2) Epidemiological investigations (EPI) are carried out after the detection of a malaria case, either active or passive. A field team takes blood smears from all residents within a kilometre radius of the home of the index case. 3) Mass blood examinations (MBE) are carried out when there is a localised outbreak of malaria, indicated by passive case detection and/or EPI. Malaria surveillance teams are then sent into the area and a blood smear collected from all residents.

The operational structure of the malaria control programme in regard to case detection was started in the 1960s. By 1976 the system as it exists today was well established and the quality of the data collected over the period from 1976 to 1988 is therefore considered to be consistent.

2.2.2 Microscopic diagnosis of malaria

In the microscopic diagnosis of malaria, thick blood smears (Shute 1988) were used. Thick blood smears have the advantage that the parasites are detected with greater frequency, so reducing the time required for the examination of each blood smear. Staining of the blood smears was as outlined by Shute (1988) for thick smears using Giemsa stain.

Microscopic examination was carried out using a compound microscope and a combination of lenses which provided a total linear magnification of *1000 for oil immersion microscopy. All the blood smears from the KwaZulu areas were examined at the KwaZulu Health Laboratory (Jozini) and quality control done at Edendale laboratory. Bloodsmears from the Natal area were screened by the Natal Regional (Pathology) Laboratory Services laboratory at Empangeni or Durban, with the latter being responsible for quality control.

2.2.3 Population data

The population census data used in this study was obtained from the Government Statistics office. The 1980 population census data was used in the analysis of the 1976-1985 malaria case data from the KwaZulu areas and the 1985 population census data for the analysis of the 1986-1988 malaria case data from both the KwaZulu and Natal areas. The 1985 population census data were adjusted for undercount prior to their use. The figures used in this adjustment were supplied by the Government Statistics office and were as follows:

Race group	% adjustment for undercount
White	7.6
Coloured	1.0
Asian	4.6
Black	20.4

2.3 RESULTS AND DISCUSSION

2.3.1 Geographical distribution of malaria cases

Of the 65 magisterial districts in Natal province, malaria cases were reported from 35 (21 districts within the Natal areas and 14 in the KwaZulu areas). In the Natal areas 91.5% of the cases came from eight districts (Table I, Figure 5). The remaining 8.5% of cases were reported from a further 13 districts of which no one district accounted for more than 0.16% of the total number of cases.

During the period 1976-1985 96.4% of the malaria cases detected in the KwaZulu areas came from : Ingwavuma district (62%), Mozambique (13.9%), Ubombo district (13.9%) and Hlabisa district (6.6%) with no other district accounting for more than 2% of cases (Figure 5). Over the three year period 1986-1988 four districts accounted for 96.1% of the malaria cases : Ingwavuma (58.4%), Ubombo (28.9%), Hlabisa (5.3%) and Nseleni (3.5%) (Figure 5). The remaining 3.9% of cases reported were spread over 10 districts.

The overall attack rate for both the Natal and KwaZulu areas using the total population figures for each area were very similar for the period 1976-1985 at 0.13 and 0.18 per 1000 head of population (Table II) (calculated using the 1980 population census data). The attack rate was markedly higher in both areas for the period 1986-1988 in relation to the attack rates from the respective areas for the previous 9 year period. Although higher, the attack rates from the two

Table I. The eight districts of the Natal area which accounted for 91.4% of the total number of malaria cases reported during the period 1986-1988, and the number of cases reported in each district.

District	Malaria cases		
	Total	Mean per annum	% of total
Lower Umfolosi	1789	596	29.3
Lower Tugela	871	290	14.3
Mtunzini	737	245	12.1
Umfolosi	660	220	10.8
Pongola	454	151	7.4
Lower Hlabisa	387	129	6.4
Durban	369	123	6.0
Hlabisa	309	103	5.1

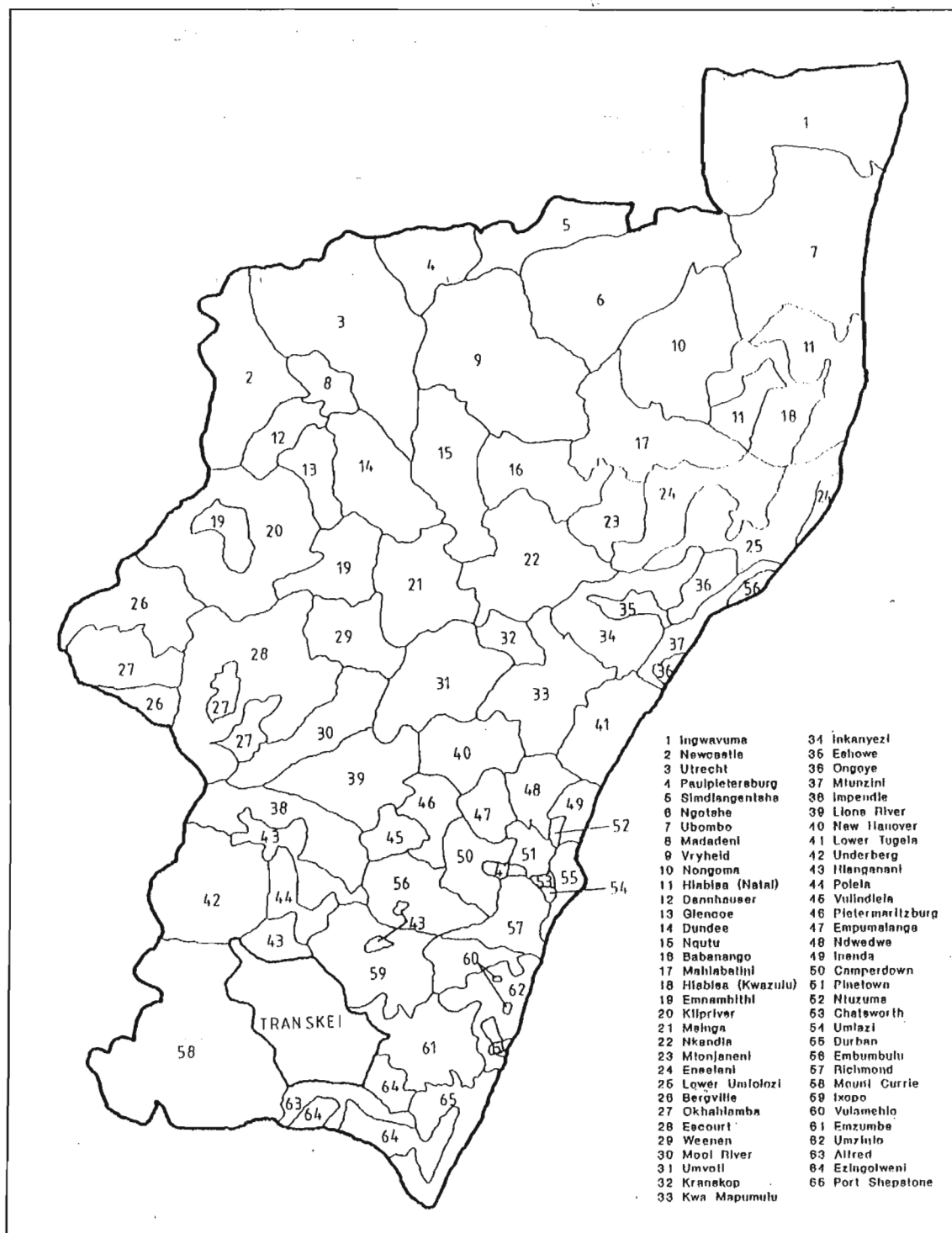


FIGURE 5. The province of Natal, showing the 65 magisterial districts.

Table II. Attack rates per annum per 1000 head of population for the Natal and the KwaZulu areas respectively and the two districts of highest risk in both regions.

Locality	Year	Attack rate /annum / 1000 head of population	
		Mean	Range
Natal	1986-1988	0.71	0.36-1.13
Lower Umfolosi	1986-1988	6.8	2.7-9.02
Lower Tugela	1986-1988	1.9	0.52-2.92
KwaZulu	1986-1988	0.70	0.15-1.07
	1976-1985	0.18	0.02-0.34
Ingwavuma	1976-1985	3.76	0.62-6.7
	1986-1988	16.6	4.2-23.34
Ubombo	1976-1985	1.09	0.02-3.52
	1986-1988	10.4	0.89-17.88

areas were again similar at 0.71 for the Natal areas and 0.70 for the KwaZulu areas (calculated using the 1985 population census data)(Table II). Although the attack rates from both the Natal and KwaZulu areas were similar, the population census data showed the population of the KwaZulu areas to be 89.5% greater than that for the Natal areas.

The overall attack rate for the KwaZulu areas showed a 3.9 fold increase for the period 1986-1988 in relation to the previous 10 year period (Table II). A marked increase was evident in the districts of Ingwavuma and Ubombo which respectively showed a 4.4 and a 9.5 fold increase in the attack rate for the 1986-1988 period in relation to the 1976-1985 period.

The attack rate in the two districts of the Natal areas with the highest malaria case rates were lower than that found in the high risk districts of the KwaZulu areas (Table II). Nevertheless the attack rate in the Lower Umfolosi district (Natal area) at a rate of 6.8 per 1000 head of population was still high.

The magisterial districts in the KwaZulu area under the attack phase of malaria vector control have been sub-divided for administrative purposes. Of a total of 227 such areas from which malaria was recorded over the 1976-1985 period, 18 accounted for 79.1% of cases. The five areas of highest risk were situated in Ingwavuma and were Mekanies Drift (11.9%), Muzi and Muzi Border Post (14.0%), Ndumu (8.5%), Sihangwane (9.3%), Shemula (5.3%), in total accounting for

49.0% of the cases. No other single area accounted for more than 5% of recorded cases. The Muzi Border Post was established by the health authorities in an attempt to assess and control the malaria parasite carriers coming into the country from Mozambique. A bloodsmear was made from all people passing through the post and each person was supplied with a single dose of chloroquine (10 mg per kg of body mass, and chloroquine plus pyrimethamine = Daraclor was used). This border post was closed by the Mozambiquen authorities on 18 March 1981. During the four years prior to its closure, the post accounted for 16.1% of the total number of malaria cases recorded in the KwaZulu areas, varying annually from a low of 9.7% to a high of 28.2% of total.

During the period 1986-1988 the distribution of the high risk areas in the KwaZulu areas changed in relation to the previous 10 years. Only five areas individually accounted for more than 5% of total malaria cases, and accumulatively accounted for 55.7% of cases. Three of these areas were in Ingwavuma district, namely: Mekanis Drift (14.9%), Ndumu (13.3%), Shemula (6.4%) and two in Ubombo district, namely: Mamfene (14.6%) and Ophansi (6.5%).

2.3.2 Annual malaria case totals

The annual numbers of malaria cases for the period 1976 to 1988 for Natal Province (KwaZulu and Natal areas) are shown in Fig. 6. The mean number of 1883 per annum (range 208-

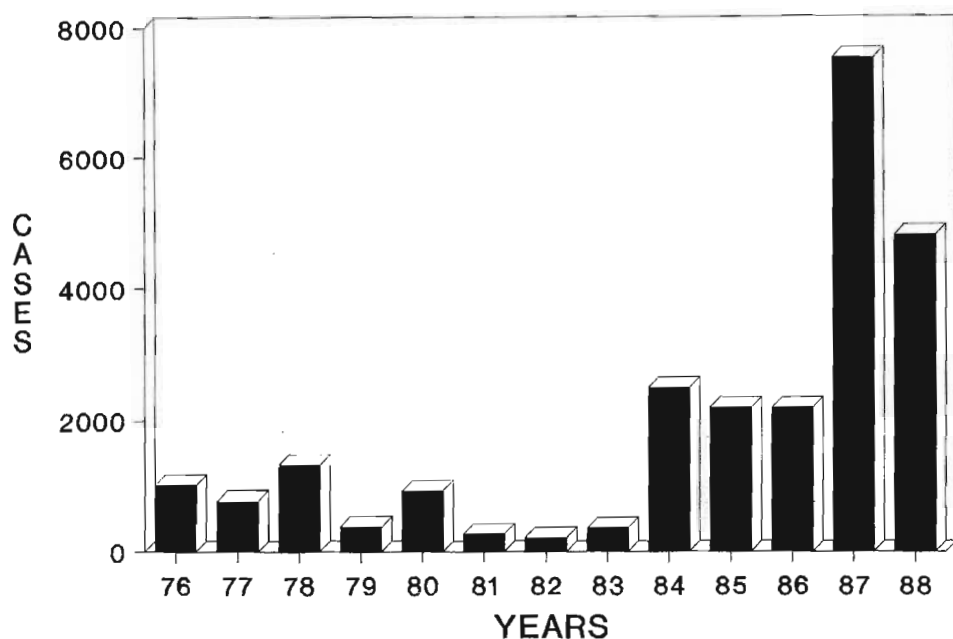


FIGURE 6. Annual totals of malaria cases for the period 1976 - 1988 for Natal province.

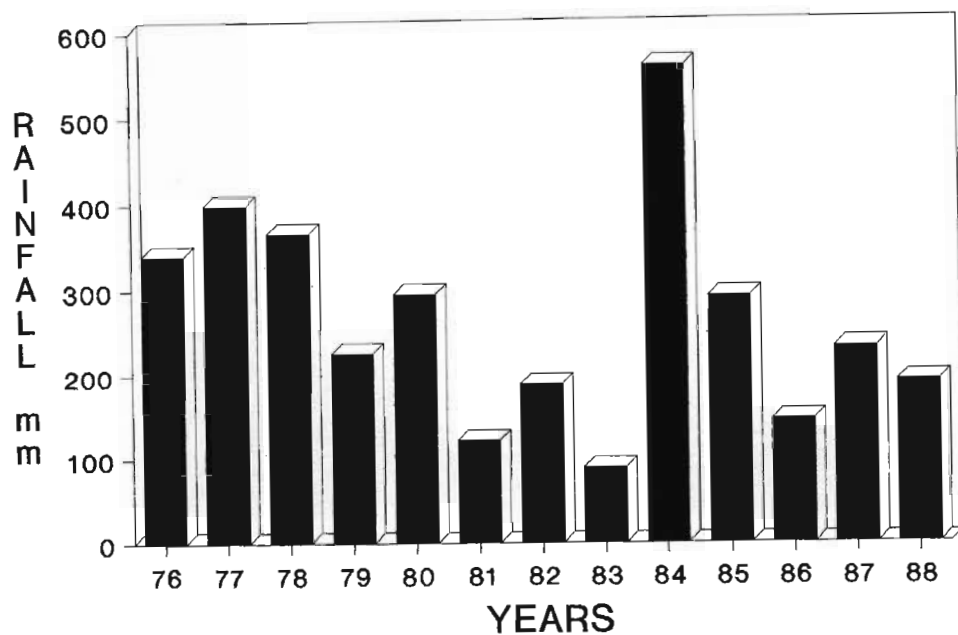


FIGURE 7. January to April accumulated rainfall data from Makhatini Research Station, Mafene area, Ubombo district, KwaZulu, for the years 1976-1988.

7530) decreased during the four drought years (1979, 1981, 1982 and 1983), ranging from 208 to 380 (mean = 305). The annual malaria case total has, however, risen markedly over the last five years (1984-1988) with a range of 2193 to 7530 cases per annum (mean= 3846)

The dramatic increase in the annual malaria case totals from 1984 to 1988 is considered to have been the result of a combination of factors. The major factors contributing to this increase are considered to have been adequate rainfall for the summer mosquito population increase and radiation of vector mosquito species, population migration into the Republic of South Africa from Mozambique and chloroquine resistant *Plasmodium falciparum*. Malaria transmission was further exacerbated on a localised scale by irrigational spillage due to agricultural development.

Good summer rains are a prerequisite for the summer radiation of the anopheline vectors from the marginal winter breeding sites (le Sueur & Sharp 1988). This is well illustrated by the low numbers of malaria cases during the drought years in the Natal province (1979, 1981, 1982 & 1983) when the annual malaria case totals were the lowest recorded for the 13 year period (1976-1988). Anopheline vector populations were further difficult to find during this drought period (Sharp 1983). Figure 7 shows the accumulated January to April rainfall recorded at the Makhatini research station, Mamfene area, Ubombo district. The year with the highest recorded rainfall was 1984, this was unusually high as a result of a cyclonic system

that moved into northern Natal province from the Mozambique channel (cyclone Demoina). The accumulated rainfall totals for 1985-1988, years with exceptionally high malaria case totals (Figure 6), were in all years lower than that recorded for the years 1976-1978 when the malaria case totals were relatively low. Rainfall is therefore not considered to be directly responsible for the dramatic increase in malaria cases during the period 1986-1988. The rainfall must, however, have been adequate for the summer radiation and population increase of the mosquito vector species.

There has been no structured malaria control programme in Mozambique for in excess of a decade and as a result of the continuing civil war in this region, increased numbers of refugees, many of whom are infected with malaria, have been crossing the border into the Republic of South Africa. This is highlighted by the large number of asymptomatic malaria cases detected during the last five years by active surveillance in the Natal area and classified as imported from Mozambique (section 2.3.5).

Chloroquine-resistant *Plasmodium falciparum* malaria was first confirmed as occurring in Natal province in 1985 (Herbst et al. 1985). During May 1987 and January 1988 Freese et al. (1988) investigated the *in vitro* chloroquine sensitivity of *P. falciparum* in isolates collected from 39 patients in the Ingwavuma and Ubombo magisterial districts of the KwaZulu areas and found 94% to be resistant. Seventy-

four percent of the tests showed inhibition to only occur at chloroquine concentrations of 32 pmol/l or greater, indicating RIII resistance. The presence of a partially chloroquine-resistant *P. falciparum* population coupled with a large mosquito vector population would most certainly lead to an upsurge in the number of cases of clinical malaria. This may have contributed to the situation in the endemic areas of KwaZulu, where 4830 cases of malaria were recorded in 1987, far in excess of the number of cases reported annually over the 11-year period 1976-1986, which ranged from 75 to 1199 with a mean of 648. In addition, the number of cases in the off-season (July-November) of 1987 was 532, which was much higher than the mean number of off-season cases (118,5; range 3-226) recorded over the same 11-year period. During January 1986, a large population of *An. arabiensis* was found breeding in the irrigational overflow from an agricultural development scheme at Mamfene, Ubombo district (Chapter 6). The adult mosquitoes of this species, which is the principal vector of malaria at numerous localities in Africa (White 1974), were found biting man both indoors and outdoors (Chapter 8). However, in 1986 only 5 cases of malaria were reported in the Mamfene area. In 1987, *An. arabiensis* was again detected in large numbers in this locality and 717 malaria cases were recorded. This was considerably higher than the annual mean case rate for Mamfene of 12,6 (range 0-34) calculated over the 11 years 1976-1986. What needs to be considered is whether this upsurge in malaria could be the result of the introduction to the area, possibly through migrant labourers, of a large

parasite reservoir which, as the study by Freese *et al.* (1988) suggests, may have consisted of an increased proportion of chloroquine-resistant parasites. If this was the case, parasite control measures involving the use of chloroquine for treatment may have been compromised in this region. As *An. arabiensis* is widely distributed within the endemic malarial areas of KwaZulu, it may be that a similar situation to that in the Mamfene area existed in the Ndumu area (where there was also chloroquine resistance, as shown by Freese *et al.* (1988)), and possibly in all the endemic malarial areas of KwaZulu in which a sudden upsurge in the number of malaria cases had been experienced. It is possible that chloroquine resistance had been exacerbating the situation in Natal province as far back as 1984, coincident with the marked increase in malaria cases.

The findings of the study by Freese *et al.* (1988) had serious implications in regard to the prophylaxis and treatment of malaria in Natal province. In view of the fact that parasite control is essential to containment of the disease, there was clearly an urgent need to consider whether the use of alternative antimalarial drugs might be appropriate. The continued use of chloroquine on a long-term basis might have resulted in an increased prevalence of malaria resistant to it and may also have given rise to a higher degree of stable resistance. In February 1989 the control authorities in both the Natal and the KwaZulu areas changed from the use of Chloroquine to Fansidar for the treatment of malaria.

The total number of malaria cases detected annually in the KwaZulu and Natal areas respectively are shown in figure 8. The mean number of cases per annum during the period 1976-1988 was higher in the KwaZulu areas (mean 1224, range 75-4835) than in the Natal areas (mean 658, range 66-2695). The annual case totals were, however, not consistently higher in the KwaZulu areas over this 13 year period. From 1976 to 1980 the annual malaria case total in the KwaZulu areas (Figure 8) (mean 744, range 297-956) was consistently higher than that from the Natal areas (mean 137, range 66-369). During the 1981 to 1983 period the mean number of cases per annum was the lowest recorded over the 13 year period for both areas. During these years the annual mean was slightly higher in the Natal areas (mean 160, range 133-184) than in the KwaZulu areas (mean 121, range 75-178). The following three years (1984-1986) saw an increase in the annual number of malaria cases to the highest levels recorded in the Natal area since 1976. In the Natal areas the annual mean rose to 1282 cases (range 1048-1295) and in the KwaZulu areas to 1010 cases (range 692-1010). The highest number of malaria cases recorded in a year in both the Natal area (2695 cases) and the KwaZulu areas (4835 cases) was during 1987. There was a dramatic reduction in the number of cases recorded in the Natal areas in 1988 (858 cases), in relation to the 1987 total. The reduction in the annual total in the KwaZulu areas during this year was not, however, as marked (3961 cases).

Overall more cases of malaria were detected annually in the

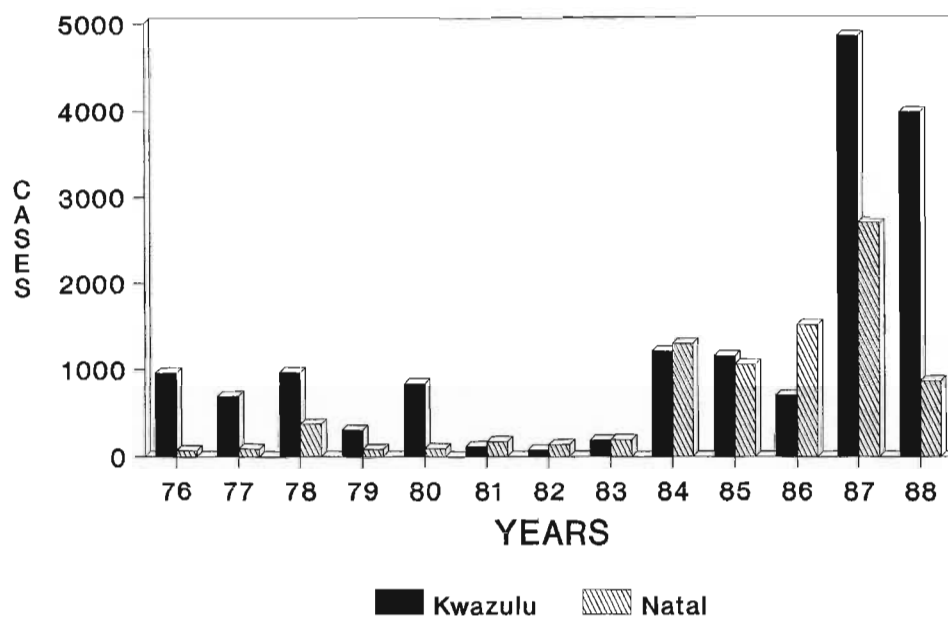


FIGURE 8. Comparative annual malaria case totals for the period 1976 - 1988 for the Natal and Kwazulu areas respectively.

KwaZulu areas than in the Natal areas over the 13 year period 1976-1988. In the KwaZulu areas the number of cases detected was 85.8% higher than in the Natal areas, but it must be borne in mind that the population according to the 1985 population census was 89.5% higher in KwaZulu.

2.3.3 Annual malaria case totals and rainfall

The annual number of malaria cases and the accumulated rainfall for the period January to April (1976-88) recorded at the Makhatini research station, Ubombo district, KwaZulu were not well correlated ($r = 0.0181$; $P > 0.9$) (Figures 6 & 7). During 1986 a large population of *An. arabiensis* was found in the Mamfene area, Ubombo district. Entomological investigation showed that large scale spillage of water from the agricultural development scheme in the area was creating ideal larval habitats. During the 11 year period 1976-1986 the mean annual malaria case rate from Mamfene was 12.6 cases (range 0-34). During 1987 and 1988 this area accounted for the highest number of cases from any KwaZulu area with 717 and 613 cases respectively being detected during the two years. Malaria vector mosquito population increases in this area were not solely a consequence of rainfall in the area but due mainly to agricultural spillage of irrigation water originating from the Pongola Poort Dam at Jozini. Excluding these two years (1987 and 1988) from the data set comparing accumulated January to April rainfall with total annual malaria cases (1976-1986) showed a high correlation ($r=0.8567$, $P<0.001$). In the drought years rainfall was less

than 230 mm from January to April, corresponding to the low prevalence of malaria recorded for these years. Rainfall from January to April during the six other years was always greater than 294 mm (mean = 420,1 mm; range 295 - 788 mm).

The relationship between rainfall measured at Ndumu and the number of cases of malaria was not simple. The cumulative January to April rainfall for Ndumu and malaria cases were not well correlated ($r = 0,643$; $P > 0,05$). Cumulative rainfall figures for the period January to April were low for 1979, 1982 and 1983 and below the average (455,6 mm) calculated for the six years when malaria prevalence was high. During 1981, however, when rainfall of 380,5 mm was recorded for January to April, only 110 malaria cases were reported; this was more rainfall than in 1985, a year with a high incidence of malaria cases (Figure 8). Furthermore, 1980 experienced low rainfall (194,3 mm from January to April) when malaria cases were high.

As outlined the relationship between cumulative rainfall and the prevalence of the disease is not straightforward. An association of this nature is of necessity influenced by the response of the anopheline vector to rainfall and the dynamics of transmission. As outlined, during 1986 there was a large population of *An. arabiensis* in the Mamfene area (Chapter 8) and yet there were only five cases of malaria reported for the area. Although the mosquito vector species was present in the area in large numbers and was biting man, it must be assumed that there were no parasite carriers available. An analysis using entomological data, rainfall

and malaria case studies on a geographically localised scale is considered necessary and might bring a more instructive relationship to the fore. The relationship between rainfall and malaria cases was not investigated for the Natal areas as a whole, due to the low numbers of indigenous cases recorded for the area (Chapter 2, section 2.3.5). This relationship was, however, investigated on a localised scale for a short period at Mtubatuba (Chapter 6).

2.3.4 Seasonal malaria

Figure 9 shows the monthly occurrence of malaria cases expressed as a percentage of the total for the period 1985-1988 for Natal province (KwaZulu + Natal areas).

This figure (Figure 9) shows that malaria cases were detected throughout the year. The total monthly malaria case data indicate that malaria transmission started to rise in December, increased to a peak in April/May, then declined gradually to June. The decline continued during August and September, to the month of October and November when the lowest monthly case totals were recorded.

To understand the seasonal malaria profile fully, it is necessary to analyse it further by including the monthly distribution of actively and passively detected cases which make up this profile (Figure 9). The actively detected cases accounted for 75.3 % of the total number of cases and this profile therefore largely mimics that of the total

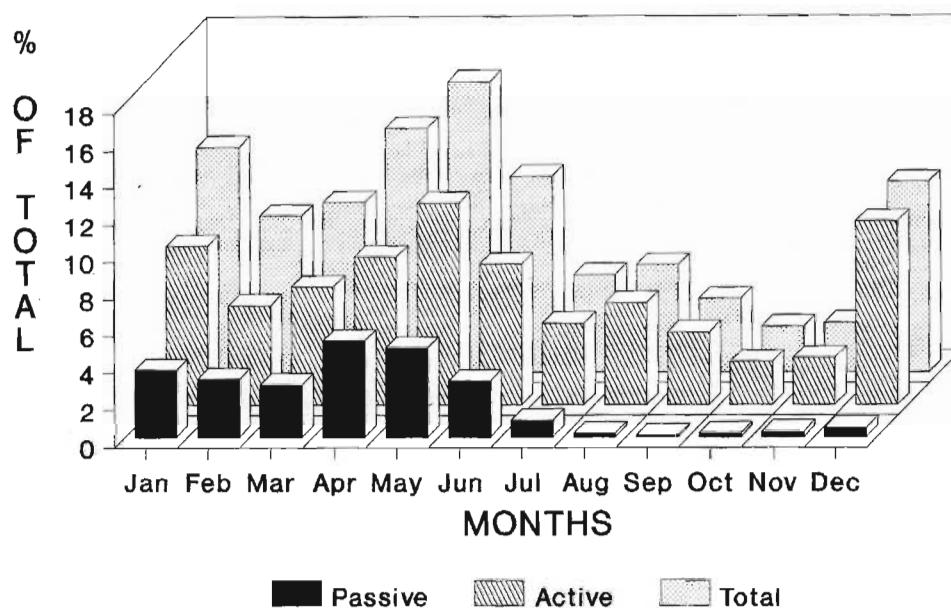


FIGURE 9. Monthly distribution of the total, the active and the passively detected malaria cases from Natal Province for the period 1985 - 1988.

number of cases. The monthly totals of passively detected cases, however, shows a somewhat different seasonal trend: the peak being in April and the numbers are extremely low from July to December. I believe that this profile represents the malaria transmission season because the passively detected cases are essentially non-immune persons who, as a result of infection, develop clinical symptoms. Since the majority of malaria cases (75.3%) were detected by active surveillance, indications are that a high level of tolerance existed in the population. This was further borne out by the fact that the detection of passive cases virtually ceased from July to December, when 93,0% of the cases were detected by active surveillance (28.5% of the annual cumulative total).

Figures 10 and 11 show the accumulated monthly malaria case profiles from the Natal and KwaZulu areas respectively. The seasonal profile of total monthly case data from these two areas is markedly different. Whereas the data from the KwaZulu areas (Figure 11) show a distinct summer/autumn malaria detection profile, the Natal areas data (Figure 10) do not. The actively detected cases from the Natal area constituted 87.9% of the total number of malaria cases detected in contrast to that from the KwaZulu areas where the actively detected cases only constituted 68.2% of total cases. The factors contributing to this difference are discussed in section 2.3.5.

Whereas the monthly malaria case totals from the KwaZulu areas reflect a seasonal profile with the monthly totals

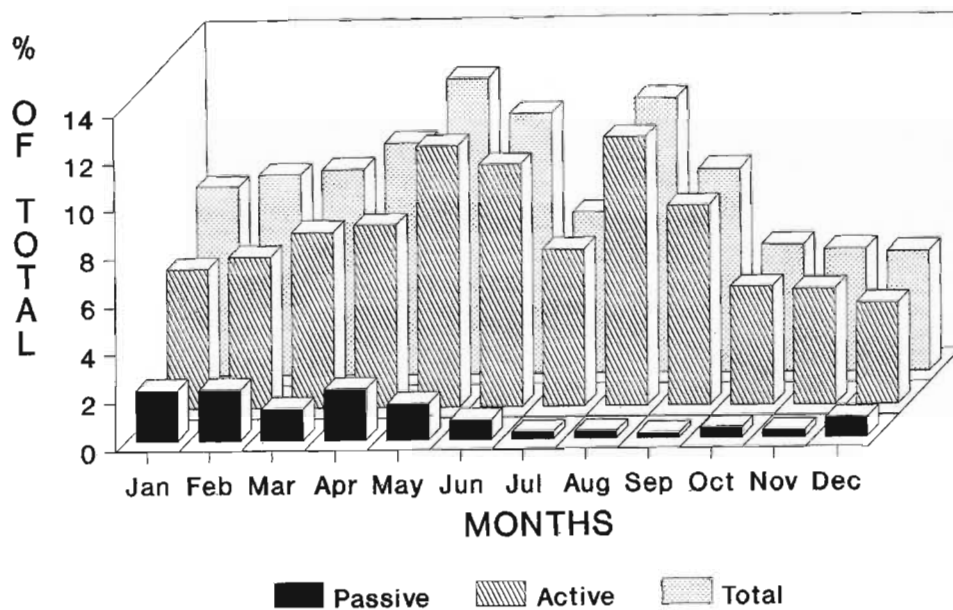


FIGURE 10. Monthly distribution of the total, the active and the passively detected malaria cases from the Natal areas for the period 1985 - 1988.

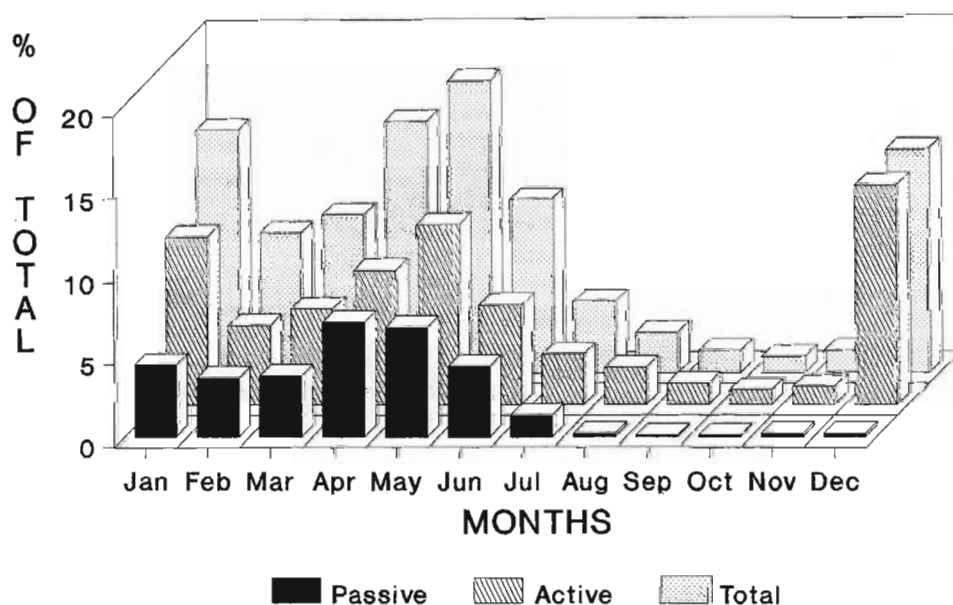


FIGURE 11. Monthly distribution of the total, the active and the passively detected malaria cases from the KwaZulu areas for the period 1985 - 1988.

decreasing in winter and only increasing again in summer, the profile from the Natal area does not show a marked seasonal profile. The Natal area data show the period of peak malaria case detection to be from April to September.

In the Natal areas all malaria patients were asked if they were or had been sick prior to treatment. Of 5839 patients asked about the state of their health, 83.3% replied that they had not felt sick and only 16.7% that they were or had been ill. Of the actively detected cases, 94.7% were classified as healthy and only 5.3% as sick (Table III).

Passively detected cases only accounted for 12.2% of the cases classified as healthy or sick, however, in contrast to the actively detected cases, 98.5% were classified as sick. These data indicate a high level of immunity or tolerance to the disease in the actively detected patients.

In the malarious districts of Ingwavuma and Ubombo there are four hospitals and nine permanent clinics, so that the higher detection of cases by active surveillance in relation to passive surveillance is not considered to be due to a lack of medical facilities in the area. It therefore seems that a high level of immunity or tolerance to the disease exists in the population, as found in a previous study (Swellengrebel et al. 1931). In their study they found that black people from the endemic malaria areas of Ingwavuma and Ubombo showed a high degree of tolerance to the disease, a condition in keeping with the situation in other endemic malaria areas in the world, a result of a high exposure to

Table III. Number of malaria patients classified as healthy or sick in relation to method of case detection.

	Method of case detection	
	Active	Passive
Healthy	4835	16
Sick	272	1057

infection from an early age (Miller 1958).

As a result of the malaria control programme in KwaZulu, the exposure of the population to the disease has been reduced. It might therefore seem surprising that an apparent high level of immunity or tolerance to the disease should still exist. Swellengrebel *et al.* (1931) concluded, however, that black people from certain areas in Natal, which were only periodically affected by epidemics, showed a higher tolerance to the disease than other population groups. This interpretation might partially explain the current finding of this study.

2.3.5 Origin of infection

Figure 12 shows the annual number of malaria cases from Natal province (1985-1988) respectively classified as indigenous to the province and those imported from other countries. During the years of 1985 and 1986 a higher number of cases were classified as imported (51% and 58%) as opposed to indigenous. During the following two years imported cases only accounted for 25% and 19% of the total number of cases detected. The imported cases came mainly from Mozambique, Swaziland, Malawi, Namibia and Angola, with a limited number from elsewhere.

The reason for the difference in the percentage of total malaria cases classified as imported between 1985-1986 and 1987-1988 can largely be attributed to the increase in indigenous cases reported during 1987 & 1988 (Figure 12).

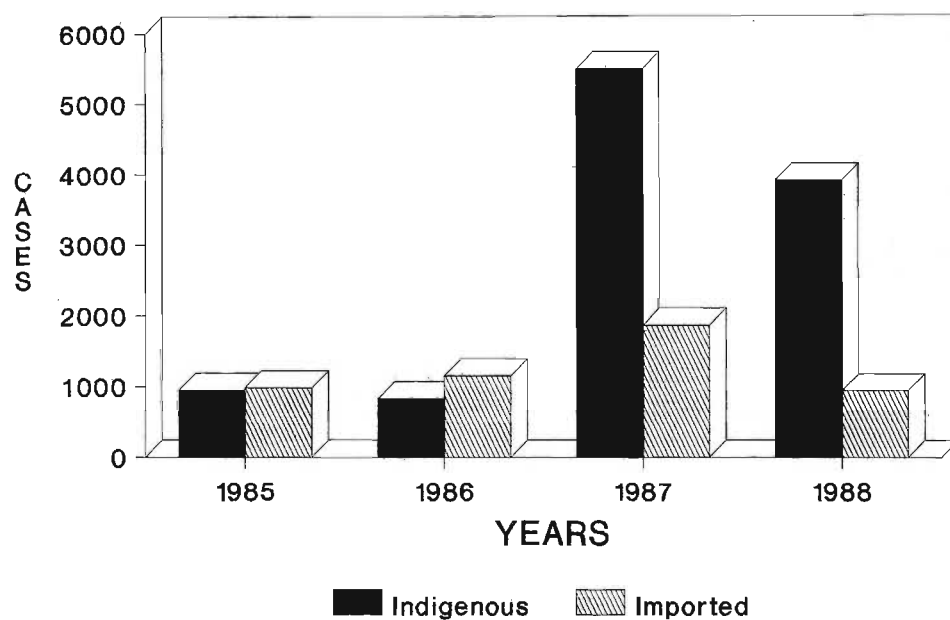


FIGURE 12. Annual number of malaria cases from Natal Province (1985 - 1988), classified as indigenous and imported.

This increase in local transmission of the disease mainly occurred in the KwaZulu areas (Figures 13 & 14). The reasons for the difference in the number of imported malaria cases detected between years must largely be determined by factors beyond the borders of the Republic of South Africa.

Figures 13 and 14 respectively show the annual number of imported and indigenous malaria cases from the Natal and the KwaZulu areas of Natal province for the period 1985-1988. In the Natal areas the majority of cases were imported in contrast to the situation in the KwaZulu area where the majority were classified as indigenous. Imported cases accounted for 82-97% of cases in the Natal area during the period 1985-1988. During this period the majority of the imported cases were classified as originating in Mozambique (mean=43% of total cases, range 19-65), followed by Swaziland (range 2-10% of total cases). These figures should be seen as minima as between 12 and 19% of total cases per annum were unclassified during this period. The mean annual number of imported malaria cases for the KwaZulu areas, expressed as a percentage of total cases for the period 1985-1988 was 18.5% (range 9.6-27% of annual total). The mean annual number of malaria cases imported into the KwaZulu area expressed as a percentage of total cases for the nine year period 1976-1984 was 17.4% and similar to that for the following four years.

In both the Natal and the KwaZulu areas in excess of 80% of the imported cases were detected by active surveillance, indicating a high level of immunity in these people.

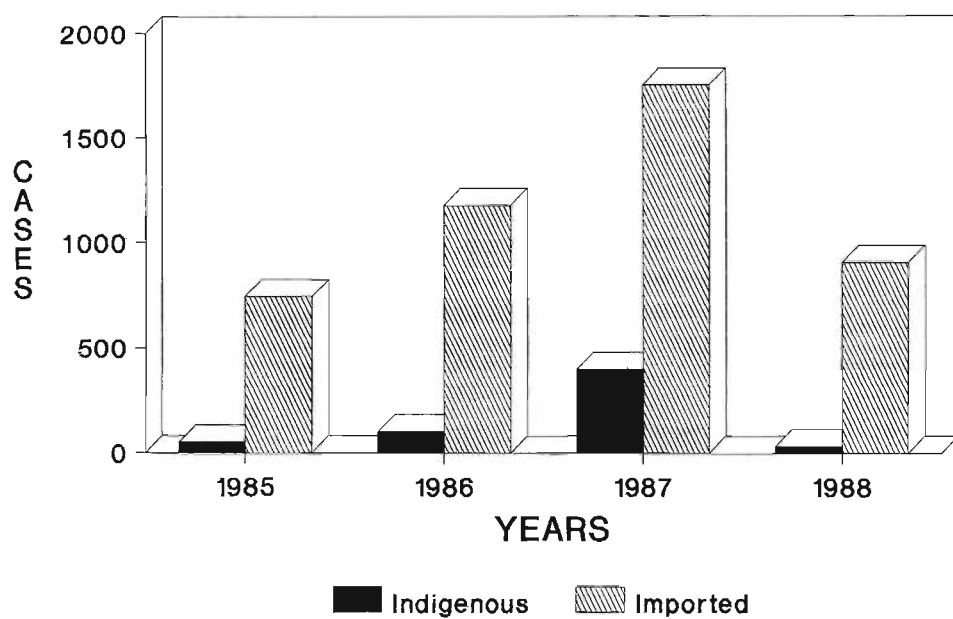


FIGURE 13. Annual number of malaria cases from the Natal area (1985 - 1988), classified as indigenous and imported.

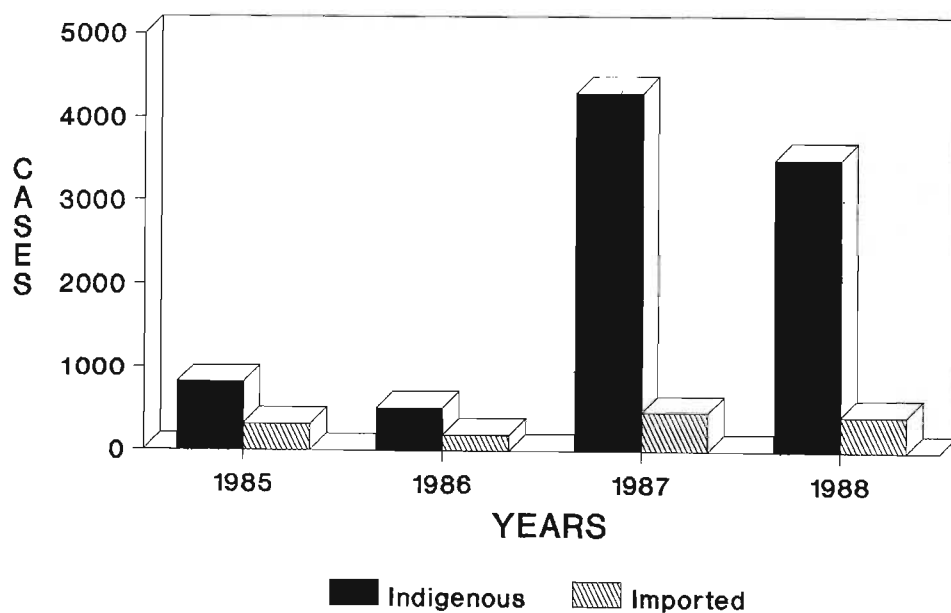


FIGURE 14. Annual number of malaria cases from the KwaZulu area (1985 - 1988), classified as indigenous and imported.

Determining the origin of an infection is not always simple and it is considered prudent to view the figures for imported cases as minima.

There was a marked difference between the Natal and the KwaZulu areas in respect of the number of indigenous and imported malaria cases and the seasonal malaria case detection profile (table IV). These differences are considered to be a direct result of differences in endemicity of the disease in the two areas. In the KwaZulu areas there is no doubt that transmission of the disease occurs seasonally, however, in Natal areas there is very little evidence to show that local transmission had been occurring. During the period 1985-1988 there were only two localised epidemics where the evidence suggested that local transmission had occurred. Evidence in relation to these epidemics which respectively occurred at Mtubatuba and Tongaat are discussed in Chapter 6. The majority of cases detected in the Natal areas were classified as imported (Table IV). Historically, the three northern districts of the KwaZulu area which accounted for >80% of malaria cases (1976-1988) were classified as an endemic malaria area whereas the rest of the province was classified as an epidemic area, where seasonal malaria occurred during years of good rain.

2.3.6 Malaria infection in relation to age and gender

Data on the age demographic profile of the population in the KwaZulu districts of Ingwavuma, Ubombo and Hlabisa were

Table IV. Comparison of the indigenous and imported malaria cases and the seasonal peak in malaria case detection in the Natal and KwaZulu areas respectively.

	Natal	KwaZulu
Indigenous cases	12.1%	86.9%
Imported cases	82-97%	13.1%
Seasonal peak	April-September	March-June

obtained from the 1985 population census. Figures 15 and 16 show the percentage of malaria cases reported for each age group relative to the age structure of the population for the period 1985-1988 for the Natal and KwaZulu areas respectively.

The majority of malaria cases from both the Natal and KwaZulu areas were accounted for by people under 25 years of age. In the KwaZulu areas 73% of cases occurred in people under 25 years of age and the population census data showed 65% of people to fall into this age category. In Natal areas 68% of the malaria cases occurred in people under 25 years of age and the population census data showed 54% of people to be in this age category. The highest number of malaria cases detected in any age category in the KwaZulu areas was in the 5-14 year old age category in contrast to that found in the Natal areas where the highest number of cases was detected in the 15-24 year old age category. In both areas the percentage of total malaria cases followed the population profile quite closely.

Figure 17 shows the percentage of total cases detected by active surveillance in specified age categories, for the Natal and KwaZulu areas respectively. In all age categories more than 58% of the cases in the KwaZulu areas and 74% of the cases in the Natal areas were detected by active surveillance. The data indicate that there is no tendency for a high percentage of cases in the young age categories to have been detected by passive surveillance due to the manifestation of severe clinical symptoms, or for people in

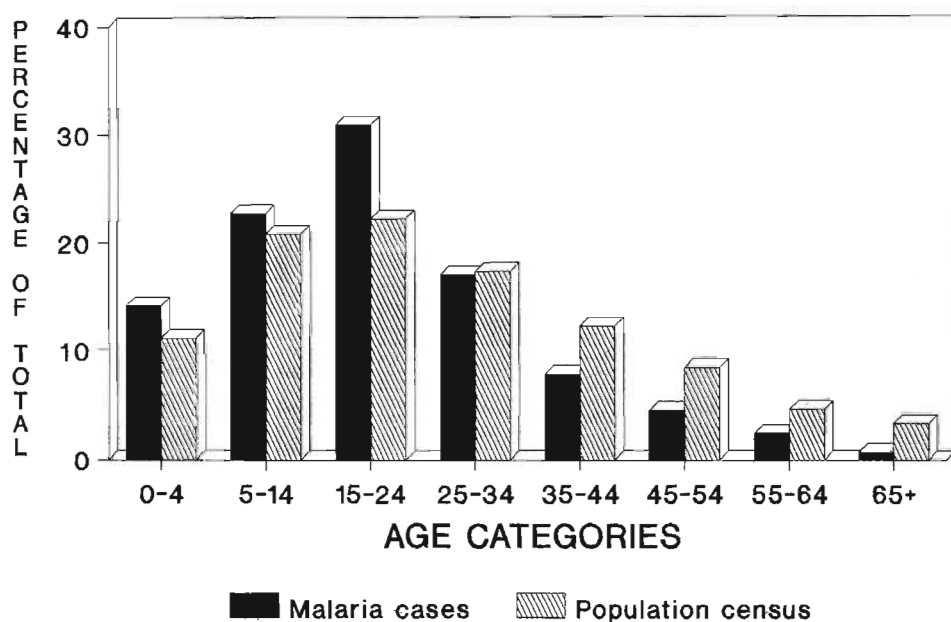


FIGURE 15. Frequency distribution of total malaria cases for the Natal area (1985 - 1988) for age-specified categories in relation to the population structure.

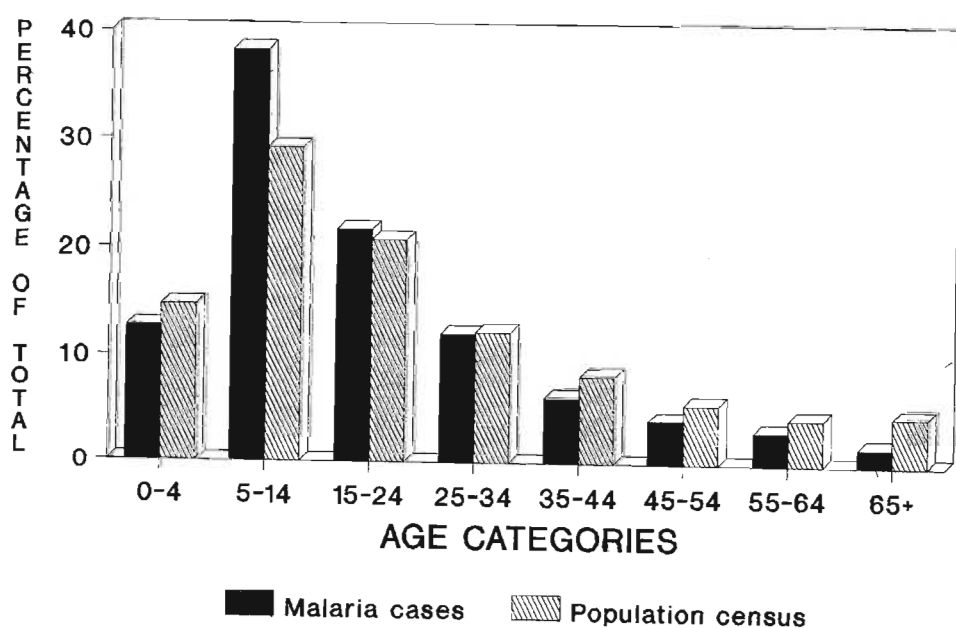


FIGURE 16. Frequency distribution of total malaria cases for the KwaZulu area (1985 - 1988) for age-specified categories in relation to the population structure.

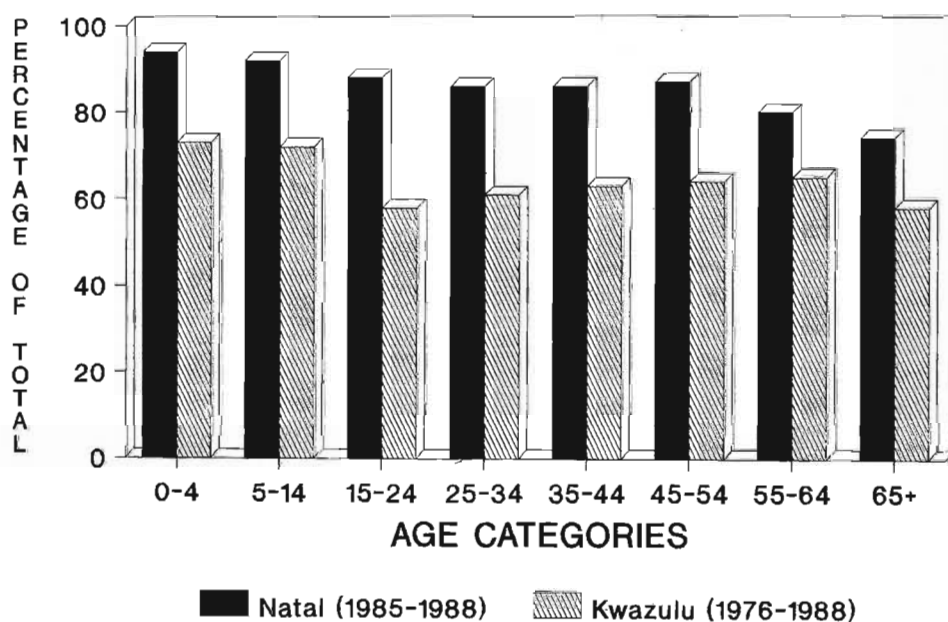


FIGURE 17. Percentage of total malaria cases detected by active surveillance in specified age categories for the Natal and KwaZulu areas respectively.

the older age categories to be identified to a greater extent by active surveillance as a result of the development of an immunity to the disease, a consequence of repeated exposure to infection from a young age. These results are in contrast to what is expected for a highly endemic malaria area. The situation in 1930 was as expected for an endemic area with the highest percentage of infected carriers being infants and toddlers and with the percentage infection decreasing with age (Swellengrebel *et al.* 1931). The severity of the disease was such that toddlers were infected almost without exception. The findings presented for the period from 1976 to 1988 for the KwaZulu areas and 1985-1988 for the Natal areas are more representative of those expected for a population subjected to epidemic malaria, for whom the percentage infection in age categories closely resembles the population age structure. This change from an endemic to an epidemic situation is considered to be the direct result of an effective malaria control programme of long standing.

In both the KwaZulu and Natal areas there was very little sexual bias in the children who contracted malaria in the under 5 age category and in both areas there was a slight bias in favour of girls in the 5-14 year old age category (Figures 18 and 19). In the KwaZulu areas there was a marked bias in the sex ratio in all age categories above 15 years of age, in favour of females. This bias was reflected in the malaria cases with females accounting for 60.2% of all malaria cases for KwaZulu areas during the period 1976-1988. In the Natal area, in contrast, there was a bias in the sex

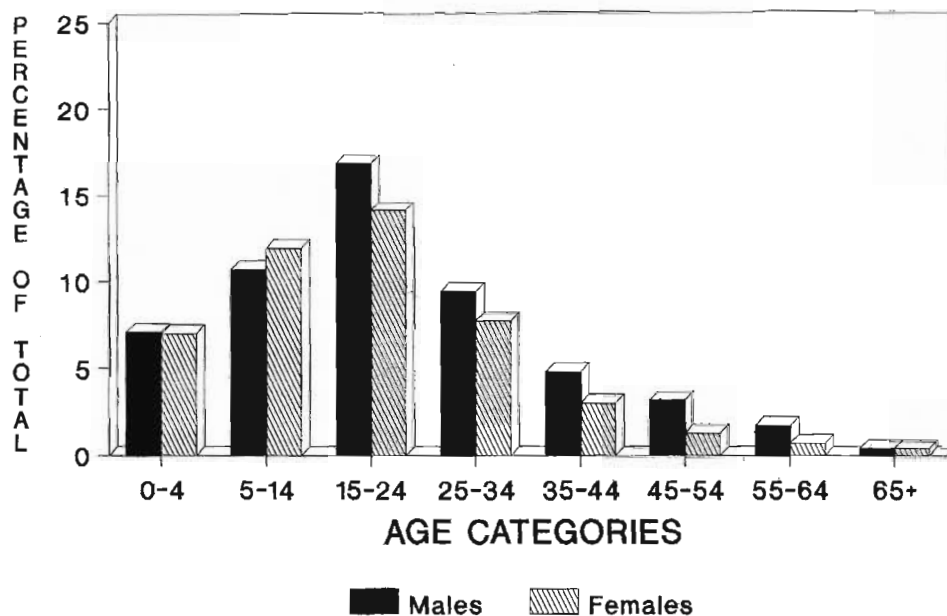


FIGURE 18. Sexual distribution of the Natal area malaria cases (1985 - 1988) in respective age categories.

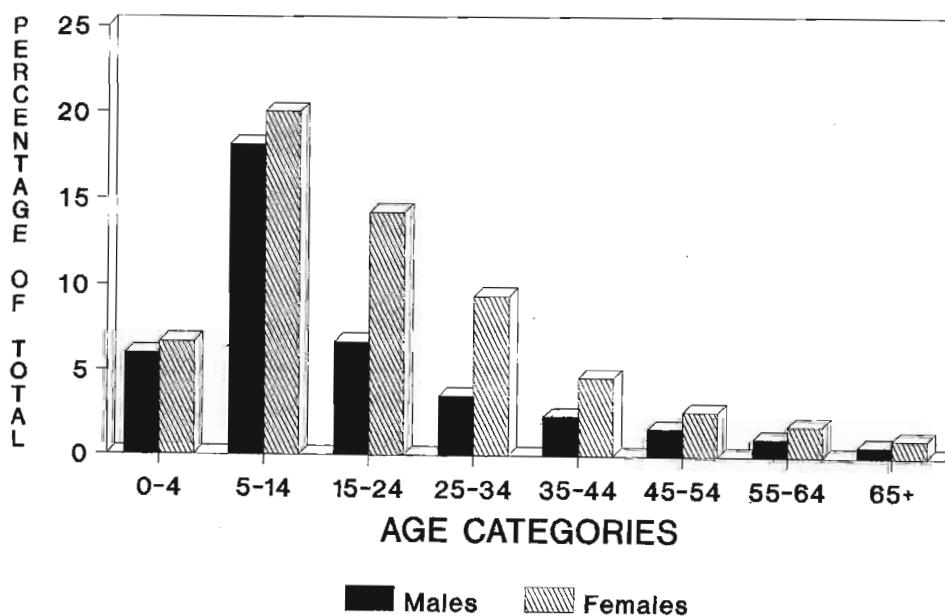


FIGURE 19. Sexual distribution of the KwaZulu area malaria cases (1976 - 1988) in respective age categories.

ratio in favour of males in all age categories above 15 years of age. Males accounting for 54% of all malaria cases from the Natal area for the period 1985-1988. This sexual bias in the malaria patients and the difference between the two areas is considered to be a reflection of a way of life in which the males move away from the malarious area of KwaZulu to seek work elsewhere, including the Natal area. According to the 1985 population census in the KwaZulu districts of Ingwavuma and Ubombo 57% of the population were female and 43% male and for the Natal region 51% of the black population was male and 49% female.

2.3.7 Parasitaemia

Asexual parasitaemia in malaria patients detected in the Natal areas ranged from <25 to 100 000 000 parasites per microlitre with a mean of 95 672. The gametocyte count ranged from <25 to 750 000 gametocytes per microlitre with 28% of the malaria patients having sexual stages of *Plasmodium falciparum* in their blood. Of those patients detected by active surveillance 28.4% were found positive for gametocytes and of the passively detected patients only 7.6%. This difference is considered to be related to the immune status of the patients. It can be expected that non-immunes, detected passively, develop clinical symptoms in the early stages of the disease and report to a hospital or clinic prior to the development of gametocytes.

Evidence exists that components of "immune" patients plasma can retard parasite development (Carlin et al. 1984,

Kharazmi & Jepson 1984, Stanley & Reese 1984, Markus 1985). Assuming that actively detected malaria patients are "immune" and passively detected patients "non-immune" the relationship between the numbers of parasites in these two groups was investigated. There was no significant difference in either the number of trophozoites (Table V) or gametocytes (Table VI) between the total active and total passive groups ($P > 0.05$).

If the infections in the upper extreme were, however, excluded from the calculations i.e. 1% of the trophozoite (Table V) and 5% of the gametocyte (Table VI) carrying patients, the data showed a highly significant difference to have existed between detection groups ($P = 0.0001$). The importance of active detection in the control of malaria in the Republic of South Africa is highlighted by the finding that the actively detected cases harbour a statistically significant higher number of gametocytes than do the passively detected patients. In the Natal and KwaZulu areas respectively 87.9 and 68.2% of cases were detected by active surveillance and the importance of this group as a source of infection to the mosquito is highlighted.

2.4 CONSTRAINTS ON MALARIA CONTROL IN NATAL PROVINCE

The malaria control programme in Natal/KwaZulu as a whole is highly effective. This is illustrated by the low mortality rate over the study period (mean 4,3; range 0 - 12) and the agricultural and industrial development of large parts of

Table V. Measures of central dispersion and statistical difference between numbers of trophozoites per microlitre of blood in actively and passively detected malaria patients.

% observations	Detection	Mean	S.D.	P
100	Active	5989.7	240337.8	
100	Passive	185355.5	3679258.9	0.1850
99	Active	1281282.1	3530.8	
99	Passive	31921.5	126935.2	0.0001

Table VI. Measures of central dispersion and statistical difference between numbers of gametocytes per microlitre of blood in actively and passively detected malaria cases.

% observation	Detection	Mean	S.D.	Prob.
100	Active	117.8	864.5	
100	Passive	2641.5	37936.9	0.0706
95	Active	21.4	55.2	
95	Passive	3.4	23.1	0.0001

the province in contrast to the situation in the late 1920s and early 1930s. The overall efficiency and success of this programme are, however, of necessity reliant on all its facets and some of these warrant special mention. The whole of the KwaZulu area under the attack phase of malaria control has been geographically divided into districts, areas and sections, with the locality of each homestead being mapped. A record card at each homestead logs its geographical position as well as its history of malaria control, such as date of DDT application, active surveillance visits and entomological investigations. All malaria cases, therefore, whether actively or passively detected, can be followed up. This documentation ensures that all homesteads are sprayed annually with a residual insecticide; in 1988, 101,647 huts were treated in the KwaZulu area. During 1988, 53,414 structures in the Natal areas were sprayed with a residual insecticide. In the Natal areas, division of the land is mainly based on private ownership, but as in the KwaZulu areas, a record card system is used. This record-keeping in both the Natal and KwaZulu areas enables the accurate mapping of all malaria cases, permitting problem areas to be highlighted such that appropriate investigation and control measures can be instituted.

Efficacy of control is further advanced by the use of mass blood examinations. During 1988, 294720 blood smears were taken during active surveillance operations in the KwaZulu areas and 100137 in the Natal areas. The efficient screening and reporting of these smears, generally within 7

days but rarely exceeding three weeks, enables timely action to be taken in problem areas.

There have been and are a number of constraints on the malaria control programme. Notable among these are the bedbug problem, chloroquine-resistant *Plasmodium falciparum*, population migration and agricultural development.

Newberry *et al.* (1984) showed a significant correlation between the occurrence of bedbugs and dwellings in the rural parts of KwaZulu which are subject to the intra-domiciliary application of DDT for mosquito control. Infestations of more than 2000 bedbugs per hut were not uncommon (Newberry and Jansen 1986). Owing to their nuisance value, the inhabitants replaster the hut walls in an attempt to cover the harbourages of the insects. The residual DDT is thereby obliterated, rendering it ineffective in mosquito control, a situation that has been of special concern to the health authorities. The large-scale application of Fenitrothion for bedbug control was started in 1986. This insecticide has been shown to be highly effective against bedbugs (Newberry, personal communication) and it is envisaged that its use will result in a large-scale reduction of hut replastering after the annual intra-domiciliary application of DDT.

In Africa, confirmed chloroquine-resistant *P. falciparum* was first reported in Kenya in 1977 (Fogh *et al.* 1979) and by 1982 the resistant parasite had gained a foothold in that

country. Of 25 *in vitro* chloroquine resistance tests done on blood specimens from infants aged between 6 and 24 months at Kisumu (Kenya) in 1982, 72% showed chloroquine resistance (Spencer et al. 1983). The chloroquine-resistant parasite is today found widely in Malawi and Tanzania, and confirmed reports exist for Mozambique (Schwalbach et al. 1985) and Zimbabwe (Dallas et al. 1984). Isaacson et al. (1984) reported chloroquine-resistant malaria from Namibia and more recently Sharp and Freese (1990) confirmed this finding. The first local cases were individually reported from Venda (Bac et al. 1985) and Louis Trichardt (Visagie and Sieling 1985). Herbst et al. (1985) collected isolates from 77 patients with *P. falciparum* in the Natal/KwaZulu area in 1985 and reported 6 cases of chloroquine resistance. During May 1987 and January 1988 Freese et al. (1988) investigated the chloroquine sensitivity of *Plasmodium falciparum* in 39 patients from the Ingwavuma and Ubombo magisterial districts of the KwaZulu areas and found 94% to be resistant. Seventy-four percent of the tests showed inhibition to only occur at (chloroquine base quantity) 32 pmol per well or greater, indicating RIII resistance (World Health Organisation 1984). Hansford (1989) retrospectively analysed hospital and field malaria treatment data pertaining to persistent parasitaemia's in the KwaZulu areas for the period 1983-1988. This investigation was not based on the standard World Health Organisation protocol for the *in vivo* or *in vitro* test determination of chloroquine susceptibility in *Plasmodium falciparum*, but on field bloodsmear retakes. The findings of the study were that persistent parasitaemia's, following treatment with chloroquine (25 mg/kg), increased

from nil in 1983 to 21.2% and 16.1% for hospital and field treatments respectively in 1987. In January 1988, both the Natal and KwaZulu health authorities decided to use Fansidar (Sulfadoxine plus pyrimethamine) in place of chloroquine for the treatment of confirmed infections. After the introduction of Fansidar in Natal province for the treatment of *Plasmodium falciparum* infections, Hansford (1989) found that persistent parasitaemia's based on field blood smear retakes dropped to 6.9% and 0.4% respectively for hospital and field treatments. This discrepancy between the success of hospital and field treated cases needs further investigation. Should this difference (6.5%) be accurate and not just a reflection of the inaccuracy of the technique employed in data collection, then standard *in vivo* or *in vitro* tests should be carried out to ascertain whether resistance to Fansidar exists or whether there is a percentage of people who do not respond to Fansidar treatment. The percentage of "natural" non-responders in Thailand has been given as 10-20% (Pinichpongse et al. 1982). The presence of chloroquine-resistant malaria in Natal province has no doubt had an exacerbating effect on the annual malaria case rate as evidenced by the high numbers of cases since its detection in 1985.

Population migration into the Natal/KwaZulu areas from Mozambique appears to be an increasing problem demanding a high degree of surveillance on the part of the health authorities. Mozambique is an endemic malaria area and there has not been a rural malaria control programme in the

country for the last decade, with the result that a large percentage of the population are carriers of the malaria parasite. As outlined, over the period 1976 to 1980 the number of malaria cases entering into the KwaZulu area through the Muzi Border Post alone accounted for 16% of the territory's malaria cases. As a result of the civil war in Mozambique and the lack of employment opportunities there, the influx of migrants is increasing and is considered to be one of the major reason for the rise in the numbers of malaria cases detected since 1986 in the non-KwaZulu areas of Natal. During the first six months of 1986, 696 malaria cases were detected in these non-KwaZulu areas; 92% of these were detected by active surveillance and more than 80% were recorded as imported cases from Mozambique.

In 1984 2.83% of all the malaria infections detected in the Natal area were due to *Plasmodium ovale* and in 1986 this figure rose to 6.56% (1987). The comment was made that cognizance should be taken of the apparent increase in occurrence of *Plasmodium ovale* infections in migrants from Mozambique (M van Rensburg, Minutes of the annual meeting, Natal province 1989). Freese and Sharp (unpublished data) investigated a malaria epidemic in the Ndumu area, Ingwavuma district, KwaZulu during March 1990 and confirmed the presence of both *Plasmodium falciparum* and *Plasmodium ovale* infections. Malaria transmission was considered to be occurring in the area and the indications are that this included the transmission of *Plasmodium ovale*. The implications of these findings are that an increased emphasise should be placed on the microscopic identification

of the *Plasmodium* species responsible for infection. In the case of *P. ovale* infection, a recurring malaria, it is necessary that additional treatment with an 8 aminoquinolone or primaquine be used against the hypnozoites.

The future success of malaria control in the Natal/KwaZulu areas is dependent on the continued coordinated effort of the KwaZulu Government Services and the Department of National Health and Population Development, an approach that to a large extent has been instrumental in the success of the malaria programme to date.

CHAPTER 3

SPECIES IDENTIFICATION OF THE *ANOPHELES GAMBIAE* GILES COMPLEX (DIPTERA:CULICIDAE) IN NATAL PROVINCE.

3.1 INTRODUCTION

3.1.1 The *Anopheles gambiae* complex

Historically the *Anopheles gambiae* s.l. are infamous as vectors of human malaria and filariasis in Africa (Gillies & De Meillon 1968, White 1974). Due mainly to their widespread distribution and morphological similarity, species diversity within the complex was not formally recognised for many years (Paterson 1964).

More recently the *An. gambiae* s.l. have been shown to consist of six morphologically cryptic species, showing pronounced ecological and behavioural diversity (Ribbands 1944, Muirhead Thomson 1948, 1951, Burgess 1960, 1962, Kuhlowl 1962, Paterson 1962, 1963, a, b, 1964, Davidson et al. 1967, Davidson & Jackson 1962, White 1985).

The sequence of events leading to the discovery of the complex are outlined by Paterson (1968). Of the member species, three have freshwater larval stages (*An. gambiae* s.s., *An. arabiensis*, *An. quadriannulatus*); one has mineral water larval stages (*An. bwambae*); and two have saltwater larval stages (*An. merus*, *An. melas*).

More recently, the situation in regards to *An. gambiae s.s.* has become more complicated. In Mali, three chromosomal forms have been described and denoted with the names, Mopti, Bamako and Savanna (Coluzzi 1988, Tour'e 1989). These three forms are characterised on the basis of specific polytene chromosome inversion polymorphisms and respectively appear to be prevalent in irrigated, riverine and savanna areas in West Africa.

3.1.1.1 The *Anopheles gambiae* complex species in relation to malaria transmission

Until the advent and use of residual insecticides in the spraying of houses for malaria vector control the presence of *An. gambiae s.l.* has almost been synonymous with the presence of malaria in Africa (Gillies & De Meillon 1968). This complex of six sibling species, species which show distinctive ecological, behavioural and vectorial capacities has of necessity been intensely investigated, mainly because it contains species that are efficient vectors of malaria and which are difficult to control.

Female *An. gambiae s.s.* show a high degree of anthropophily, and are generally considered to be the species with the highest vectorial potential of the *An. gambiae s.l.*. Sporozoite rates in excess of 9% have been recorded for this species (White & Rosen 1973). *Anopheles arabiensis* is a primary vector of malaria at numerous localities throughout

Africa, infection rates, however, tend to be lower in this species than found in *An. gambiae s.s.*. This difference is considered to be due to the greater zoophily of *An. arabiensis* (White 1974, Gillies & Coetzee 1987). Infection rates as high as 7.8% have, however, been recorded for *An. arabiensis* (Joshi et al. 1975). The third freshwater breeding species *An. quadriannulatus* is predominantly zoophilic and of no direct medical importance (White 1974, Sharp et al. 1984, Hunt & Mahon 1986, Gillies & Coetzee 1987).

Anopheles bwambae is a local vector of malaria among the Bambute pygmies of Bwamba, Uganda, and are limited in their distribution to the mineral-water breeding sites that occur in the area (White 1985). Salivary gland dissections by White (1973) showed 0.7% of *An. bwambae* to be infected with sporozoites, compared with 7.2% of *An. gambiae s.s.* in the same area.

Both saltwater breeding species, *An. merus* and *An. melas* have been implicated in the transmission of malaria. *An. melas* shows mixed feeding with little discrimination between man and animal (Muirhead Thomson 1948), *An. merus* in contrast has a consistent preference for cattle (Gillies 1968). Both species show lower sporozoite rates than do the freshwater breeding vector species. Bryan (1983) found a sporozoite rate of 0.32% in *An. melas*, although 3.5% of freshwater *An. gambiae s.l.* were infected in the same area. Muirhead Thomson (1951) investigating *An. gambiae s.l.* caught resting in and leaving houses found only 0.8% of *An.*

merus to be sporozoite infected in contrast to 9.8% of the freshwater *An. gambiae s.l.*. Mosha & Petrarca (1983), however, found sporozoite infection rates of 3.3% in *An. merus*, indicating that this species can play an important role in malaria transmission.

3.1.2 Mosquito species identification in medical entomology

In applied vector research, definitive species identification is essential. With the known behavioural differences between cryptic species (White 1974), there is little point in defining host preference or investigating infection rate, when it is not certain which species is being dealt with. For efficient vector control it is essential to understand the behaviour of the vector species concerned and this necessitates accurate species identification. Records exist where confusion has arisen in respect to interpretation of the efficacy of control strategies when specific species identification was not applied (Paterson 1963). Green (1981) cites an example where lack of species identification would have had disastrous consequences for malaria control: Three *An. gambiae s.l.* populations in Zimbabwe gave 98, 43 and 0.8% mortality in susceptibility tests against dieldrin. Using electrophoresis the populations were identified as a mixture of largely susceptible *An. quadriannulatus* (a species with a preference for feeding on cattle and of no direct medical importance) and resistant *An. arabiensis* (a primary vector of human *Plasmodium* spp. at numerous localities in Africa).

Based solely on ovarian polytene chromosomes of survivors in the susceptibility tests, the populations could have been misidentified as *An. arabiensis*, and HCH used for residual spraying without achieving control.

3.1.3 Species identification of the *Anopheles gambiae* s.l.

Numerous studies have investigated the potential of morphological characteristics for primary identification of *An. gambiae* s.l. individuals at the sibling species level, but they remain of limited value (Coluzzi 1964, Davidson et al. 1967, Gillies & Coetzee 1987).

There are, however, two morphological characters of the saltwater (SW) species which are of diagnostic value and useful in separating them from the freshwater (FW) species. The eggs of both *An. melas* and *An. merus* are characterised by being longer and the deck opening on the dorsal surface broader than those of FW *An. gambiae* s.l. and this characteristic was used with success by Muirhead Thomson (1945, 1948) and Bryan (1983) to distinguish *An. melas* from the FW forms. Similarly, Paterson (1962, 1964a) and Kuhlow (1962) used this characteristic to distinguish *An. merus* from *An. gambiae* s.s.. Paterson (1964a), however, found some overlap in egg size with the FW species, but members of an egg batch from an individual female could always be identified.

The second feature concerns the markings on the labial palps. In adults, the dark area on the apical pale band of

the female palp (4-banded palp) shows a higher frequency in *An. melas* and *An. merus* and although not specific to the SW species, this feature can serve as a useful indicator of species composition in the field situation . This phenotype varies with species, but is generally low in freshwater *An. gambiae* s.l.: 3,7% (Holstein 1952); 2.1-21.7% (Paterson 1963c); 0% in *An. gambiae* s.s., 4.6% in *An. arabiensis* and 26.9% in *An. quadriannulatus* (Coetzee 1986) and more common in the saltwater forms: 50 to 90% incidence in *An. merus* (Muirhead Thomson 1951, Kuhlow 1962, Halcrow 1957, Paterson 1963c, 1964a, Sharp 1983); and 15 to 90% reported for *An. melas* (Ribbands 1944a, Muirhead Thomson 1945, Bruce-Chwatt 1949).

Bushrod (1981) successfully separated *An. merus* from the FW breeding members of the *An. gambiae* s.l. complex (*An. gambiae* s.s. and *An. arabiensis*) in Tanzania by plotting the number of coeloconic sensilla against the palpal ratio. Coetzee (1986) found that *An. quadriannulatus* and *An. merus* could be separated using this technique.

More recently Coetzee et al. (1982) and Coetzee (1986, a) demonstrated that *An. gambiae* / *An. arabiensis* could be distinguished from *An. merus* / *An. quadriannulatus* by the width of the pale band at the apex of hind tarsomeres 3 and 4. Coetzee (1986, a, 1989) examined the external morphology of four members of the *An. gambiae* complex from southern Africa and published a key for the separation of these species. Using this key based on the width of the pale band

at hind tarsomeres 3 and 4 and the palpal ratio, 100% of the *An. merus* and *An. quadriannulatus* families studied could be correctly identified. Based on the same key and using the pale band at hind tarsomeres 3 and 4 and the sum of coeloconic sensilla on flagellomeres 5+6+9 of both antennae, 94% of *An. gambiae* s.s. and 87.5% of *An. arabiensis* families used in the study could be correctly identified. Coetzee (1986, 1989) further found that the use of multivariate discriminant function analysis of 13 variables from all the life stages correlated for each individual entered into the programme, had an obvious advantage over the key as 97% of *An. gambiae* s.s. and *An. arabiensis* individuals were correctly identified.

Physiological tolerance of 75 percent saltwater by first stage larvae of *An. melas* and *An. merus* forms the basis of a valuable test devised by Ribbands (1944a) and Muirhead Thomson (1951) to distinguish between the saltwater and freshwater members of the complex. Sharp (1983) using colonised *An. merus*, *An. arabiensis* and *An. gambiae* s.s. extended this test in the laboratory to include all instars.

Crossbreeding is a technique that has been used with much success in elucidating cryptic species. The freshwater species of the *An. gambiae* complex were originally identified through crossing experiments (Davidson 1962, Davidson & Jackson 1962, Paterson et al. 1963, Davidson & White 1972). Here species identification is based on hybrid sterility of interspecific hybrids (Paterson 1964). Davidson et al. (1967) outline the results that can be

expected using this technique and emphasises its importance in definitive works aimed at elucidating potential genetic species. However, this technique is scarcely practical for the routine identification of field samples and in this role has largely been superseded by cytogenetic and electrophoretic techniques.

With the development of cytological techniques for the study of the *An. gambiae* complex (Coluzzi & Sabatini 1967, 1968, 1969) polytene chromosome differences could be correlated with mating types. This advance together with the discovery of ovarian polytene chromosomes in the *Anophelinae* (Coluzzi 1968) and a technique for the collection and preservation of ovaries in the field (Hunt 1973), made this technique a most efficient means of identification for field samples.

Work on chromosomally (polytene) identified members of the *An. gambiae* complex further resulted in an electrophoretic technique whereby species-specific iso-enzyme frequencies were used successfully for species identification (Mahon et al. 1976, Miles 1978, 1979). This technique has also been used with much success in field studies.

Carlson & Service (1979) investigated the possibility of identifying both sexes of *An. gambiae* s.s. and *An. arabiensis* by extracting and analysing their cuticular hydrocarbons. The preliminary findings of this study merit a more detailed appraisal of the non-volatile and chemically inert cuticular hydrocarbons for the separation of *An.*

gambiae s.s. and *An. arabiensis* and the other species of the *An. gambiae* complex.

More recently DNA probes have been developed for species identification of the *An. gambiae s.l.*. Collins et al. (1987) showed that a DNA fragment cloned from an rRNA gene of *An. gambiae* was capable of distinguishing three members of the *An. gambiae* complex, namely *An. gambiae s.s.*, *An. arabiensis* and *An. melas*. Gale & Crampton (1988) developed a DNA probe capable of identifying *An. arabiensis* males. Due to the sophisticated nature of the laboratory and the level of expertise necessary for use of these techniques, it is unlikely, that they will be utilised in the near future for the routine identification of the *An. gambiae s.l.*.

3.1.4 Correlation of polytene chromosome and electrophoretic techniques for identification of the *An. gambiae s.l.*

In the *An. gambiae s.l.* species criteria are currently differences in chromosomal rearrangements as seen in the polytene chromosomes (Green 1981). The application of this technique is, however, restricted to specific stages of the life cycle and development of the mosquito (World Health Organisation 1975), and this can severely restrict species identification in field population studies. The use of isoenzyme electrophoresis has the advantage that an adult in any stage of development or sex can be identified. This provides a distinct advantage when investigating hut resting or leaving behaviour in an area where the huts are sprayed with a residual insecticide and exit trap caught

mosquitoes might be unfed and/or die prior to reaching the correct gonotrophic state for polytene chromosome species determination. In areas where the gonotrophic cycle is 48 hours and pyrethrum knockdown catches are being done during the day, it is unlikely that any females killed will have ovaries at the correct stage of development for chromosomal species identification.

Due to the emphasis placed on species identification in this study, a number of specimens from each species were identified by both polytene chromosome banding patterns and iso-enzyme electrophoresis. This was done to verify the use of the biochemical key of Miles (1979) for the identification of *An. gambiae s.l.* populations in Natal. As outlined by Miles (1978); "because of the limited sample sizes, (used by Miles (1978) to establish the biochemical key) the allelic frequency data give only an indication of which loci may be useful in further studies in the various localities sampled". There are examples of closely related species which are indistinguishable in their electromorphic variation and as chromosomal rearrangements are the benchmark for the *An. gambiae s.l.* species, it was considered necessary to ascertain that the electromorph frequencies of the diagnostic loci (Miles 1978, 1979) would correlate to the genetic species.

3.1.5. The value of hind leg banding patterns in the identification of the species of the *Anopheles gambiae* complex in Natal

Coetzee et al. (1982) and Coetzee (1986, a) demonstrated that *An. gambiae* / *An. arabiensis* could be distinguished from *An. merus* / *An. quadriannulatus* by the width of the pale band at the apex of hind tarsomeres 3 and 4. Due to the practical implications of this technique for the field entomologist, an investigation was launched to evaluate 1) the effectiveness of this method of identification of *An. gambiae* s.l. species in Natal 2) the sensitivity of the technique, due to loss of scales in wild caught adult mosquitoes it was decided to compare newly erupted F1 individuals of wild caught females to the free flying female population.

3.2 MATERIALS AND METHODS

3.2.1 Morphological species identification

Adult mosquitoes caught in the field were transported to the field laboratory in clean gauze covered 500ml ice cream containers which were held in a cooler box covered by wet Muslim cloth. A freezer block was placed in the cooler box on hot days to prevent death of mosquitoes from heatstroke during transportation.

All adult anopheline mosquitoes caught were individually identified using morphological criteria outlined by Gillies & De Meillon (1968) and Gillies & Coetzee (1987). These field identifications were done using a Wild stereo dissecting microscope at 100x magnification. Adult

mosquitoes morphologically identified as *An. gambiae s.l.* were subsequently handled according to the requirements of the particular species identification techniques to be employed.

3.2.2 Polytene chromosome species identification

A portion of the *An. gambiae s.l.* identifications were done by ovarian nurse cell polytene chromosome analysis (Coluzzi 1968, Green 1972, Hunt 1973). Adult *An. gambiae s.l.* females for identification by this technique were bloodfed if they had not taken a bloodmeal prior to capture. These mosquitoes were held in gauze covered 500ml ice cream containers in a cooler box covered by wet muslin cloth until half gravid i.e. Sella's stage III. The ovaries of anaethetised mosquitoes were removed by squeezing the thorax between the thumb and forefinger of the left hand and at the same time pulling away the terminal segment of the abdomen with forceps and so withdrawing the ovaries (Green 1972). These were washed off using Carnoy's fixative (1 part glacial acetic acid: 3 parts absolute alcohol) into 1 ml bottles and held in a cooler box or refrigerator for transporting back to the laboratory. Laboratory processing of the ovaries followed the technique of Green (1972). Reading of the processed samples was done using a Zeiss phase contrast microscope and a combination of lenses which provided a total linear magnification of 1000* for oil immersion microscopy. The polytene chromosome arm designation and inversion notations used followed that of Green & Hunt

(1980).

3.2.3 Electrophoretic species identification

The majority of the *An. gambiae s.l.* caught in this study were identified to species level by iso-enzyme electrophoresis (Mahon et al. 1976, Miles 1978, 1979). *An. gambiae s.l.* adults for species identification were killed and individually placed in numbered gelatin capsules. These capsules were held in a liquid nitrogen flask (-170°C) such that enzyme integrity would be maintained and only removed prior to use in electrophoresis. In the performance of iso-enzyme electrophoresis at least two controls were used on each gel. The controls came from colonised mosquitoes maintained at the R.I.D.T.E.; namely *An. merus* from Ophansi ($28^{\circ} 34'S$ $31^{\circ} 56'E$), Natal, Republic of South Africa and *An. arabiensis* from Kanyembe ($15^{\circ} 40'S$ $30^{\circ} 20'E$), Zimbabwe.

3.2.4 Physiological method of *Anopheles merus* identification

This technique introduced by Muirhead Thomson (1951) is based on an observed differential response to saline waters and distinguishes the first instar larvae of the freshwater breeding from those of the saltwater breeding species of the *An. gambiae* complex (Section 3.1.3). This method was used to a limited extent to identify *An. gambiae s.l.* caught at localities where electrophoretic identification showed *An. merus* to be the predominant species.

In view of the high numbers of *An. gambiae s.l.* caught at Nkunduse (Chapter 5), a subsample were subjected to species identification by this technique. The technique was further used to specifically select for *An. merus* family groups for use in the investigation of hind leg banding patterns. The mosquitoes for identification were bloodfed in captivity if they had not already fed and placed in breeding tubes to oviposit. The breeding tubes were made up using 40 ml plastic specimen bottles. A small wad of wet (H₂O) cotton wool was placed in the bottom of the jar and covered with filter paper cut to the same diameter as the bottle. To complete the breeding tube the top of the bottle was covered with gauze which was held in place by an elastic band. Individual bloodfed female mosquitoes were placed in tubes, with subsequent oviposition occurring on the damp filter paper. The first instar larvae obtained from eggs deposited from the wild caught *An. gambiae s.l.* were treated as families and each family placed in a bowl containing 75% saltwater (24g NaCl/L). The larvae were examined after three hours by which time all larvae of the freshwater species (*An. quadriannulatus*, *An. arabiensis*, *An. gambiae s.s.*) would be dead, whereas *An. merus* larvae would survive for at least six hours.

3.2.5 Identification of the *Anopheles gambiae* species using hind leg banding patterns

Family groups were raised by placing individual female

mosquitoes that had been allowed to feed, into breeding tubes to oviposit. The eggs obtained from each female were then placed in separate bowls of distilled water to be raised as individual family groups. The larvae were fed three times per day, taking special care not to overfeed, which would result in high mortality due to the water becoming scummed. The larval food was a mixture of 30 g of activated yeast, 10 g of brewers yeast tablets and 150 g of Wests dog biscuits. This mixture was preground in a liquidiser followed by grinding with a mortar and pestle to a powder of particle size of <67 microns. The rearing of the family groups as outlined was done by D. le Sueur.

The legs of wild caught females, their adult female progeny and adult females raised from wild caught larvae, were mounted according to the technique of Hunt & Coetzee (1986). The legs, wings and palps were carefully removed with forceps and arranged on a dry microscope slide. A clean coverslip was placed on top of these and a small drop of mounting medium (e.g., Depex) was placed at each corner of the coverslip. No more than 5 F1 females per family or 5 females from larvae collected in any one pool, were used in scoring the leg bandings.

Measurements were taken of the pale band at the junction of hind tarsomeres 3 and 4 using a compound microscope (magnification x100) fitted with an eyepiece micrometer. The maximum and minimum length of pale scaling were measured and the mean calculated. For each species, specimens were treated as two groups; (a) wild caught adults (WCA) and (b)

eruptions from wild caught larvae or adult progeny of wild caught females (LE).

3.2.5.1 Collection sites

All material was collected in the Natal province of the Republic of South Africa at the following grid references: *An. arabiensis*, Dondota ($31^{\circ} 58'E$, $28^{\circ} 34'S$), Mamfene ($32^{\circ} 15'E$, $27^{\circ} 22'S$); *An. merus*, Ophansi ($32^{\circ} 16'E$, $27^{\circ} 32'S$), Chubu ($32^{\circ} 51'E$, $28^{\circ} 2'S$); *An. quadriannulatus*, Numaneni ($32^{\circ} 16'E$, $26^{\circ} 58'S$), Reservoir Hills ($30^{\circ} 57'E$, $30^{\circ} 48'S$), Ophansi ($32^{\circ} 16'E$, $27^{\circ} 32'S$), Mamfene ($32^{\circ} 15'E$, $27^{\circ} 22'S$), Lumbongwenya stream ($32^{\circ} 12'E$, $27^{\circ} 51'S$), Numaneni ($32^{\circ} 15'E$, $26^{\circ} 58'S$), Buwensi ($32^{\circ} 11'E$, $27^{\circ} 12'S$), Makhani's Drift ($32^{\circ} 17'E$, $27^{\circ} 1'S$), Sihangwane ($32^{\circ} 33'E$, $27^{\circ} 5'S$).

3.3 RESULTS AND DISCUSSION

3.3.1 Correlated electrophoretic and polytene chromosome identifications

Ninety-eight *An. arabiensis*, 28 *An. quadriannulatus* and 18 *An. merus* from Natal were identified by both polytene chromosome banding patterns and electrophoresis (Table VII). All 98 correlated chromosomal and electrophoretic identifications of *An. arabiensis*, irrespective of collection site, had allelic frequencies of: Superoxide dimutase (SOD, E.C. 1.15.1.1.) 100/100, Aspartate aminotransferase (AAT, E.C. 2.6.1.1.) 100/100 and Octonal

Table VII. Collection areas and numbers of paired chromosomal and electrophoretic species identifications of the *An. gambiae* complex species found in Natal.

Collection Area	Species	Number identified by both polytene chromosomes and iso-enzyme electrophoresis
Mamfene 36° 16'E, 27° 23'S	<i>An. arabiensis</i>	83
	<i>An. quadriannulatus</i>	1
Dondota 31° 58'E, 28° 34'S	<i>An. arabiensis</i>	7
Mtubatuba 32° 9'E, 28° 24'S	<i>An. arabiensis</i>	6
	<i>An. quadriannulatus</i>	2
Tongaat 31° 6'E, 29° 34'S	<i>An. arabiensis</i>	2
Numaneni 32° 15'E, 26° 58' S	<i>An. quadriannulatus</i>	25
Ophansi 32° 16' E, 27° 32' S	<i>An. merus</i>	18
Total	<i>An. arabiensis</i>	98
	<i>An. quadriannulatus</i>	28
	<i>An. merus</i>	18

dehydrogenase (ODH, E.C. 1.1.1.73) 95/95. The 28 correlated identifications of *An. quadriannulatus* specimens had allelic frequencies of SOD 100/100, AAT 95/95, and ODH 95/95 and for the 18 correlated identifications of *An. merus*, the allelic frequencies were: SOD 95/95, AAT 100/100, and ODH 95/95.

The allelic frequency data of chromosomally identified *An. arabiensis*, *An. quadriannulatus* and *An. merus* obtained in this study correlated well with the electromorph frequencies of the diagnostic enzyme loci of Miles (1979) and Marchand & Mnzava (1985), for specimens of the *An. gambiae s.l.* from East Africa. These data validate the use of the Miles (1979) key for the electrophoretic identification of the *An. gambiae* complex species from the collection areas.

3.3.2 Polytene chromosome study

All three populations of *An. arabiensis* investigated chromosomally were polymorphic for the 2b inversion system. The only other polymorphic inversion was a 2bc heterozygote from Mamfene and only one was seen in 68 preparations. The other chromosomal arms scored were in the configuration 3a, 4, 5 and Xbcd. The results of the chromosomal examinations of this species in Natal are in contrast to the findings for the species from West Africa, among which no fewer than eight floating inversions are commonly detected (Coluzzi et al. 1979). The 2a arrangement was found to be associated with exophagy and exophily in West Africa and reached

frequencies between 50-100% in many localities (Coluzzi et al. 1977) and showed the standard arrangement in indoor collections in Ethiopia (Mekuria et al. 1982). The only other study done in Southern Africa was that of Shelley (1973) in the Zambesi valley, and 2a heterozygotes were also not detected, as was the case in this study. Two heterozygous inversion systems were detected by Shelley (1973), both on autosomal arm 2. The 2c inversion was common and the 2cd inversion rare.

All five chromosomal arms were only investigated in the sample of *An. quadriannulatus* from Numaneni (Table VII). The only polymorphic inversion seen was the f inversion of the X chromosome, the other four chromosome arms conformed to the standard arrangement as outlined by Coluzzi et al. (1979). The only other floating inversion (2i) documented for this species (Coluzzi et al. 1979), was not detected in the sample investigated.

All *An. merus* chromosomes investigated conformed to the standard arrangements of Xa, 2op, 3a, 4, 5 (Coluzzi & Sabatini 1969, Coluzzi et al. 1979). This finding conforms to that of previous studies in that this species shows no inversion polymorphism.

3.3.3 The value of hind leg banding patterns in the identification of species of the *Anopheles gambiae* complex.

A total of 440 females viz 206 *An. arabiensis*, 118 *An. merus* and 116 *An. quadriannulatus* were examined. These measurements were subjected to the Coetzee (1986a) model in order to determine percentage correct identification. In this model the pale band at the junction of hind tarsomeres 3 and 4 measures less than 0,099 mm in the case of *An. gambiae* / *An. arabiensis*, and more than 0,1 mm in the case of *An. merus* / *An. quadriannulatus*.

The percentage of *An. merus* correctly identified was >92% for both larval erupted and wild caught females and >79% for *An. quadriannulatus* (Table VIII). The Dondota *An. arabiensis*, population (A), revealed correct identification in excess of 86%, whereas only 19,6% were correctly identified in the case of the Mamfene *An. arabiensis*, population (B).

The distribution of leg banding measurements for *An. arabiensis* populations (A) and (B) are presented in Figure 20. Peak frequency was at 0,07 mm in the case of population (A) and at 0,1 mm in population (B). Using the Coetzee (1986a) method sixty-eight percent of the leg-band measurements in population (B) were wrongly identified in contrast with only 7.6% of population (A). This difference was statistically significant (Chi squared = 136.842, $P < 0.001$).

The percentage WCA and LE legs incorrectly identified according to the Coetzee (1986a) method is shown in Table IX. Within species, the number of specimens in the WCA and

Table VIII. Percentage correct identification of *An. gambiae* complex species using the Coetzee (1986a) method.

WCA = Wild caught adult mosquitoes.

LE = Imagoes from wild caught larvae or adult progeny of wild caught females.

Species	Status of adults	Number of adults	% correct identification
<i>An.</i>			<i>merus</i>
	WCA	52	92.3
	LE	66	93.9
	Total	118	93.2
<i>An. quadriannulatus</i>			
	WCA	48	81.3
	LE	68	79.4
	Total	116	80.2
<i>An. arabiensis</i> (A)			
	WCA	33	93.9
	LE	76	86.8
	Total	109	89.0
<i>An. arabiensis</i> (B)			
	WCA	97	19.6

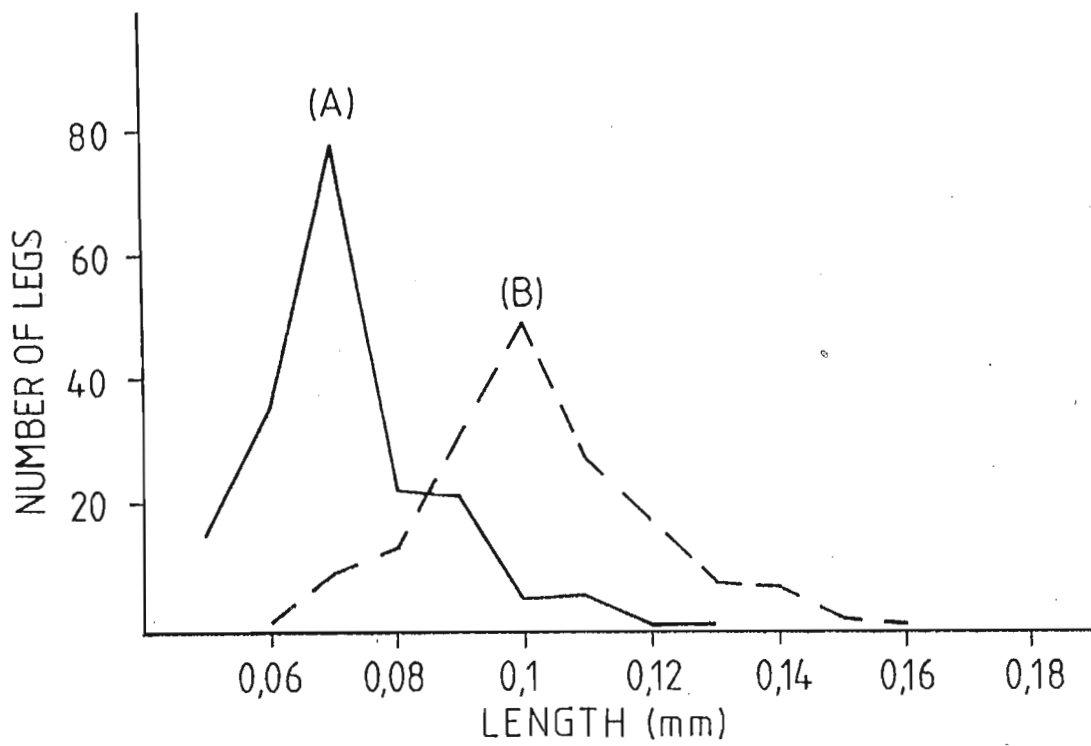


FIGURE 20. Distribution of the leg-banding measurements of *Anopheles arabiensis* populations (A) and (B).

Table IX. Comparisons of WCA and LE leg measurements within species wrongly identified by the Coetzee (1986a) method.

WCA = Wild caught adult mosquitoes.

LE = Imagoes from wild caught larvae or adult progeny of wild caught females.

NS = Statistically not significant

Species	% of leg measurements wrongly identified			
	WCA	LE	Chi sq.	p=
<i>An. merus</i>	4.2	3.2	0.009	NS
<i>An. quadriannulatus</i>	6.1	13.6	2.599	NS
<i>An. arabiensis</i> (A)	4.6	7.8	0.142	NS

LE groups wrongly identified by the method were not significantly different (Table IX). A high percentage of measurements conformed to the Coetzee (1986a) criteria in *An. merus*, *An. quadriannulatus* and *An. arabiensis* population (A). However, in all cases this was lower than the 94,0% correct grouping found by Coetzee (1986a). This may be due to the use of greater magnification, method of measurement or the use of a calculated mean measure in this study. Since the scaling of the leg-band may become rubbed with age the possibility that this might affect identification, was investigated. All samples within species were separately treated as freshly erupted and wild caught adults prior to lumping. Within any of the species there was no statistical difference between these groups in respect of those specimens that were wrongly identified by the Coetzee (1986a) method.

Two populations of *An. arabiensis* were investigated during this study: population (A) from an area that had never been subjected to the intra-domiciliary application of D.D.T. and population (B), from an area which is sprayed annually. It is interesting that a much higher percentage of measurements from population (A) fitted the Coetzee criteria than from population (B). The peak in distribution of the leg-banding measurements in population (B) was at 0,1 mm, coincident with the measurement used by Coetzee (1986a) to separate the species groups *An. gambiae* / *An. arabiensis* from *An. merus* / *An. quadriannulatus*.

Coetzee (1986a) states that of the four species examined by her, *An. arabiensis* showed the greatest variability. This finding is highlighted by the results of this study but it is considered noteworthy that the major difference was between two distinct populations of *An. arabiensis*, separated by approximately 120 kilometres. The major difference between these two collection areas being the application of DDT to the inner walls and roof surfaces of houses at Mamfene for mosquito vector control. The majority of the areas from which *An. arabiensis* were collected by Coetzee (1986a) were not subject to a malaria vector control programme, and her data compare well with those of this study for mosquitoes collected from the unsprayed area.

The pooling of the *An. arabiensis* data from this study resulted in only 56% correct identification. This result seriously detracts from the use of leg-banding for the separation of the *An. gambiae* complex member species in the Natal region and negates the usefulness of the key outlined by Coetzee (1989) based on this and other morphological characters and multivariate discriminant analysis for *An. gambiae* s.l. species separation. This finding highlights the necessity of confirming morphological identifications by biochemical and/or cytological means.

CHAPTER 4

THE DISTRIBUTION OF THE *ANOPHELES GAMBIAE* GILES COMPLEX (DIPTERA:CULICIDAE) SPECIES IN NATAL PROVINCE

4.1 INTRODUCTION

The members of the *An. gambiae* complex only occur in the Afro-tropical region and broadly show a tropical and subtropical distribution, with numbers diminishing and finally ceasing in temperate areas (Gillies & De Meillon 1968). The individual species are however not uniformly distributed and a number of abiotic and eco-physiological restraints are known to effect species distribution.

Anopheles bwambiae is only known from the Buranga mineral springs in the Semliki forest, Uganda (White 1985). The distribution of this species appears to be limited by it's dependence on the mineral waters in the area as a larval habitat. Attempts to breed *An. bwambiae* larvae in tap or distilled water were unsuccessful (Davidson & White 1972).

Both *An. melas* and *An. merus* utilise saline waters as larval breeding sites, and their distribution is of necessity limited by the availability of suitable saline breeding sites. The physiological tolerance of the first instar larvae to salinity is a further limiting factor on the suitability of specific saline waters for the utilisation by *An. merus* larvae. Whereas the first instar larvae of *An. melas* can complete development in 150%

seawater (Ribbands 1944a), *An. merus* first instar larvae cannot tolerate salinities approximating seawater (Mosha & Mutero 1982, Sharp 1983) and this limits the species from utilisation of true seawater habitats, in contrast to *An. melas*. le Sueur (personal communication) recorded first instar larvae of *An. merus* in pools of up to 111% seawater. However, second instar larvae were absent from the pools when the salinity exceeded 94% seawater. This indicates that the first instar larvae could not complete development at salinities greater than 94% seawater. The number of individuals at these high salinities were, however, low and the optimal salinity range was between 26 and 52% of seawater. This agrees with the findings of Mosha & Mutero (1982); that wild *An. merus* larvae could complete development in 100% seawater, although a salinity of 60% appeared to be optimal. *An. melas* is confined to the West coast of Africa (Muirhead Thomson 1945, 1948, 1951) and *An. merus* to the East Coast, adjacent inland areas, coastal islands and at inland localities in association with salt pans (Mackay 1935, Gebert 1936, Jepson et al. 1947, Muirhead Thomson 1951, Halcrow 1957, Maffi 1960, Iyengar 1962, Kuhlow 1962, Paterson 1962, Gillies & De Meillon 1968, White 1974, Bushrod 1981, Mosha & Mutero 1982, Coetzee & Cross 1983, Mosha & Petrarca 1983, Muspratt & Henning 1983, Sharp 1983, le Sueur & Sharp 1988).

Studies indicate that there are no major differences in the utilisation of transient rain pools or more permanent water bodies by the freshwater breeding members of the *An. gambiae*

complex. *An. gambiae* and *An. arabiensis* larvae have been found in the same pools by Service (1970b) and Service et. al. (1978) in Nigeria and Kenya, and by White & Rosen (1973) in Nigeria. le Sueur and Sharp (1988) in an investigation of the breeding requirements of the *An. gambiae* complex in Natal found *An. quadriannulatus* and *An. arabiensis* to utilise the same pools, and concluded that the larval site requirements of these two species were essentially the same.

An. quadriannulatus appears to have a very disjunct distribution on the Eastern side of the continent with records from the Republic of South Africa, Zimbabwe, Mozambique, Tanzania and Ethiopia (Gillies & Coetzee 1987). The factors limiting *An. quadriannulatus* distribution are not understood, however White (1974) has pointed out the tolerance of this species to relatively cool conditions on highland plateaux, in contrast to the other *An. gambiae* complex species.

An. gambiae and *An. arabiensis* are the most widely distributed of the *An. gambiae* complex species on the African continent and the adjacent oceanic islands. Discontinueties in their distribution in East and West Africa do occur and although humidity appears to play an important role in this regard, with *An. gambiae* s.s. predominating in the high humidity and forest areas, these discontinueties cannot be explained purely by this factor (Gillies & Coetzee 1987).

Abiotic factors known to play an important role in the abundance and occurrence of the *An. gambiae s.l.* include temperature, rainfall, topography and geology (Gillies & De Meillon 1968, Sharp 1983, le Sueur & Sharp 1988). In particular, temperature has been shown to play a major role in controlling distribution (Leeson 1931, De Meillon 1934a). Leeson (1931) working in Zimbabwe found that *An. gambiae s.l.* were never present when the minimum temperature in winter fell below five degrees Celsius, but could be found at lower altitudes where temperatures were higher.

De Meillon (1934a) divided the Transvaal province, Republic of South Africa into a number of thermal regions based on range of temperature and the number of days of frost per year. Winter and summer collections of *An. gambiae s.l.* showed that they were, as a rule, only present during both winter and summer in areas where the range of temperature was less than forty degrees Fahrenheit and where there was no frost. Areas where they were present in the summer only, showed a temperature range of forty-one degrees Fahrenheit to forty-five degrees Fahrenheit with nought to 50 days of frost per year. Thermal region three experienced a temperature range of 46 to 50 degrees Fahrenheit and a frost period of 100 days. De Meillon (1934a) found that *An. gambiae s.l.* only invaded this region in some years when temperature conditions and rainfall were favourable. A similar expansion and contraction of *An. gambiae s.l.* populations occurs in Natal province. Within the Ubombo and Ingwavuma districts of KwaZulu (Chapter 1,

Figure 2), such populations are present throughout the year and expansion and contraction occurs on a more localised basis. During the summer months, *An. arabiensis* and *An. quadriannulatus* larvae can be found in many of the temporary water bodies resulting from rain. During winter, the temporary sites dry up and breeding is confined to more permanent, marginal sites such as the floodplain lakes and disused borrow-pits (le Sueur & Sharp 1988).

The relationship between rainfall and mosquito numbers has been the subject of numerous studies and qualitative and quantitative relationships have been shown to exist (Lamborn 1925, De Meillon 1934a, Ribbands 1944, Haddow 1945, Mattingly 1949, Breeland 1972, Molineaux & Gramiccia 1980). Breeding pools utilised by freshwater breeding members range from temporary rain pools to semi-permanent pools and it might well be that a quantitative relationship exists between mosquito numbers and rainfall in areas when and where temporary rain pools are used, and a qualitative relationship in areas when and where more permanent pools are predominantly exploited.

4.2 DISTRIBUTION OF THE *ANOPHELES GAMBIAE* COMPLEX SPECIES IN NATAL PROVINCE

Distribution records from published data and collections made as part of this study for the *An. gambiae* species from Natal province are presented in Appendix 2 and are shown in Figure 21. With the exception of the data collected by le Sueur & Sharp (1988) the distribution records to a large

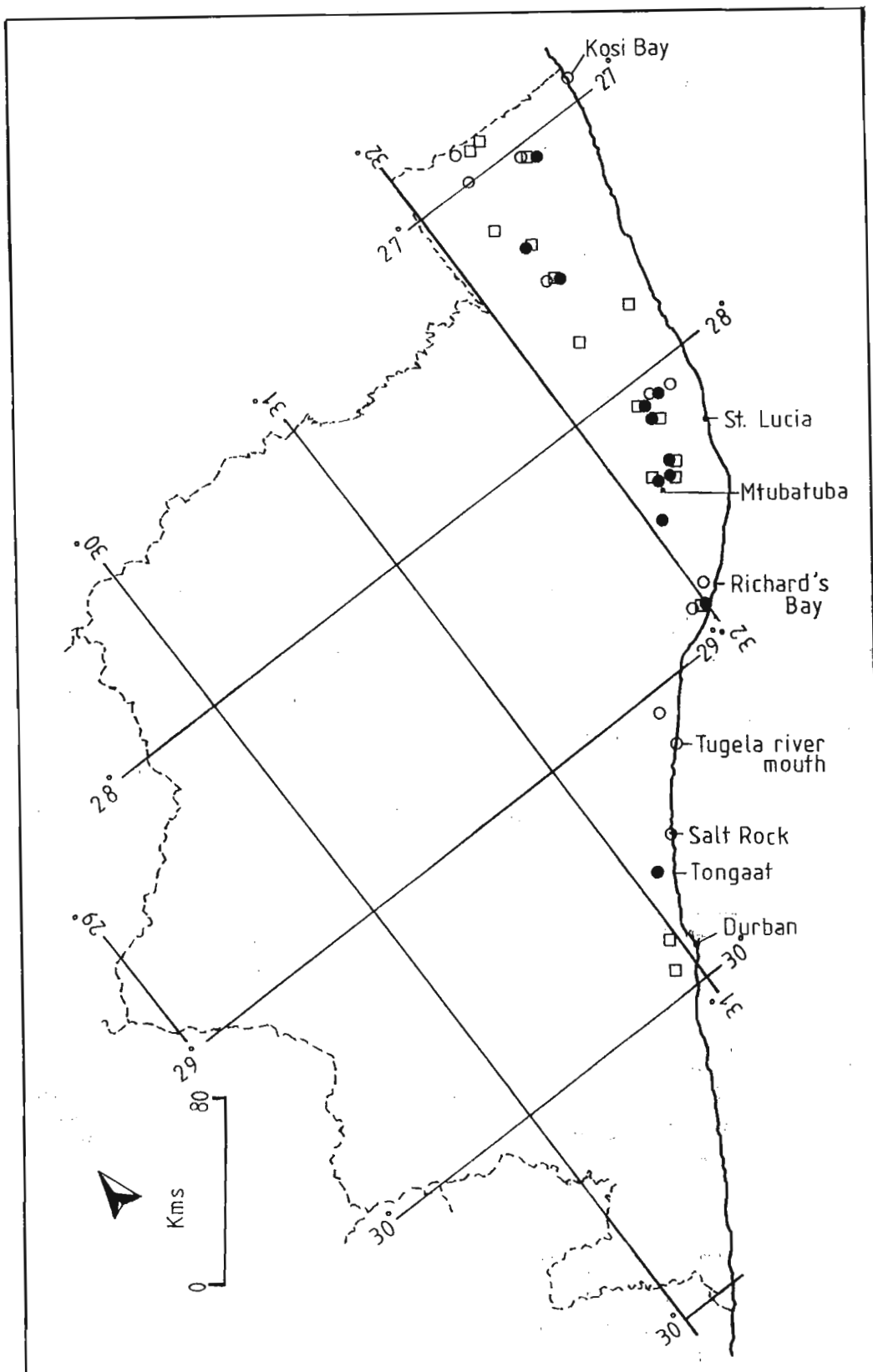


FIGURE 21. The distribution of the *Anopheles gambiae* complex species in Natal province.

- *An. arabiensis*.
- *An. merus*.
- *An. quadriannulatus*.

extent mirror localities where field studies have been carried out and are not the result of systematic collections done purely to ascertain species distribution. From a total of 24 collection sites stretching from just south of the Mozambique border to Durban, three species of the *An. gambiae* complex namely, *An. arabiensis*, *An. quadriannulatus* and *An. merus* were found to occur. The only record not in keeping with this trend was one specimen of *An. gambiae* s.s. collected in a cattle kraal at Pelindaba (Miles 1978). This specimen is the only specifically identified *An. gambiae* s.s. ever to be identified from Natal province.

Sympatric association was found to occur between all three species at three localities, between the freshwater breeding species *An. arabiensis* and *An. quadriannulatus* at nine localities and between *An. merus* and one of the fresh water breeding species at four localities. *An. arabiensis* was identified from 12 localities, *An. quadriannulatus* from 13 and *An. merus* from 12. The two freshwater breeding species appear to be widely distributed in the Natal coastal belt. In the more northern areas of the province where *An. quadriannulatus* has been found, it is considered likely, that more intensive surveillance in these areas would in many cases detect *An. arabiensis*. At both Ophansi and Nkunduse the predominant species in the area was *An. merus* (Chapter 5), however, intensive sampling coupled with species identification showed *An. arabiensis* to be present in low numbers at both localities. Of 78 *An. gambiae* s.l. specimens collected from huts at Nkunduse and identified, 2 were *An. arabiensis*. Had a smaller number of specimens been

identified to species level it is possible that only *An. merus* would have been found and recorded as occurring in the area. It is believed that *An. arabiensis* populations are generally much smaller than those of the other two species in those areas subject to DDT house spraying, a direct result of their more anthropophilic behaviour and consequent control by the intra-domiciliary D.D.T..

The most southern distribution record for *An. arabiensis* is from Tongaat and for *An. quadriannulatus* those collected from the Durban area, namely Reservoir hills and Chatsworth. During the last six years *An. gambiae* s.l. specimens collected from the Reservoir hills area have been identified on 5 different occasions and from Chatsworth twice, and in all instances only *An. quadriannulatus* were identified. White (1974) has pointed out the tolerance of *An. quadriannulatus* to relatively cool conditions in the highlands of Zimbabwe (Muirhead Thomson 1960a) and it has also been found to occur in the Transvaal highveld (Paterson pers. comm.). It's occurrence in the Durban area, and the absence of *An. arabiensis* from these collections, might reflect a potential in the species to adapt to cooler climates. The demonstration by Hill & Haydon (1905) of sporozoite infected *An. gambiae* s.l. caught during the malaria outbreak of 1905 in Durban suggests that *An. gambiae* s.s. or *An. arabiensis* can occur this far south. The possibility, however, exists that their occurrence this far south might have been the result of dispersal into the area during a period of suitable rainfall and temperature, the

climate this far south not being suitable to sustain a population over winter. A laboratory based study to assess the temperature and saturation deficit tolerances in relation to r (intrinsic rate of increase) of the three species of the *An. gambiae* that occur in Natal province would be enlightening in terms of summer and winter distributional ranges.

An. merus larvae have never been found in freshwater pools in the wild, the availability of suitable saline pools must therefore be considered as an major factor determining the distribution of this species. Due to the larval tolerance of the species (Mosha & Mutero 1982, Sharp 1983) true seawater habitats cannot be utilised and rainfall is essential for the establishment of temporary pools in areas of saline substrate or to dilute waters of high salinity (Mosha & Mutero 1982, le Sueur & Sharp 1988). The coastal plain of Natal province is ideally suited to the distribution of *An. merus* due to the extensively exposed marine cretaceous deposits in the area (Heeg et al. 1978). The addition of rainwater to exposed deposits creates saline pools. The suitability of the Natal region is highlighted by the high number of distributional records for the area (12) in comparison to only two for the rest of the country (Cross & Theron 1983, Muspratt & Henning 1983).

returns (Snow 1980, Gillies & Wilkes 1981, Sharp 1983a). Snow (1980) found catch rates to decrease when the wind speed reached 1.4 m/s. These data compared well with the finding of Gillies & Wilkes (1981) that mosquito flight speeds were in the range of 1.4 - 1.8 m/s. Sharp (1983a) found man-baited net catches of *An. merus* to drop off steeply from wind speeds of 0 m/s to zero catch at 2.5 m/s. Sharp (1983a) also showed rain to disrupt the biting cycle of *An. merus* with a resultant effect on catch returns. When the minimum, daily ambient temperature exceeded 23°C (Sharp 1983a) the biting cycle of *An. merus* was found to be similar to that for the *An. gambiae s.l.* (Kerr 1933, Haddow 1942, 1945, Haddow et al. 1947, Mattingly 1949, Holstein 1952, Gillies 1957). In contrast when the minimum ambient temperature was 16°C the cycle was markedly different with the biting peak shifted to an earlier period.

Due to the effects of wind, rain and temperature on trap return, comparison of efficiency of traps should be based on data collected on the same nights, in order to standardise these environmental effects, or during periods of optimal conditions.

The most fundamental methods of catching adult female mosquitoes are to i) Attract hungry host-seeking mosquitoes to human or animal bait for capture. ii) Capture bloodfed female mosquitoes after leaving a host. iii) Capture mosquitoes resting in natural sites.

Numerous types of traps which use human or animal bait as the attractant have been designed (Service 1976). Due to the importance of mosquito species showing anthropophilic biting behaviour in the transmission of human malaria, human bait catches have been used for many years. Mosquito surveillance teams in the Republic of South Africa work mainly in areas where the homes are subject to the annual application of residual DDT to the inner walls and roof surfaces of huts. Residual insecticide control programmes have in many cases eradicated or reduced indoor resting behaviour by repelling and/or killing members of the *An. gambiae* complex (Muirhead Thomson 1951). In consequence outdoor trapping using man-baited nets (Shannon 1939) is widely used for mosquito detection/surveillance.

Two basic types of trap have been used to capture mosquitoes leaving huts after feeding on the inhabitants and/or having rested indoors. These are window egress traps and verandah traps (Service 1976). Huts in a rural area vary tremendously in size, type of construction, number of inhabitants and the possessions they contain. These factors and the behaviour of the inhabitants can make it difficult to use local huts for experimental purposes. To overcome such difficulties and to enable comparison of results, Haddow (1942) built huts especially designed for mosquito experimentation. The use of experimental huts to evaluate house entering and leaving behaviour and the reaction of mosquitoes to residual insecticides have since been widely used. Sharp (1983) using a specially designed tent with entry slits and window traps (Muirhead Thomson 1947) showed it to be an efficient means

of trapping *An. merus*.

Adult mosquitoes probably spend more time resting in natural or man-made shelters than in flight (Service 1976). In the 1930's, such observations prompted De Meillon to the pioneering use of pyrethrum spray catches in huts as a means of controlling malaria through attack on the indoor resting mosquitoes. This resulted in a marked interruption of transmission in rural areas. It also laid the foundation for the later universal implementation of residual insecticide treatment of houses as the standard control measure against vectors of human *Plasmodia*. As outlined above this can lead to low indoor catches and places an increased emphasis on the mosquitoes resting outdoors as indices of population abundance and for the collection of material. Outdoor resting sites utilised by the *An. gambiae s.l.* include bushes and coarse fern, the bases of uprooted coconut palms, crannies at the base of large trees, the base of heavily shaded mangrove and mango trees, under corralin rocks, under fallen trees, in small and large holes in the ground and in eroded cavities (Muirhead Thomson 1951, 1958, Iyengar 1962, Paterson 1964a, Sharp 1983). The resting sites utilised by the *An. gambiae s.l.*, as outlined, are very diverse, but they all obviously provide a micro-climate suitable to the mosquito. Sharp (1983) described the physical conditions constituting the micro-environment in huts and some out-door resting sites utilised by *An. merus* in Natal. Temperatures were found to be lower and the saturation deficit reduced in these sites in relation to recordings done outdoors in the

shade. These differences were particularly pronounced in the heat of the day. Pit shelters (Muirhead Thomson 1951) were found to be up to 6°C cooler and with a saturation deficit up to 12 torr lower than found in the shade.

The investigations described in this chapter were included as knowledge of the efficiency of different trapping techniques in the capture of specific mosquito species is a prerequisite to any behavioural study, and an important tool in a malaria vector control programme. Furthermore an alternative technique (the experimental tent (Sharp 1983)) for the capture of adult mosquitoes, was investigated and its efficiency in the capture of *An. arabiensis* compared to that of the man-baited net.

5.2 MATERIALS AND METHODS

5.2.1 Study areas

A number of study areas have been utilised over the last six years in the collection of data, and these are outlined in Table X.

5.2.2 Mosquito capture techniques

5.2.2.1 Exit trap catches

Exit traps (ET) of the Muirhead-Thomson (1948) design were used on the experimental tent and huts with suitable windows. All-night catches were conducted and the exit traps

Table X. Study areas and periods of data collection.

Study Area	Data Collection Period.
Sihangwane 32°33'E, 27°03'S	March-June 1984
Dondota 31°58'E, 28°34'S	October, November 1986 April 1987
Mamfene 32°15'E, 27°22'S	1984-1989
Nkunduse 32°20'E, 28°05'S	November, December 1983 January 1984
Ophansi 32°18'E, 27°34'S	December 1985
Qhubu 31°58'E, 28°51'S	April 1984
Numaneni 32°16'E, 26°58'S	May, August 1985 August 1986

cleared prior to 08h00 each day.

5.2.2.2 Indoor resting catches

Indoor resting (IR) catches were carried out during daylight hours and accomplished by: i/ Covering the floor and furniture in the hut with white sheets ii/ Spraying around the eaves of the house, followed by indoor space spraying using a Rega pump and a 4% pyrethrum and paraffin solution iii/ A 10 minute period was allowed to elapse for the pyrethrum to kill the mosquitoes iv/ The dead mosquitoes were subsequently collected off the sheets in the house using jewellers forceps.

5.2.2.3 Huts used for indoor resting and exit trap catches

The huts used were well constructed timber and daub huts with thatch or in some cases corrugated iron roofs. Catches were carried out in:

- i/ Control huts. Huts that had not been sprayed with a residual insecticide.
- ii/ DDT sprayed huts. Huts where the inner wall and under-roof had been sprayed with water-wettable DDT at 2gm per M².
- iii/ Replastered huts. Sprayed huts in which the inner walls had been replastered after spraying, thereby covering the residual DDT. The under-roof DDT was, however, still exposed.

Only one hut in a homestead comprising two to six huts was used for either exit trap or indoor resting catches during any 24h00 period.

5.2.2.4 Baited net catches

The baited nets used in this study closely followed the design of Shannon (1939). For the duration of the study man-bait comprised two adult *Homo sapiens* and bovine-bait a one to two year old Nguni steer. The human (MN) or bovine (BN) bait were readied in the nets prior to last light and remained there for the duration of the catch. Duration of catch varied according to the nature of the data required and were either from last light to 21h00 or 24h00, or for the duration of the night.

All mosquitoes were caught on the inside of the net and the catches treated as hourly totals, with the exception of those catches carried out to compare the man-baited net to the experimental tent. In these catches the two men in the baited net actively caught all mosquitoes on themselves or resting inside the net from last light to 2100h. From 2100h the two men slept until 0500h when they awoke and caught all mosquitoes inside the net.

Comparative baited net catches were performed by placing the two baited nets, one human baited and one bovine baited, on open ground approximately 30 m apart. The bait was readied in the nets prior to last light and remained there

for the duration of the night. Hourly catches were made for five minutes prior to the hour in the man-baited net and for five minutes after the hour in the bovine-baited net.

5.2.2.5 Cattle kraal catches

Cattle enclosure (CK) catches were done by collecting anopheline mosquitoes found resting on the inside of the enclosure fences. These catches were carried out between 21h00 and 24h00.

5.2.2.6 Experimental tent catches

The experimental tent was a frame tent 3.65 metres long by 2.75 metres wide, with a minimum height of 1.9 metres (Plate 1). The ground sheet was separate from the tent, but made to the same internal dimensions and overlaid on a fifteen centimetre flap on the wall bottoms. There were two funnelled entry slits on either side of the tent, situated under the roof overhang. The outer dimensions of these were one metre by 7.5 centimetres (Plate 2). Illustration 1 depicts the funnel from the entry slit with the relevant dimensions. The roof overhang (Illustration. 1) stopped light being visible from inside the tent through the entry funnel. Plate 3 shows the entry funnel viewed from the inside of the tent. The leading edge of the funnel was pocketed to take two ten millimetre by one metre dowels, which served to keep the funnel edge from sagging when tied in place. The leading edge of the funnel and the ties are visible in Plate 3. The tent was fitted with four 30.5 cm³



Plate 1. A side view of the experimental tent showing three of the four window traps and the roof overhang covering the entry slits.



Plate 2. An external view of the entry slits situated under the roof overhang are shown.

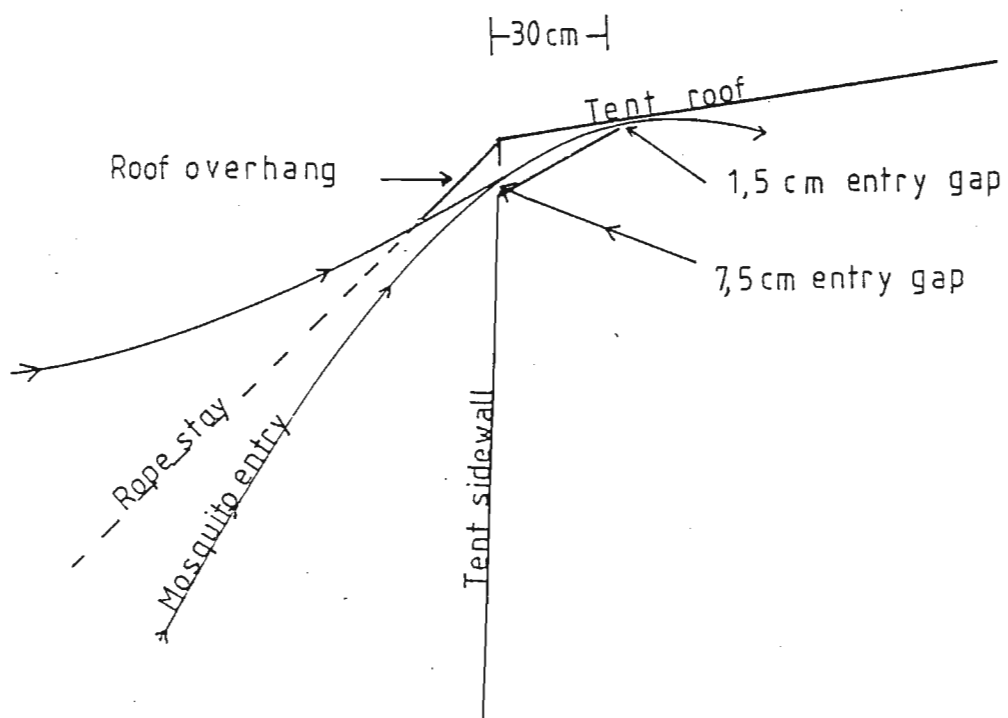


Illustration 1. Depicts the experimental tent entry funnel and it's dimensions.

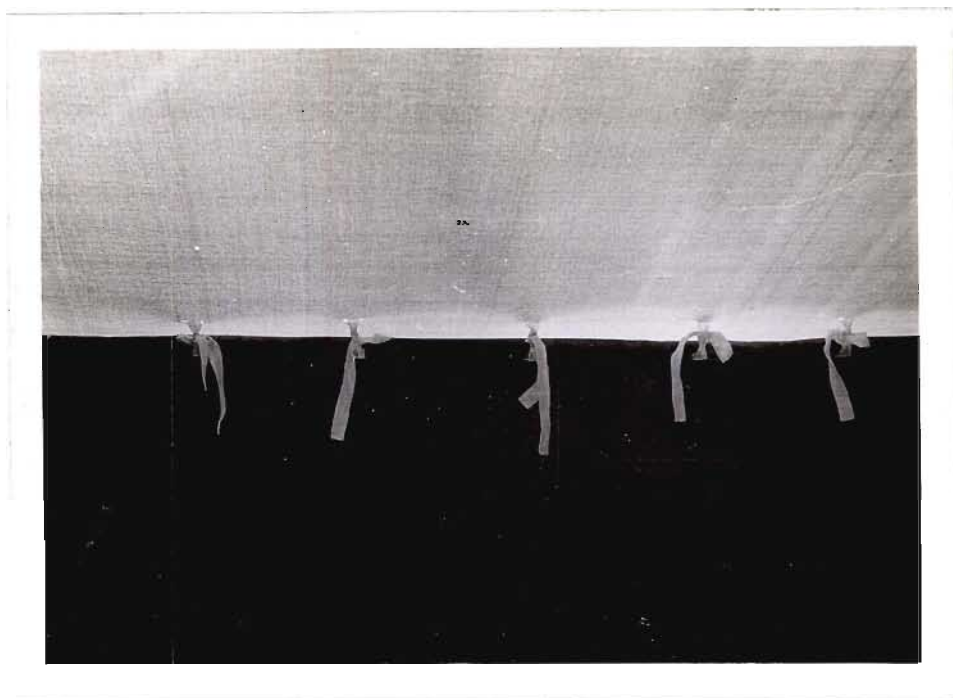


Plate 3. A view of the entry funnel from inside the experimental tent showing the gap between the leading edge of the funnel and the roof.

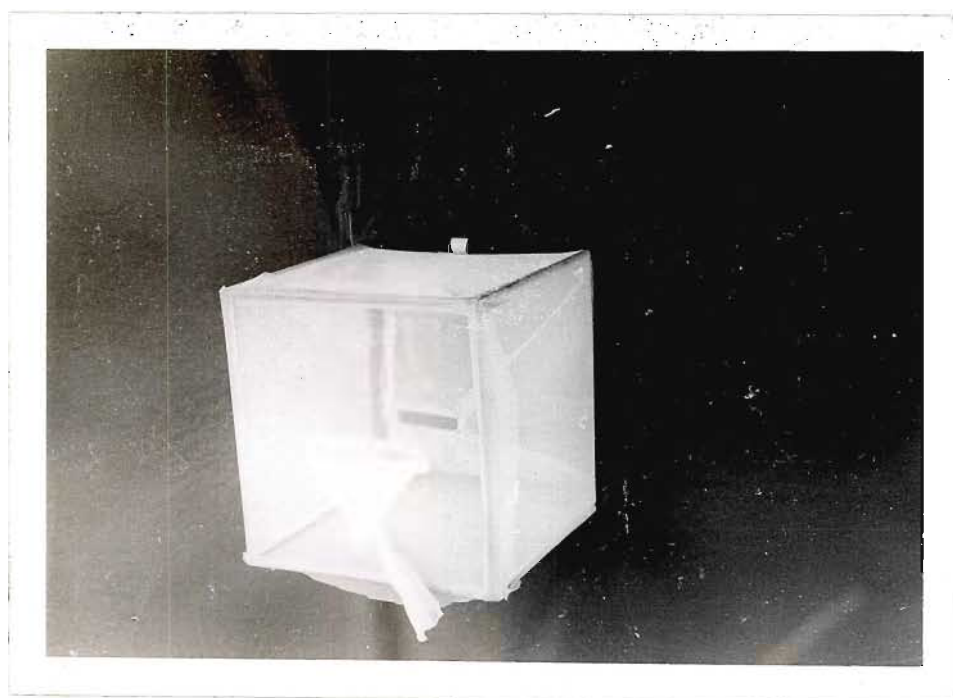


Plate 4. A close-up of a window trap fitted to the experimental tent.

window traps of the Muirhead-Thomson (1948) design, one to each wall (Plate 4). These traps were held in place by velcro on the inside of the window. Two men were always used as bait inside the tent. These men entered the tent at 18h00 and remained inside until first light (05h00). The collection of mosquitoes was made each morning from the window traps by means of an aspirator (Reproduced from Sharp 1983).

5.2.2.7 Pit shelter collections

Pit shelters were dug to conform to the Muirhead Thomson (1951) design. These were holes (approximately) 2 metres in depth and 1.5 x 1 metre in topview dimension. Smaller lateral holes (approximately 150 x 150 x 150 mm) were made in the sides to provide sites for mosquito resting. Mosquito collections from these pit shelters were made prior to 0800h.

5.3 RESULTS AND DISCUSSION

The five year time period (1984-1989) which was needed to collect these data and the number of localities from which data were collected, is a reflection of the difficulty in locating large enough populations of specific species of the *An. gambiae* complex in Natal to enable an assessment of the different trapping techniques.

5.3.1 *Anopheles arabiensis* and *Anopheles quadriannulatus*

The numbers of *An. gambiae s.l.* caught by different trapping techniques at two localities are outlined in Table XI. Species identification of *An. gambiae s.l.* caught (Table XII) at both localities and during different times at Mamfene showed both *An. arabiensis* and *An. quadriannulatus* populations to occur sympatrically. Both species were caught by the trapping techniques employed, however, with differential success.

5.3.2 Comparison of indoor resting, exit trap and pit shelter collections

At mamfene, all 198 of the indoor resting caught *An. gambiae s.l.* were identified to species level and of the 464 *An. gambiae s.l.* caught by exit trap, 194 were identified to species level. *An. arabiensis* accounted for 96% and 95% of the indoor resting and exit trap identifications respectively.

No statistical difference was found between the numbers of *An. arabiensis* caught resting indoors in replastered and control huts (Chapter 7). Both data sets were, however, statistically different to that for DDT sprayed huts. Of 71 indoor resting catches from fully DDT sprayed huts only seven *An. arabiensis* were caught. It was also found that there was no statistical difference between the numbers of *An. arabiensis* caught exiting replastered, control or DDT sprayed huts, and this factor need therefore not be borne in

Table XI. Numbers of *An. gambiae s.l.* caught by different trapping techniques during three periods at two localities.

<i>An. gambiae s.l.</i> caught					
Locality	Trap	n Traps	Total	Mean(S.E)	Range
Mamfene ¹	I.R.	17	198	11.6(4.4)	0-69
Mamfene ¹	E.T.	38	464	12.2(2.1)	0-59
Mamfene ¹	Pit	7	31	4.4(0.9)	1-8
Sihangewane ²	I.R.	16	172	10.7(2.4)	4-39
Sihangewane ²	Pit	4	11	2.8(0.9)	1-5
Mamfene ³	M.N.	5	47	9.4(2.8)	3-18
Mamfene ³	B.N.	5	73	14.6(4.6)	3-29
Mamfene ³	E.T.	21	267	12.7(4.2)	0-86

I.R. = Indoor resting.

E.T. = Exit trap.

M.N. = Man-baited net.

B.N. = Bovine-baited net.

Data collection periods

¹ 1/01/86 - 30/03/86

² 22/03/84 - 17/04/84

³ 4/02/86 - 18/03/86

Table XII. Electrophoretic and chromosomal species identification of *An. gambiae* s.l. caught by different trapping techniques at different localities and/or times.

Locality	Trap	<i>An. arabiensis</i>			<i>An. quadriannulatus</i>		
		Elect.	Polyt.	Total	Elect.	Polyt.	Total
Mamfene ¹	I.R.	190	4	190	8	0	8
Mamfene ¹	E.T.	184	40	184	10	0	10
Mamfene ¹	Pit	4	2	4	27	1	27
Sihangwane ²	I.R.	100	0	100	2	0	2
Sihangwane ²	Pit	5	0	5	1	0	1
Mamfene ³	M.N.	13	1	14	1	0	1
Mamfene ³	B.N.	11	12	16	4	1	5
Mamfene ³	E.T.	130	30	128	7	0	7
Numaneni ⁴	C.K.	0	0	0	39	25	39

Elect. = Electrophoresis.

Data collection periods

Polyt. = Polytene chromosome.

¹ 1/01/86 - 30/03/86

I.R. = Indoor resting.

² 22/03/84 - 17/04/84

E.T. = Exit trap.

³ 4/02/86 - 18/03/86

M.N. = Man-baited net.

⁴ 24/07/85 - 20/08/85

B.N. = Bovine-baited net.

C.K. = Cattle kraal.

mind when utilising this trapping technique. The data in table XII show that indoor resting catches in control and replastered huts and exit trap catches, irrespective of the DDT status of the huts, are highly successful in the capture of *An. arabiensis*.

Of those *An. gambiae s.l.* caught resting indoors (control huts) and in the pit shelter respectively at Sihangwane and identified, 98% and 83% were *An. arabiensis* and two percent and 17% were *An. quadriannulatus* (Tables XI and XII). Indoor resting catches were more successful in the capture of *An. gambiae s.l.* and more specifically *An. arabiensis* than was the pit shelter. A Mann-Whitney test found this difference in the numbers of *An. gambiae s.l.* caught by the two trapping techniques to be significantly different ($P=0.0067$).

The seven pit shelter collections made at Mamfene returned a total of 31 *An. gambiae s.l.*, of which 13% were *An. arabiensis* and 87% *An. quadriannulatus*. Paired T tests showed no significant differences between the numbers of *An. gambiae s.l.* caught in the pit shelter in relation to both the indoor resting and exit trap samples from this locality. *An. arabiensis*, however, only constituted 13% of the *An. gambiae s.l.* collected from the pit shelter. If the mean catch of *An. gambiae s.l.* from the pit shelter is corrected using this percentage, then the mean return of *An. arabiensis* was less than one per pit per day. The data for specifically identified *An. arabiensis* from the pit shelter collections at Mamfene showed a significant difference using

a Mann-Whitney test to those from the exit trap collections ($P=0.0016$), but not from the indoor resting collections ($P=0.0748$).

The pit shelter collections from both Mamfene and Sihangwane (Table XII) were made during periods when there were large *An. arabiensis* populations in the areas, based on the high indoor resting catches. At both localities the mean catch per pit of *An. gambiae s.l.* was <37% of the mean indoor resting catch (Table XII). These data clearly show the inadequacy of using Muirhead Thomson (1951) pits for the collection of *An. arabiensis* or for the estimation of the relative abundance of this species in an area.

Species identification of exit trap and indoor resting collections of *An. gambiae s.l.* from Mamfene would seem to indicate that the *An. quadriannulatus* population in the area was small (Table XII). *An. quadriannulatus* only constituted 4% of indoor resting and 5% of exit trap identifications. In contrast, species identification of pit shelter collected *An. gambiae s.l.*, seems to indicate that the *An. quadriannulatus* population at Mamfene was larger than that of the *An. arabiensis* population in the area. *An. quadriannulatus* constituted 87% of the *An. gambiae s.l.* identified from the pit collections.

Sharp et al. (1984) using the same pit shelter collected up to 318 *An. gambiae s.l.* in one collection and the mean number collected per day was 128/pit/day. Of a total of 72

An. gambiae s.l. collected from the pit shelter and identified to species level, they found 97% to be *An. quadriannulatus*. These data indicate that pit shelter collections are highly suitable for the collection of *An. quadriannulatus* in comparison to indoor resting and exit trap collections.

5.3.3 Comparison of man-baited net, bovine-baited net and exit trap collections

Both *An. arabiensis* and *An. quadriannulatus* were caught in the man-baited net, bovine-baited net and exit trap catches. Of 15 from man-baited net catches identified to species level and 21 from the bovine-baited net catches, 93% and 76% respectively were *An. arabiensis*. The highest percentage of *An. arabiensis* identified was obtained from the exit trap catches (95%).

A comparison of the mean number of *An. gambiae s.l.* caught per night at Mamfene by the respective methods (Table XI) showed the bovine-baited net catches to be the most successful. Paired T tests on all permutations, however, showed no significant differences between these data ($P>0.05$). Of those specimens caught by bovine bait and identified electrophoretically, only 76% were *An. arabiensis* in contrast to the 93% in the man-baited net catches. These data show *An. arabiensis* to be caught by both human and bovine bait and indicate a preference by *An. quadriannulatus* for the bovine baited trapping technique. Due to the relatively low numbers of *An. quadriannulatus* caught by

these "outdoor" trapping techniques at Mamfene (Table XI) it is not possible to gauge the relative efficiency of the different trap types in catching this species. The zoophilic host preference of *An. quadriannulatus* (White 1974, Sharp et al. 1984), however, suggests that animal-baited trapping techniques should be the most successful in the capture of this species. Animal baited traps have been successfully used in Natal to capture *An. quadriannulatus*. Of 39 *An. gambiae* s.l. identified from a cattle kraal catch at Numaneni, *An. quadriannulatus* was the only species identified (Table XII). Identification of 35 *An. gambiae* s.l. caught in a goat-baited net at Mamfene during a period when large numbers of *An. quadriannulatus* were collected in pit shelters, showed all 35 to be *An. quadriannulatus* (Sharp et al. 1984).

5.3.4 Man-baited net and experimental tent catches

Iso-enzyme electrophoresis of 123 specimens of *An. gambiae* s.l., showed 67 of 70 collected from the man-baited net catches to be *An. arabiensis* and three to be *An. quadriannulatus*. Of the 53 identified from the experimental tent, 50 were *An. arabiensis* and three were *An. quadriannulatus*.

During the course of 17 nights a total of 92 *An. gambiae* s.l. were caught in the 18h00-21h00 man-baited net catches, 23 in the 21h00-05h00 man-baited net catches and 564 in the experimental tent catches (Table XIII). Wilcoxon signed rank

Table XIII. Comparative man-baited net (18h00 - 21h00 and 21h00 - 05h00) and experimental tent catches of *Anopheles gambiae* s.l. on 17 nights.

Collection Date	Man-baited net		Experimental tent
	18h00 - 21h00	21h00 - 05h00	18h00 - 05h00
88/03/15	5	0	21
88/03/16	6	0	6
88/03/17	4	0	32
88/03/21	0	11	155
88/03/22	19	2	70
88/03/23	8	1	153
88/03/24	14	0	48
88/03/28	9	0	15
88/03/29	21	0	23
88/03/30	6	0	4
88/04/05	0	0	1
88/04/06	0	9	1
88/04/07	0	0	6
88/04/11	0	0	0
88/04/12	0	0	3
88/04/13	0	0	18
88/04/14	0	0	8
Total	92	23	564
Mean	5.4	1.4	33.1

tests showed the catches of *An. gambiae s.l.* from both the 18h00-21h00 and the 21h00-05h00 man-baited net catches to be significantly lower than those from the experimental tent ($P=0.0095$ and $P=0.00003$ respectively).

The accumulated totals of *An. ziemanii* and *An. tenebrosus* from 13 catches in both the man-baited nets and the experimental tent showed the baited nets to be the preferred trapping technique for these species (Table XIV). A Wilcoxon signed rank test found the combined catch of *Anopheles ziemanii* and *Anopheles tenebrosus* from both the 18h00-21h00 and the 21h00-05h00 man-baited net catches to be statistically higher than those from the experimental tent catches ($P=0.0003$ and $P=0.0015$ respectively). *Anopheles squamosus* was the only other species complex caught over the 13 night period. A total of five were caught in the 18h00-21h00 man-baited net, 4 in the 21h00-05h00 man-baited net and none in the experimental tent.

Both the man-baited net catches and the experimental tent were successful in the capture of *An. arabiensis*. Electrophoretic species identification of a subsample of the *An. gambiae* caught found the majority (>95%) to be *An. arabiensis*. Of the total numbers of mosquitoes caught by the respective methods the experimental tent was the most successful and on all but two nights recorded the higher catch. The efficiency of the experimental tent in the capture of *An. gambiae s.l.* in relation to the baited net catches is emphasised by the ratio, experimental tent: man-baited net 18h00-21h00: man-baited net 21h00-05h00 which was

Table XIV. Comparative man-baited (18h00 - 21h00 and 21h00 - 05h00) and experimental tent catches of *Anopheles ziemanni* and *Anopheles tenebrosus* (presented as a combined total) from 13 night catches.

	Man-baited net		Experimental tent
	18h00 - 21h00	21h00 - 05h00	18h00 -05h00
Total	129	80	5
Mean	9.9	6.2	0.4

24:4:1. The efficiency of the experimental tent in relation to the other two capture techniques is considered in part to be due to it being in operation for the full duration of the night and the chances are high that exiting mosquitoes will be caught in the exit traps.

For sampling purposes there are a number of further advantages of the experimental tent over the baited net for sampling purposes. The tent uses passive bait whereas in the baited net people have to actively catch the mosquitoes. This is highlighted by the low catch from the man-baited net (21h00-05h00) when continuous catching was not done. The inefficiency of this method is further highlighted when it is taken into account that the period of peak biting of the *An. gambiae s.l.* (Gillies & De Meillon 1968) is mainly encompassed by the trapping time of 21h00-05h00. The baited net requires personnel skilled in the capture of mosquitoes and sleep is not possible, and this limits the capture time. The experimental tent remains effective as a trap for the duration of the biting cycle, does not require skilled or active people to be effective and on nights of inclement weather, is still effective, should the weather abate. On two occasions during data collection the 21h00-05h00 baited net catching was abandoned due to rain with a resultant negative catch, whereas in both instances, the experimental tent showed a positive return.

5.3.5 *The capture of Anopheles merus*

The numbers of *An. gambiae s.l.* caught by five different

trapping techniques during two periods at two localities are outlined in table XV. Species identification by electrophoresis showed both *An. merus* and *An. arabiensis* to occur at Nkunduse during the study period. *An. merus* was, however, the predominant species caught in all traps and accounted for >97% of identifications irrespective of trapping technique (Table XVI).

All *An. gambiae s.l.* identified from both the man-baited and the bovine-baited net at Ophansi were found to be *An. merus* (Table XVI), 196 were identified electrophoretically and 19 were also identified chromosomally. Due to the predominance of *An. merus* in the sample of *An. gambiae s.l.* identified, irrespective of trapping technique, the results from both Nkunduse and Ophansi are considered to reflect the behaviour of *An. merus*.

The mean catch returns for different trapping techniques at Nkunduse are shown in table XV. The highest return was from the man-baited net, followed by the pit shelter collections. These catch returns were far higher than those from either the exit trap or indoor resting collections. There was no significant difference between the data from the man-baited net and the pit collection using a Mann-Whitney test ($P>0.05$). These catch returns were, however, both higher and significantly different to both the exit trap and the indoor resting catch data ($P<0.005$). A students T test further showed the exit trap and indoor resting catch data to be significantly different ($P=0.0198$), with the exit trap

Table XV. A comparison of the capture efficiency of *An. gambiae s.l.* by different trapping techniques.

<i>An. gambiae s.l.</i>					
Area	Trap	n Traps	Total	Mean(S.E.)	Range
Nkunduse	Pit	7	1039	148.4(33.6)	18-278
Nkunduse	I.R.	32	70	2.2(0.9)	0-23
Nkunduse	E.T.	40	312	7.8(2.2)	0-54
Nkunduse	M.N.	3	957	319.0(77.1)	174-437
Ophansi	M.N.	2	40	20.0	
Ophansi	B.N.	2	156	78.0	

I.R. = Indoor resting.

E.T. = Exit trap.

M.N. = Man-baited net.

B.N. = Bovine-baited net.

Table XVI. Species identification of *An. gambiae s.l.* caught by five different trapping techniques.

Area	Trap	Species identified	
		<i>An. merus</i>	<i>An. arabiensis</i>
Nkunduse	Pit	98	0
Nkunduse	I.R.	33	1
Nkunduse	E.T.	71	2
Nkunduse	M.N.	46	0
Ophansi	M.N.	40	0
Ophansi	B.N.	156	0

I.R. = Indoor resting.

E.T. = Exit trap.

M.N. = Man-baited net.

B.N. = Bovine-baited net.

catches returning a higher number of mosquitoes.

The mean number of *An. gambiae s.l.* caught in the bovine-baited net at Ophansi was far higher than that from the man-baited net and a χ^2 test of heterogeneity showed these data to be significantly different ($P=0.0046$).

These data indicate that the most efficient means of catching *An. merus* is to use bovine bait. Both pit shelter and man-baited net catches are, however, also efficient means of catching this species. Although *An. merus* is caught in and leaving huts, indoor resting and exit trap catches are the least efficient means of catching this species. Sharp (1983) showed that the experimental tent was effective in the capture of *An. merus*. Its efficiency in the capture of *An. merus* was greater than that of the exit trap catches but less than that of the man-baited net.

5.4 CONCLUSIONS

For surveillance, collection of material and estimation of relative abundance of a species, it is essential to have an understanding of the efficiency of any capture method used. Table XVII is included to serve as a guideline towards the comparative effectiveness of seven different trapping techniques in the capture of three species of the *An. gambiae* complex. The data for cattle kraal catches is largely based on the assumption that if the bovine-baited net method is an effective means of trapping a zoophilic species, so will the cattle kraal.

Table XVII. Comparative effectiveness of seven different trapping techniques in the capture of three species of the *An. gambiae* complex.

Trap	<i>Anopheles</i> <i>arabiensis</i>	<i>Anopheles</i> <i>quadriannulatus</i>	<i>Anopheles</i> <i>merus</i>
Exit trap	+++	+	++
Indoor resting	+++*	+	+
Man-baited net	+++	+	++**
Bovine-baited net	+++	+++	+++**
Cattle kraal	+++	+++	+++
Pit shelter	+	+++	+++
Experimental tent	+++	+	++

* excluding DDT sprayed huts

** refers to trapping over the period 2100h-0300h.

All seven trapping techniques were effective in capturing the three species in question, however, they showed considerable variation in capture efficiency within and between species. These data highlight the importance of appropriate trapping technique selection, for the capture of specific species. No trapping technique was found to be species-specific and these data emphasise the necessity of species identification in the Natal region, irrespective of the trapping method(s) used.

For *An. arabiensis*, exit trap and experimental tent catches are considered the best means of capturing this species in a DDT sprayed area. The reasons for this are 3 fold:

- i) The bait/people do not need to remain awake in the experimental tent during the catching period and when using exit traps, the local population serve as the attractant.
- ii) Traps remain effective for the full active period of the species (21h00 - 05h00).
- iii) In the case of exit traps, a number of these can be fitted and used on one night.

In non DDT sprayed areas indoor resting catches by pyrethrum space spraying would be an additional efficient technique for the collection of *An. arabiensis*.

A number of techniques are efficient in the capture of *An. merus* and *An. quadriannulatus*, but pit shelter collections have the advantage of requiring very little input from man

to be effective. Once the basic pit has been dug, only a low level of maintenance is required and collections are made during daylight hours.

CHAPTER 6

THE MOSQUITO VECTORS OF MALARIA IN NATAL PROVINCE

6.1 INTRODUCTION

6.1.1 Vectors of malaria in Natal province.

The first documented evidence incriminating an anopheline species in malaria transmission in South Africa was by Hill & Haydon (1905), during the malaria epidemic of that year in Durban. During their investigation of the epidemic, they dissected out the salivary glands and found 16 of 28 *Pyretophorus costalis* (*Anopheles gambiae* Giles (1902)) to be sporozoite infected.

Indoor resting members of this species were again incriminated in malaria transmission by Swellengrebel et al. (1931) working in the Transvaal. They also showed indoor resting *An. funestus* to be a malaria vector. These findings were later confirmed by Ingram & De Meillon (1927) who expressed the opinion that the two principle carriers of malaria in South Africa were *An. gambiae* (*Costalis*) Giles and *An. funestus* Giles, and that *An. gambiae* was more important in this respect than was *An. funestus*, on account of their larger numbers during the malaria transmission season. This opinion was based on the result of extensive field research in the malarious areas of South Africa including Natal.

The knowledge in respect of malaria transmission accumulated prior to the 1960's has been significantly affected by at least two subsequent developments, namely:

- i) the effects of the use of intra-domiciliary residual insecticide (DDT) introduced in the late 1940's and 1950's for the control of malaria vector species; and
- ii) the elucidation of the *An. gambiae* in the 1960's and later *An. funestus* as complexes of morphologically cryptic species.

The use of intra-domiciliary DDT has been extremely efficient in reducing or eliminating the endophagic/endophilic members of these species complexes, i.e. *An. gambiae s.s.* and *An. funestus s.s.*, at numerous localities in eastern Africa (Gillies & De Meillon 1968). The events leading to and associated with the elucidation of the *An. gambiae* complex are well documented (Gillies & De Meillon 1968, White 1974, Chapter 3). The complex is presently known to comprise six species, namely; *An. gambiae s.s.*, *An. arabiensis*, *An. quadriannulatus*, *An. merus*, *An. melas* and *An. bwambae*. These species show pronounced behavioural and ecological diversity (White 1974, Gillies & Coetzee 1987). After the elucidation of the complex it was not known to which species earlier studies had related. This situation was further complicated when it became known that these species occurred sympatrically. Species identification is essential, when the direct effect of their species specific behavioural diversity is related to

their vectorial efficiency and thus to the introduction of appropriate control measures.

6.1.2 Present day malaria transmission

Since the elucidation of *An. gambiae* and *An. funestus* as species complexes, there have been no published studies on the mosquito species responsible for malaria transmission in the Republic of South Africa. Local vector control strategies are still largely based on the findings obtained prior to the introduction of control measures and on data from studies completed elsewhere in Africa.

Malaria transmission still occurs in Natal province (Chapter 2), irrespective of the activities of the control programme over the last 30 years. There is thus clearly a need for data pertaining to the present day dynamics of transmission of the disease and its control in Natal province. This chapter reports on entomological investigations which were carried out at three localities in Natal Province during or shortly after reported local transmission of malaria had occurred. The aim of these investigations was to ascertain which mosquito species were present and possibly involved in the transmission of malaria. In addition, entomological investigations were carried out at a further two localities to assess the possible role of *An. merus* in malaria transmission.

6.2 MATERIALS AND METHODS

6.2.1 Study areas

The study areas utilised in these investigations are outlined in table XVIII.

Mamfene area is in the Ubombo district, KwaZulu (Figure 5, Chapter 2). This district falls into that portion of Natal that is historically considered to be an endemic malaria area. Irrigational overflow from the Mjindi agricultural development scheme was instrumental in creating suitable larval pools which resulted in extensive breeding of *An. gambiae s.l.* in the area (Sharp et al. 1984).

Both Mtubatuba and Tongaat are rural towns situated in the portion of Natal Province historically classified as the epidemic malaria area of Natal (Figure 21, Chapter 4). Malaria epidemics had not been recorded in either of these two towns for more than 20 years prior to these investigations.

Ophansi area is situated in the Ubombo district, and borders on the Mamfene area. A large waterbody, the Muzi swamp, occurs in this area of exposed marine cretaceous deposits. These cretaceous deposits are responsible for the creation of saline pools which are ideally suited to utilisation by *An. merus* larvae (le Sueur & Sharp 1988).

Nkunduse is situated between the Nyalazi and the Hluhluwe rivers and borders on False Bay, Lake St. Lucia (Figure 21,

Table XVIII. Study localities and dates of entomological collections.

<u>Locality</u>	<u>Data collection</u>
Mamfene (27° 23'S, 32° 16'E)	January 1986 - December 1988
Mtubatuba (28° 24'S, 32° 9'E)	February, March 1987
Nkunduse (28°05'S, 32°20'E)	November 1983 - January 1984
Ophansi (27°34'S, 32°18'E,)	December 1985
Tongaat (29° 34'S 31° 6'E)	May 1988

Chapter 4), in the Hlabisa district. The area has extensive saline swamps, as a result of exposed marine cretaceous deposits in the area. These swamps create ideal larval habitats for *An. merus* (Sharp 1983).

6.2.2 Identification of mosquitoes

All Anopheline mosquitoes were morphologically identified as outlined in chapter 3, section 3.2.1. The *An. gambiae* s.l. specimens were identified to species level by isoenzyme electrophoresis and polytene chromosome analysis as described in chapter 3, section 3.2.2. Identification of the *An. funestus* s.l. was by the morphological criteria for partial species identification of imagoes outlined by Gillies & De Meillon (1968).

6.2.3 Mosquito trapping techniques

The trapping techniques utilised included baited net catches, exit trap catches, indoor resting catches and pit shelter collections as described in chapter 5, section 5.2.2.

6.2.4 Bloodmeal analysis

Bloodfed mosquitoes were anaesthetised using ether, the portion of the mosquito abdomen containing the bloodmeal was dissected from the anaesthetised mosquito, squashed onto filter paper, numbered and allowed to dry before storage. In the laboratory the blood was eluted from these filter paper

collections with 50ul of distilled water. The bloodmeal analyses were performed by using the Ouchterlony double diffusion gel technique and each sample was tested against bovine and human anti-serum. The test plates were 8*4 cm glass plates on which a 1 mm agar gel was poured and overlayed with a perspex template. The template contained six rosettes of 2mm holes, with each rosette in the configuration of the number five on a gambling dice. In the test, 10ul of anti-serum was placed in the central well of a rosette and surrounded by three specimens and a positive control.

6.2.5 Confirmation of human malaria infection

Confirmation of malaria was by microscopic investigation of a bloodsmear for malaria parasites (Chapter 2, section 2.2.2). This was performed by the Regional Laboratory Services (Natal) and the Kwazulu Health Department laboratories (Jozini) depending on whether the case was detected in the Natal or the KwaZulu regions of the province.

6.3 RESULTS AND DISCUSSION

6.3.1 Investigations of local transmission of malaria

6.3.1.1 Microscopic confirmation of malaria infections

All microscopically confirmed malaria cases from Mamfene,

Mtubatuba and Tongaat were a result of infection with *Plasmodium falciparum*.

6.3.1.2 Mamfene malaria outbreak.

The number of malaria cases from Mamfene area was low in 1986 with <10 indigenous cases being reported. For 1987 and 1988, however, there were >700 and >600 malaria cases respectively reported for the area. Figure 22 shows the monthly distribution of malaria case totals from this area for these two years. The Anopheline species and/or species complexes caught at Mamfene by both indoor and outdoor trapping techniques are shown in table XIX.

Of the eight mosquito species and/or species complexes caught at Mamfene only three were caught resting indoors or leaving huts, the majority were caught outdoors. Five comparative all night bovine-baited net and man-baited net catches (table XX) indicated that the majority of these species were more zoophilic than anthropophilic, in all instances in excess of 82% of the catch was made in the bovine-baited net. Anthropophilic biting behaviour by a mosquito species is a prerequisite for a vector of human malaria.

An. quadriannulatus was only caught in low numbers leaving or resting in huts. Of 22 caught, 11 were bloodfed and nine of these tested against both human and bovine antisera were shown to have fed on bovine and none on man. *An. arabiensis* was caught in large numbers by both exit trap and indoor

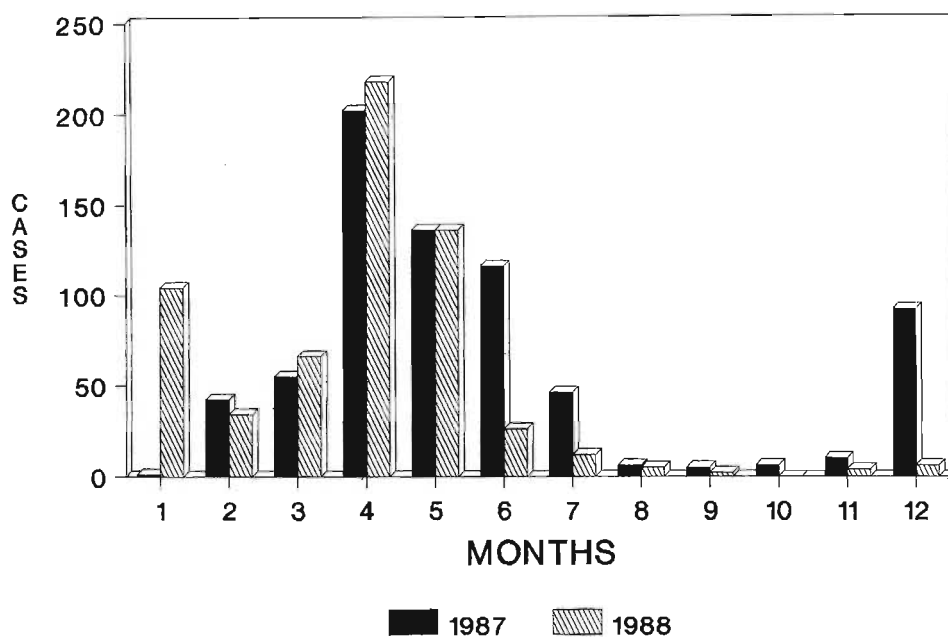


FIGURE 22. Monthly distribution of malaria cases from Mamfene for 1987 and 1988.

Table XIX. Anopheles species and/or species complex's caught by five different trapping techniques at Mamfene.

Species	Exit trap	Indoor resting	Man- baited net	Bovine- baited net	Pit shelter
<i>An. arabiensis</i>	x	x	x	x	x
<i>An. quadriannulatus</i>	x	x	x	x	x
<i>An. funestus s.l.</i>	x	x		x	x
<i>An. tenebrosus</i>			x	x	
<i>An. ziemanii</i>			x	x	
* <i>An. pharoensis</i>			x	x	
* <i>An. squamosus</i>			x	x	
* <i>An. rufipes</i>			x	x	x

x denotes a positive catch.

* denotes a species complex (Gillies & Coetzee, 1987).

Table XX. Comparative man-baited and bovine-baited net catch totals for six species from five nights of trapping at Mamfene.

Species	Total number of mosquitoes caught in the	
	Man- baited net	Bovine- baited net
<i>An. tenebrosus</i> + <i>An. ziemanii</i>	203	933
<i>An. squamosus</i>	11	116
<i>An. pharoensis</i>	2	46
<i>An. rufipes</i>	5	72
<i>An. funestus s.l.</i>	6	46

resting collections and bloodmeal analysis showed feeding on man to be common (Chapter 8). Bloodmeal analyses on 26 *An. funestus* s.l. caught by exit trap or indoor resting collections revealed that 24 had fed on cattle and two on man.

6.3.1.3 Mtubatuba malaria outbreak.

The first case of malaria amongst the white population of the town was diagnosed on 15.1.87. Subsequently, during the period 20.1.87 to 28.2.87 numerous cases were treated by local practitioners. Table XXI shows the number of medically diagnosed cases that were treated and the number of malaria cases confirmed by bloodsmear analysis. The majority of the malaria infections in the rural population confirmed by microscopy were detected by mass blood examinations conducted by the Natal Regional authorities. Two mass blood examinations (Sharp et al. 1988) were conducted in the area at the end of January.

Among 600 black people of which + 90% live approximately three kilometres out of the town, there was one positively confirmed malaria case. Among the 1137 blacks living + one kilometre south of the town centre and investigated for malaria there were 32 confirmed cases. House visits were made in the town to question those individuals who had been confirmed as, or suspected of having had malaria, and it was found that the majority had been permanently resident in the town for the two month period prior to infection.

Table XXI. Clinically diagnosed and microscopically confirmed cases of malaria from Mtubatuba.

Town residents		Rural population	
Clinically diagnosed	Confirmed by microscopy	Clinically diagnosed	confirmed by microscopy
62	26	numerous	61

The first malaria cases indigenous to the town, were diagnosed in mid-January. Allowing 9-14 days for the incubation of the disease in man and 10-15 days for the sporogonic cycle in the mosquito, it is assumed that transmission was initiated between 17 and 25 December 1986. This extrapolation correlates with the rainfall data for this period which is depicted in figure 23. There was 118mm of rain during December 1986, an amount that it is considered would have been adequate for the creation of suitable transient pools for the breeding of potential vector mosquitoes. Rainfall was again high during January 1987 (146 mm). During the period 20.1.87 to 19.2.87 only 7 mm of rain was recorded. It is assumed that as a consequence numerous breeding sites would have dried up by the 16.2.87 when entomological collections were started, with a resulting reduction in the vector population as reflected in the low catches of *An. gambiae s.l.* (Table XXII). By mid March the Mtubatuba malaria epidemic had practically ceased. Low rainfall between the 20.1.87 and the 28.2.87, coupled with focal larviciding had reduced the larval sites suitable for *An. gambiae s.l.*. Mass prophylaxis and mass blood examinations together with the treatment of patients by the local medical practitioners, had reduced the presence of the parasite.

Twenty-eight searches were made in houses in Mtubatuba for indoor resting mosquitoes, and only one female specimen belonging to the *An. funestus* complex was caught. The upper branch of the fifth vein of this mosquito had two pale

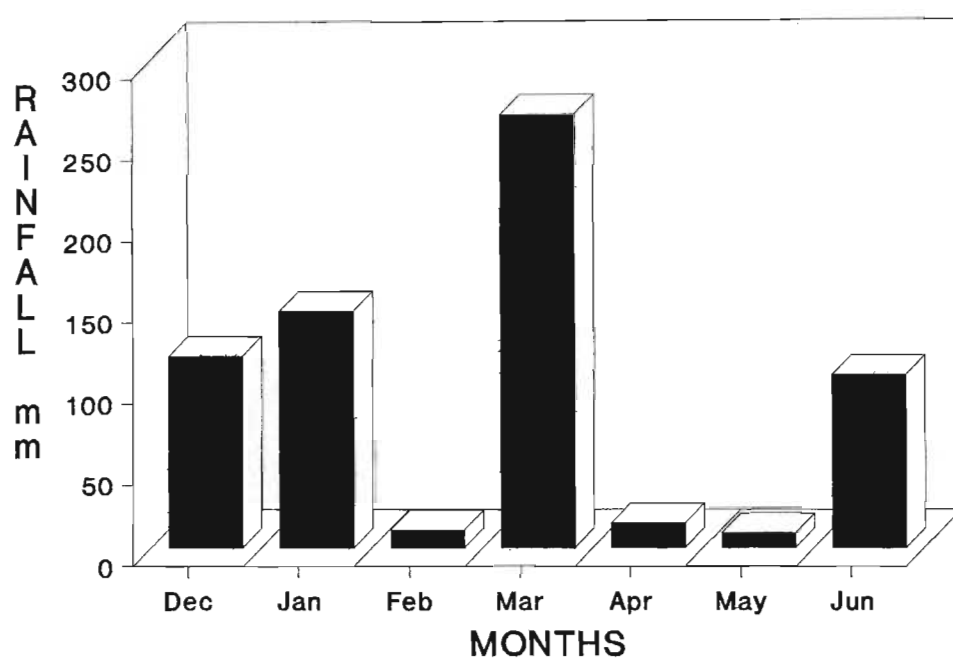


FIGURE 23. Rainfall recorded at Mtubatuba for the months of December 1986 to June 1987.

Table XXII. Anopheline species caught by man-baited net traps during the period 16-19 February and 3-5 March 1987 at Mtubatuba.

* denotes a species complex or evidence in this regard (Gillies & Coetzee 1987).

Species	no. mosquitoes	Mean no. per catch
* <i>An. gambiae s.l.</i>	11	2.2
* <i>An. pharoensis</i>	21	4.2
<i>An. tenebrosus</i>	39	7.8
* <i>An. coustani</i>	21	4.2
* <i>An. funestus/demeilloni</i>	7	1.4
* <i>An. squamosus</i>	1	0.2
<i>An. rufipes</i>	4	0.2
* <i>An. marshalli</i>	1	0.2

spots. A feature which reliably showed the female to be one of two species, ie *An. brucei* or *An. rivulorum* (Gillies & De Meillon 1968).

Anophelines from six known species complexes and two other *Anopheline* species were caught in the man-baited net (Table XXII).

The number of specimens of each particular species or species complex caught was low, with the mean catch per baited net, from a total of five trapping episodes, ranging from 0.2 for the *An. squamosus* complex to 7.8 for *An. tenebrosus*.

An. gambiae s.l. larvae were collected at four localities within the environs of the town itself and at three localities on the outskirts of the town. Of the *An. gambiae* s.l. collected, 54 were identified electrophoretically and eight both electrophoretically and chromosomally (Table XXIII).

Both *An. arabiensis* and *An. quadriannulatus* were identified from the man-baited net catches. Species specific identification showed both *An. arabiensis* and *An. quadriannulatus* larvae to occur in the rain puddles. Larval collections were made at six sites and *An. arabiensis* was found to have occurred alone in two, in conjunction with *An. quadriannulatus* at three, and with one site yielding only *An. quadriannulatus*.

Table XXIII. Species identification of 54 *An. gambiae* s.l. caught by man-baited net traps and by larval collections in Mtubatuba town

Species	no. Identified	no. Identified
	by electrophoresis	by electrophoresis
	only	and chromosomes
<i>An. arabiensis</i>	36	7
<i>An. quadriannulatus</i>	18	1

6.3.1.4 Tongaat malaria outbreak

During May 1988, eight cases of human malaria were detected at Tongaat. D. Eckard of the National Institute for Tropical Diseases investigated these cases and deduced that local transmission of the disease had occurred. Indoor resting catches were carried out and *An. gambiae s.l.* found by D. Eckard. D. Eckhard consulted me and it was decided that species identification should be carried out on specimens from this locality. Further indoor catches were made and 11 *An. gambiae s.l.* made available to me. Of these specimens nine were subjected to species identification using electrophoresis and two by polytene chromosome analysis and all 11 were identified as *An. arabiensis*. An analysis of the bloodmeals of five of these specimens showed that they had all fed on man.

6.3.2 Investigations of the possible role of *Anopheles merus* in malaria transmission

Entomological investigations at Nkunduse during each of the years 1981, 1982, 1983 (Sharp 1983) and 1984 found a large population of predominantly *An. merus* to occur in the area. No indigenous cases of malaria were, however, reported from this area during this four year period, and there were only 2 cases reported in the following four years. Although not conclusive evidence the implication of this eight year virtually malaria free period at Nkunduse is that *An. merus* is not a primary vector of malaria. It must be borne in

mind, however, that it is possible to have a large population of a vector species in an area and not to experience large scale transmission as outlined in chapter two (Section 2.3.2) for Mamfene during 1986. *An. merus* was further found to occur in large numbers at Ophansi during the period 1985 to 1987 (le Sueur unpublished data) an area that experienced 50, 34 and 459 malaria cases for the three years respectively. The initial indications were thus that *An. merus* might have been responsible for malaria transmission in the area. Intensified collections coupled with species identification of *An. gambiae* s.l. from the area, however, showed *An. arabiensis* to occur sympatrically (Chapter 4) with *An. merus* in the area.

The most successful means of trapping *An. merus* in this study was to use bovine bait (Chapter 5). This technique was statistically more efficient in the capture of *An. merus* than the man-baited net ($P < 0.005$). Outdoor trapping techniques were generally more successful than hut resting or leaving collections. Both outdoor resting (pit shelter) and man baited net catches were statistically more efficient than were both indoor resting and exit trap catches ($P < 0.005$). Comparison of host preference of *An. merus* using comparative man-baited net and bovine-baited net catches not only showed a statistical difference in the totals caught but also in the percentage bloodfed. Of those caught in the man-baited net 35% were bloodfed in comparison to the bovine-baited net where 90% had fed. Table XXIV shows the result of bloodmeal analysis of those *An. merus* caught in

Table XXIV. The bloodmeal analysis of *An. merus* caught by five trapping techniques.

Trap	Total collected	Bloodmeal origin	
		Human	Bovine
Man-baited net	92	84	0
Bovine-baited net	61	0	61
Exit trap + Indoor resting	73	71	2
Pit shelter	65	2	63

the man-baited net and bovine-baited net and in both cases those that had fed had done so predominantly on the bait in the respective net. Bloodmeal analysis of those identified and caught by exit trap and indoor resting had fed mainly on man (97%), however, pit shelter resting caught specimens showed 97% to have fed on bovine and only 3% on man.

6.4 CONCLUDING DISCUSSION

Historically the primary vectors of malaria in Africa are species that feed and rest indoors, and early work in South Africa showed the local vector species to have been no exception. This finding formed the basis for the use of intra-domiciliary residual insecticides for the control of malaria vector mosquitoes. Predominantly outdoor biting mosquitoes are generally not malaria vectors and if they do play a role in this regard it is generally as secondary vectors. The majority of the people in rural Natal sleep indoors and it follows that mosquitoes biting indoors are more likely to be the local vectors of the disease.

The Mamfene study found only *An. arabiensis* to predominantly bite man indoors and this species was found to occur at both Mtubatuba and Tongaat. The Tongaat specimens were found to have fed on man. *An. arabiensis* is a primary vector of malaria in Africa and its presence is not uncommon in areas with a house spraying programme (Chapter 8). Salivary gland dissections were not carried out as part of any of these studies, but the occurrence of this species of known malaria vectorial efficiency at all three localities and in the

absence of any other potential vector species suggests *An. arabiensis* to be the primary vector of malaria in Natal.

The following discussion considers the medical importance of the other species complexes caught in these studies and their potential importance as secondary vectors of malaria.

An. quadriannulatus is strongly zoophilic and therefore of no direct medical importance (White 1974).

An. pharoensis Chromosomal evidence (Miles et al. 1983) indicates that there might be two distinct species within the taxon. This species complex has been shown to be a vector of malaria in Egypt (Gillies & De Meillon 1968). In the province of Natal, the species is highly zoophilic as shown in the comparative man-baited and bovine-baited net catches in this study and by Sharp et al. (1984). It is therefore highly unlikely to be involved in the transmission of human *Plasmodium* spp..

An. tenebrosus, *An. ziemanii*, *An. rufipes* and *An. squamosus* have previously been shown to be predominantly zoophilic (Gillies & De Meillon 1968) and this is borne out by the comparative man and bovine baited net catches of this study.

An. coustani is predominantly zoophilic and of no known medical importance (Gillies & De Meillon 1968).

Of the member species of the *An. funestus* complex, *An. funestus* s.s. is a primary vector of malaria in Africa (Gillies & De Meillon 1968). Evidence suggests that this species was widely distributed in the malarious areas of the Republic of South Africa prior to the widespread application of intra-domiciliary residual insecticides for malaria vector control (Swellengrebel et al. 1931). It is generally believed, however, that *An. funestus* s.s. no longer occurs in the Republic of South Africa in those areas which are under the attack phase of malaria control as is the case elsewhere in Africa. The effectiveness of control of *An. funestus* s.s. by a house spraying programme is due to the highly endophilic resting behaviour of this species (Gillies & De Meillon 1968). Aboul-Nasr (1970) suggests that *An. funestus* s.s. was replaced in South West Uganda by *An. rivulorum* when the former species was eliminated by house spraying (cited in Gillies & Coetzee 1987).

Of the 26 bloodmeals from indoor resting *An. funestus* s.l. caught at Mamfene, 92% had fed on cattle. This finding is in contrast to what would be expected from *An. funestus* s.s., a highly anthropophilic species. Comparative man and bovine baited net catches found that 88% of the *An. funestus* s.l. were caught in the bovine bait. Of eight *An. funestus* s.l. caught at Mtubatuba, five were identified as *An. rivulorum*. In two of these cases it was necessary to raise family groups to arrive at a morphological identification. Three specimens could not be identified further than belonging to the *An. funestus* complex and/or *An. demeilloni*.

An. rivulorum is distributed in East Africa but shows a highly zoophilic biting preference and is of no known malaria vectorial importance (Gillies & De Meillon 1968).

An. demeilloni shows a highly zoophilic biting behaviour and is not considered to be of medical importance (Gillies & De Meillon 1968).

An. marshalli s.l. is of no known medical importance (Gillies & De Meillon 1968, Gillies & Coetzee 1987).

Although the indications are that *An. funestus* s.s does not occur in those areas of Natal under the attack phase of malaria vector control, a more in depth morphological and chromosomal study would be necessary to conclusively prove this. None of the other species or species complexes caught are considered to be of any importance as potential secondary vectors of malaria in Natal. The only other species distributed in Natal (Chapter 4) that has been shown to be important in this regard elsewhere in Africa is *An. merus*.

The susceptibility of *An. merus* to infection with *P. falciparum* under laboratory (Pringle 1962) and field conditions (Muirhead Thomson 1951, Mosha & Petrarca 1983) suggests that this species might play a role in malaria transmission. The host preference investigation conducted in this study (Chapter 5) and the low human blood index of natural resting *An. merus* concur well with the findings of

other studies. Iyengar (1962) working in Tanzania found that only 1.6% of outdoor resting *An. merus* had fed on man. Gillies (1968) concluded from laboratory experiments with *An. merus* that the zoophilic tendencies of *An. merus* reported from field studies are an inherent biological characteristic of this species. In the rural areas of Natal/KwaZulu, the majority of homesteads have cattle kraals. It is probable that under these conditions, the cattle serve as a form of zooprophylaxis, inhibiting the transmission of malaria by *An. merus*. This species was not exclusively caught in areas where local transmission was occurring, but always found in sympatric distribution with *An. arabiensis*.

CHAPTER 7

EFFECT OF DDT ON SURVIVAL AND BLOOD FEEDING SUCCESS OF *ANOPHELES ARABIENSIS* PATTON (DIPTERA:CULICIDAE).

7.1 INTRODUCTION

The efficient control of vector species is central to the containment of malaria. The development of low cost residual insecticides after the second World War revolutionized rural malaria control (Davidson 1982). The use of intra-domiciliary insecticides for malaria vector control has, however, met with variable success, depending on logistical and social factors, finance, vector behavior and the development of insecticide resistance (Fontaine 1983). It still, however, remains an important component of malaria control in some rural areas (Gratz 1985). The assessment of the efficiency of this measure in an area is essential to planning an effective malaria control strategy.

Three species of the *Anopheles gambiae* Giles complex, *An. quadriannulatus* Theobald, *An. arabiensis* Patton and *An. merus* Donitz occur in KwaZulu (le Sueur & Sharp 1988), an area experiencing seasonal malaria transmission (Sharp et al. 1988). The malaria case rate has increased over the past 2 years, in part due to chloroquine resistance in *Plasmodium falciparum* Welch (Freese et al. 1988).

Malaria transmission occurs in Natal Province (Chapter 2)

despite the presence of a longstanding malaria vector control programme. The efficacy of intra-domiciliary DDT for vector control in the malaria area of KwaZulu was therefore investigated.

7.2 MATERIALS AND METHODS

7.2.1 Sampling site

Mosquitoes for the study were collected at Mamfene (36°16'E 27°23'S), Ubombo district during the period January 1986 to April 1988.

7.2.2 Sampling techniques

Exit traps of the Muirhead-Thomson (1947) design were used and cleared before 08h00 each day as outlined in Chapter 5, section 5.2.2.1. Indoor resting catches were by space spraying with a 4% pyrethrum solution in kerosene as outlined in Chapter 5, section 5.2.2.2. On each day only one hut in a homestead comprising two to six huts was used for exit trap or indoor resting collection.

7.2.3 Species identification

Identification of the *An. gambiae* sibling species was by polytene chromosome analysis and iso-enzyme electrophoresis as outlined in Chapter 3, sections 3.2.2 and 3.2.3.

7.2.4 D.D.T. susceptibility of mosquitoes

After capture, the mosquitoes to be used for D.D.T. susceptibility testing were blood fed, if not already fed, placed in individual breeding tubes and held in an insulated container for transfer to an insectary ($25 \pm 1^{\circ}\text{C}$, $75 \pm 5\%$ RH) where individual family broods were reared. Susceptibility tests (World Health Organisation 1975) were performed using 1-4 day old adult females.

7.2.5 Exit trap survival

After removal from the exit traps the mosquitoes were stored in collecting cups in an insulated container covered with damp muslin cloth. Mortality counts were done periodically until 12-16 hours after collection (08h00) and the gonotrophic status of both the surviving and dead mosquitoes was scored.

7.2.6 Contact Bioassays

Contact bioassays (World Health Organisation 1975) were done on both DDT sprayed and non-sprayed (controls) walls of houses using 2-5 day old *An. arabiensis* from a colony strain (KANB) that originated from Kanyembe, Zimbabwe ($15^{\circ} 40'S$, $30^{\circ} 20'E$). In addition to the one hour exposure required by the standard World Health Organisation bioassay, shorter exposure times were also employed.

7.2.7 Classification of abdominal appearance of mosquitoes

The classification of the abdominal appearance of the mosquitoes caught was done according to the criteria of Sella (World Health Organisation 1975), using a dissecting microscope. The classifications used were: unfed, blood fed and gravid.

7.3 RESULTS

Seventy-nine *An. arabiensis* were identified by polytene chromosome analysis, 65 caught by exit trap and 14 by indoor resting collections. Sixty-five were identified by both polytene chromosomes and enzyme electrophoresis. A further 559 specimens were only identified by enzyme electrophoresis (Table XXV).

Identification of indoor resting and exit trap caught *An. gambiae s.l.* showed >96% to be *An. arabiensis* with very low numbers of *An. quadriannulatus* present (Table XXV).

Both exit trap and indoor resting catches were successfully used to collect *An. gambiae s.l.* (Tables XXVI and XXVII). The mean number caught per hut was higher in exit trap collections than in the indoor resting collections, but this difference was not significantly different ($t=1.608$, $P>0.1$).

Anopheles gambiae s.l. were caught leaving huts irrespective of the presence or absence of DDT (Table

Table XXV. The species composition of *Anopheles gambiae* s.l. caught in exit traps from huts and those resting indoors in huts. Identification of species carried out by iso-enzyme composition.

Trap	<i>An. arabiensis</i>	<i>An. quadriannulatus</i>
Exit trap	301	12
Indoor resting	236	10
Total	537	22

Table XXVI. Numbers of *Anopheles gambiae s.l.* caught in exit traps located in control, replastered and DDT sprayed huts.

	Control	Replastered	DDT	Total
no. huts	60	150	155	365
no. <i>An. gambiae s.l.</i>	252	512	312	1076
mean / hut	4.2	3.4	2.0	2.9
S.E. / hut	1.3	0.7	0.5	0.4
range	0 - 59	0 - 86	0 - 68	0 - 86

Table XXVII. Numbers of *Anopheles gambiae* s.l. caught resting in control, replastered and DDT sprayed huts.

	Control	Replastered	DDT	Total
no. huts	23	57	79	159
no. <i>An.gambiae</i> s.l.	68	213	7	288
mean / hut	2.9	3.7	0.1	1.9
S.E. / hut	1.0	1.5	0.1	0.5
Range	0 - 17	0 - 69	0 - 6	0 - 69

XXVI). There was however an inverse relationship between the presence of DDT and the numbers of *An. gambiae s.l.* caught, with the highest numbers per hut being caught in control huts. The range in numbers caught was high irrespective of hut status and pairwise comparisons with a Mann-Whitney test showed no significant difference between catches from huts of differing DDT status ($P>0.5$).

Indoor resting occurred mainly in replastered and control huts (Table XXVII). Only 7 *An. gambiae s.l.* were caught resting indoors in a total of 79 DDT sprayed huts and six of these were caught in one hut. The Mann-Whitney test showed no significant difference between catches from control and replastered huts ($P>0.7$), but in both cases these data were significantly different to that from the DDT sprayed huts ($P<0.003$).

A greater percentage of the *An. gambiae s.l.* caught leaving huts (Table XXVIII) were unfed, followed by freshly fed and lastly a low number of gravid mosquitoes. There was an inverse relationship between the percentage of freshly bloodfed mosquitoes and the presence of DDT. The highest percentage of freshly bloodfed specimens were caught leaving control huts, while an increased proportion of unfed mosquitoes left the DDT sprayed huts (Table XXVIII). The percentage of gravid females caught in all huts was extremely low. Chi squared tests (2×3) of heterogeneity showed that the gonotrophic status of *An. gambiae s.l.* from the different hut categories were significantly different ($P<0.0001$).

Table XXVIII. The gonotrophic status of *Anopheles gambiae* s.l.. caught in exit traps from huts.

	%	%	%	no.	no.
	Bloodfed	Gravid	Unfed	<i>An.gambiae</i> s.l.	huts
DDT	32.6	0.3	67.1	310	153
Replastered	41.9	5.5	52.5	489	146
Control	65.5	2.5	32.0	237	58
Total	44.7	3.4	51.9	1036	357

The majority of *An. gambiae s.l.* caught resting indoors were freshly bloodfed, followed by unfeds, with the lowest numbers being in the gravid state (Table XXIX). The percentage bloodfed was high irrespective of the DDT status of the hut. The percentage gravid was higher than the percentage unfed in both the DDT sprayed and the control huts. Only 6 *An. gambiae s.l.* were caught in the DDT sprayed huts. In the replastered huts the percentage unfed was greater than the percentage gravid. The Mann-Whitney test showed no significant difference between the total numbers of *An. gambiae s.l.* caught in the control and replastered huts ($P>0.7$), but a Chi-square test of heterogeneity showed the gonotrophic status of the mosquitoes from these to be significantly different ($P<0.005$). Data collected from the DDT sprayed huts were excluded from statistical analysis due to the low numbers of mosquitoes caught ($n=6$).

Survival of exit trap caught mosquitoes was highest in the control huts, less in the replastered huts and lowest in DDT sprayed huts (Table XXX).

Overall survival of the mosquitoes caught 8-12 months after DDT application was marginally increased ($<7\%$) in comparison to those caught 1-3 months after spraying. Overall survival of the mosquitoes caught in control huts was 16 to 18.8% higher than from replastered huts and approximately 40% higher than from DDT huts. The percentage of total mosquitoes caught and classified as bloodfed was highest in

Table XXIX. The gonotrophic status of *Anopheles gambiae* s.l. caught resting indoors in huts.

	%	%	%	no.	no.
	Bloodfed	Gravid	Unfed	<i>An. gambiae</i> s.l.	Huts
DDT	66.6	33.3	0.0	6	78
Replastered	61.5	13.8	24.6	195	53
Control	52.0	36.0	12.0	50	19
Total	61.0	18.9	20.0	251	150

Table XXX. The survival of *Anopheles gambiae* s.l. caught in exit traps from control, replastered and DDT sprayed huts in relation to the age of the DDT.

A) 1-3 months after DDT application.

B) 8-12 months after spraying.

	Hut	Total		%	% survival		
	status	huts	mosq.		bloodfed	notfed	overall
	DDT	49	222	31.5	72.9	30.9	44.1
A	Repl.	13	91	53.8	93.9	38.1	68.1
	Control	15	82	86.6	88.7	54.6	84.1
	DDT	6	65	26.2	88.2	37.5	50.8
B	Repl.	21	119	54.6	93.8	44.4	71.4
	Control	14	82	86.6	95.8	54.5	90.2

the absence of DDT (86.6%), markedly reduced in the replastered hut catches (53.8 and 54.6%) and lowest in the DDT sprayed hut catches (31.5 and 26.2%). Survival of bloodfed mosquitoes was high (>72%) irrespective of the DDT status of the huts from which they were caught, whereas survival of unfed mosquitoes was low, even in the control hut catches (<55%).

Twenty-five and 17 colony derived female *An. arabiensis* were respectively used in a 0.5 and a 1.0 hour control bioassay carried out in a non-DDT sprayed hut, as part of the three month post-DDT spraying control bioassays. Twenty and 36 mosquitoes were respectively used in a 10 and a 30 minute bioassay as part of the 9-month post-spraying control bioassays. There was no mortality in any of these control bioassays. Five minutes (n=54), 10 minutes (n=53) and 30 minutes (n=67) of contact on 3 month old DDT was sufficient for 100% kill in all cases. In the case of nine month old DDT five minutes of contact only effected 26% mortality (n=19), however, 15 minutes or more (n=54) of contact effected 100% mortality.

Families raised from 21 individually identified wild caught *An. arabiensis* totaling 339 individual mosquitoes were tested for DDT susceptibility. These mosquitoes and samples of colony derived *An. arabiensis* were respectively exposed to five standard concentrations of DDT for one hour and the 24 hour mortality plotted on logarithmic probability paper to construct dosage mortality regressions (Figure 24). Neither graph indicates insecticide resistance in the

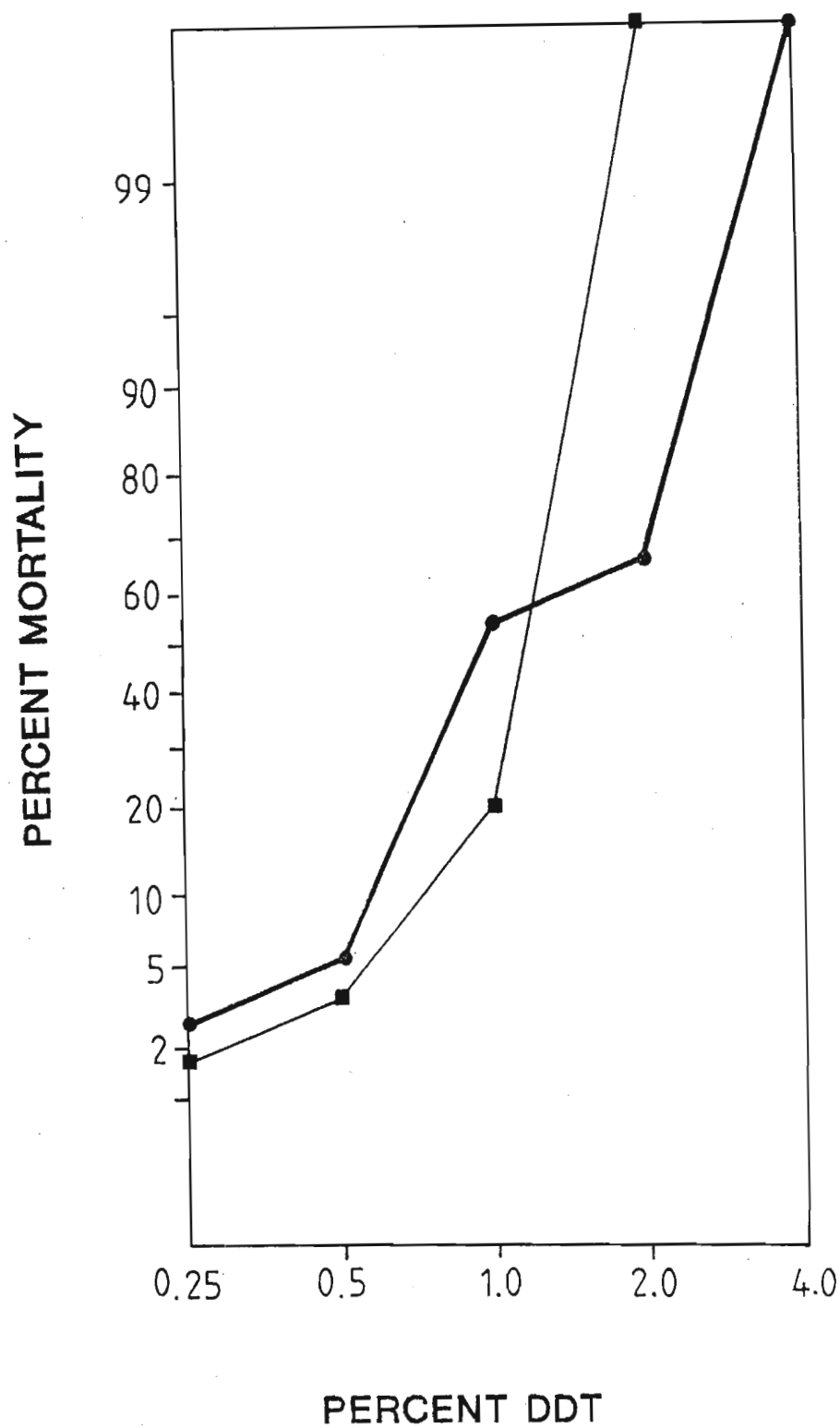


FIGURE 24. DDT dosage mortality regression of colonised *An. arabiensis* and wild caught *An. arabiensis*.

■ = Colonized *Anopheles arabiensis*.
● = Wild caught *Anopheles arabiensis*.

populations concerned at a discriminating dosage of 4.0% DDT. The figures indicate that the wild population (Figure 24) had increased vigour tolerance relative to the colony population. In the colonized population 100% mortality was obtained at a concentration of 2.0% DDT, whereas in the wild population, this occurred at 4.0% DDT.

7.4 DISCUSSION

Anopheles arabiensis and *An. quadriannulatus* were caught both resting indoors and leaving huts in the study area. *An. arabiensis* was, however, the dominant species, accounting for >96% of the identified specimens. As a consequence, these data are considered to reflect the behaviour of *An. arabiensis*.

Overall, higher numbers were caught leaving huts than resting indoors. A direct relationship did, however, exist between the numbers caught leaving and the presence of DDT. These data indicate the expected, namely, with the highest numbers being caught leaving the control hut, a reduced number from the replastered huts where the underside of the roof had exposed DDT and with the lowest numbers being caught leaving the fully DDT sprayed huts. Extremely low numbers were found resting in DDT sprayed huts, and of a total of 79 huts investigated, only 2 were positive. Indoor resting occurred commonly in both replastered and control huts. The indications from these data are that the mosquitoes were resting on the DDT-free walls of the replastered huts. Had they have been mainly resting on the

underside of the roof, then results comparable to those from both the DDT sprayed huts would be expected. The lack of physiological tolerance to DDT by the wild caught *An. arabiensis* population further precludes survival after resting on DDT sprayed roofs. These data are in contrast to that of Brun (1973) from Burkina Faso where he found that in excess of 70% of the indoor resting *An. gambiae s.l.* were resting on the roof.

The fed : gravid ratio of the mosquitoes caught varied tremendously between the window trap and the indoor resting catches and between catches in the same trap type depending on the DDT status of the hut from which the collections were made (Table XXXI). The fed:gravid ratios of indoor resting caught mosquitoes from control and replastered huts were similar to those found for *An. arabiensis* caught resting in DDT sprayed huts and outdoors by Haridi (1972) and to indoor resting collections made in control huts by Shelley (1973) (Table XXXI). In the replastered huts there was, however, an increase in the bloodfed component of the ratio in relation to that from the control huts. This ratio was markedly different in the exit trap caught mosquitoes, with a marked increase in the percentage bloodfed and particularly so in the case of the fully DDT sprayed huts (101:1). From these data it is clear that a high percentage of bloodfed mosquitoes were leaving the huts and not resting indoors. This tendency for bloodfed mosquitoes to leave huts was particularly marked in the fully DDT sprayed huts and could be related to an irritational effect by the DDT

Table XXXI. The fed:gravid ratio of *Anopheles arabiensis* caught in exit traps from huts and those resting indoors in huts in this study and that of Haridi (1972) and Shelley (1973).

Collection site	Fed:Gravid ratio	Study
I.R. DDT huts	3.7:1	Haridi (1972)
Outdoors	2.2:1	Haridi (1972)
I.R. Control huts	3.9:1	Shelley (1973)
I.R. Control huts	1.4:1	Present
I.R. Replastered huts	4.5:1	Present
E.T. Control huts	25.8:1	Present
E.T. Replastered huts	7.9:1	Present
E.T. DDT huts	101:1	Present

I.R. = Indoor resting

E.T. = Exit trap

on the mosquito, a phenomena that Muirhead Thomson (1960) suggested was a drawback of using this particular insecticide.

The overall survival of mosquitoes caught leaving huts 1-3 months after DDT spraying was not markedly different to that 8-12 months after application. The survival of mosquitoes varied depending on the fed status of the mosquito and depending on whether the hut was not sprayed, replastered or fully DDT sprayed. The percentage of mosquitoes that were bloodfed showed an inverse relationship to the amount of DDT in the hut and suggests that the presence of DDT hampered the taking of a bloodmeal. The percentage survival of bloodfed mosquitoes caught in exit traps fitted to DDT sprayed huts (72.9% from huts 1-3 months after application and 88.2% from huts 8-12 months after spraying) compares well with similarly treated data collected by Haridi (1972) who found an 88% survival. The greater survival of engorged females is further in keeping with the findings of Trapidio (1954). The survival of engorged *An. arabiensis* in this study were far higher than that found by Mpofu et al (1988). They recorded 4% survival of bloodfed mosquitoes 3 months after DDT spraying and 30% survival at 8 months post spraying, in contrast to the 72.9 and 88.2% found in this study. The reason for the higher survival recorded in this study can in part be attributed to the method of calculation of percentage survival and the mosquitoes used. In this study, only exit trap caught mosquitoes were used in this calculation, whereas Mpofu et al. (1988) used both exit trap and hut floor mortalities in their calculation. The

mosquitoes used by Mpofu et al. (1988) were derived from a colony strain, whereas in this study, wild mosquitoes were used. Susceptibility tests done in this study on both *An. arabiensis* collected from the wild and on colony derived *An. arabiensis* showed the wild population to have increased DDT vigour relative to the laboratory colony material. Overall survival of exit trap catches (all gonotrophic stages) in the study by Haridi (1972) was 94% and far higher than the 44.1 and 50.8% of this study. It is however expected that the proportion leaving and surviving will vary considerable according to the dosage, coverage, formulation, nature of the wall surface and behavior of a particular species (Muirhead-Thomson 1950, Hadaway 1951, Wilkinson 1952, Haridi 1972). The percentage mortality in this study is considered to be exaggerated as a result of high mortality in the unfed component of the exit trap catches. Unfed mosquitoes collected in exit traps from control huts only showed a 54.6 and 54.5% survival. Overall, survival of exit trap caught *An. arabiensis* showed an inverse relationship to the presence of DDT with the lowest survival (44.1%) from the DDT sprayed huts 1-3 months after spraying. If the mortalities of the unfed component of the exit trap catches are corrected using Abbott's formula, bearing in mind the limitation of using this correction with such a high mortality in the control, mortality of unfed mosquitoes caught by exit traps on replastered and DDT huts respectively was as low as 30.2 and 43.4%.

Bioassays in DDT sprayed huts indicate minimal loss of

effectiveness of the DDT deposits over time in killing *An. arabiensis*. In fact, 8-12 months after application an exposure time of 15 minutes was sufficient to effect 100% mortality. Dose mortality curves further showed no marked increased vigour tolerance or physiological resistance to DDT in the wild *An. arabiensis* with 100% mortality occurring at a discriminating dosage of 4% DDT. Overall, a higher percentage of *An. arabiensis* were leaving huts than were resting inside. The mean number caught leaving replastered huts compared to control huts was reduced by 19% and in DDT huts by 52%. Assuming random entry of huts, this reduction in exit trap catch is taken to represent the numbers killed indoors. The findings of this study have implications for the malaria control program. Transmission was occurring in the study area, and during 1987 there were 709 malaria cases and during the period January to June 1988, 595 cases. The inverse relationship between the percentage of mosquitoes bloodfed and presence of DDT in a hut clearly indicates behavioural avoidance. This could be counter-balanced by an increase in biting of man outdoors. The replastering of the inner walls of huts by the inhabitants for social and practical reasons is a further impediment to control of *An. arabiensis* by house spraying with DDT; the percentage of bloodfed *An. arabiensis* caught leaving these huts and surviving was high. Surveys in the area subjected to the DDT vector control programme showed that the percentage of huts replastered varied from 1.7 to 72% at different localities and times (K. Newberry personal communication). Surveys in the study area found 25.7% of huts to be replastered in April/May 1981 and 46.6% in April/May 1982. The exit trap

survival data indicate that there was a 20 to 24% greater survival of mosquitoes leaving replastered huts than from DDT sprayed huts. Overall this figure is expected to be in excess of 50% should the differential kill in replastered and DDT huts prior to capture in the exit trap be taken into account. A reduction in the replastering of huts should therefore result in more efficient vector control. K. Newberry (personal communication, 1989) found that replastering of huts was to a large extent carried out to combat biting by bed bugs and that their control by insecticidal spraying has led to a significant decrease in the replastering rate in the districts of Ubombo and Ingwavuma.

The data presented strongly suggest that optimal control of *An. arabiensis* will not be achieved using the current control strategy of the annual intra-domiciliary application of DDT. To increase the effect of vector control in the study area would require an integrated approach utilizing additional measures. In this respect the use of an alternative insecticide with a reduced irritant effect on the vector species warrants investigation.

CHAPTER 8

BEHAVIOURAL TRAITS OF *ANOPHELES ARABIENSIS* PATTON (DIPTERA:CULICIDAE) POPULATIONS

8.1 INTRODUCTION

An. arabiensis is a principle vector of malaria at numerous localities throughout Africa (White 1974). The human blood index (H.B.I.) of this species varies markedly between and within localities depending on the site of capture (Gillies & Coetzee 1987).

It has been suggested that this variation in biting behaviour is coupled to the high degree of inversion polymorphism in the species (White 1974). Correlation has been reported between chromosome inversion frequency and behaviour (Coluzzi et al. 1977, 1979, Petrarca et al. 1987). Coluzzi et al. (1977, 1979) reported statistically significant differences in inversion frequency between individuals caught resting indoors and those caught outdoors. Mosquitoes caught exiting after feeding had the same inversion frequency as those caught outdoors. An important conclusion has been drawn from the polymorphism theory, that any house spraying programme, however efficient the coverage and the chemical, would be bound to fail in the Sudan-Savannah zone of Africa because of the genetically exophilic fraction of the population (Molineaux & Grammiccia 1980).

The high degree of behavioural diversity of *An. arabiensis* throughout its geographical distribution, suggests that one should investigate this species at each locality at which it occurs so as to assess the effectiveness of regional control measures. Most studies on this species have been carried out in East and West Africa (White 1974). The only study carried out in Southern Africa is that of Shelley (1973) in Zambia. No such behavioural studies have been conducted in the Province of Natal, which is the southernmost limit of distribution for *An. arabiensis*.

The human malaria vectorial importance of a mosquito species is directly related to the degree of anthropophilic biting behaviour of the species. The anthropophilic biting behaviour of *An. arabiensis* as evidenced by its HBI (Gillies & Coetzee 1987) varies within and between localities, and this study reports on the variation in this characteristic of the species in Natal Province.

8.2 MATERIALS AND METHODS

8.2.1 Sample sites

Specimens for the study were collected at four localities all more than 50 kilometres apart (Table XXXII).

8.2.2 Sampling techniques

Table XXXII. Sampling sites, dates sampled and presence or absence at the locality of intra-domiciliary DDT for malaria vector control.

Locality	Area	DDT	Collection Dates
1	Mamfene (36°16'E 27°23'S)	Annually for >10 years	Jan 86-Jan 87
2	Pelindaba (32°33'E 27°05'S)	None for > than 3yrs	Feb/March 84
3	Dondota (31°58'E 28°34'S)	Never	April 87
4	Chubu (32°51'E 28°02'S)	Never	April 84

Exit traps of the Muirhead-Thomson (1947) design were used and cleared before 08h00 each day as outlined in Chapter 5, section 5.2.2.1. Indoor resting catches were by space spraying with a 4% pyrethrum solution in kerosene as outlined in Chapter 5, section 5.2.2.2. On each day only one hut in a homestead comprising two to six huts was used for exit trap or indoor resting collection.

8.2.3 Species identification

Identification of the *An. gambiae* sibling species was by polytene chromosome analysis and iso-enzyme electrophoresis as outlined in Chapter 3, sections 3.2.2 and 3.2.3.

8.2.4 Bloodmeal analysis

The origin of bloodmeals was determined using the Ouchterlony double diffusion technique and human and bovine antisera to whole blood as described in Chapter 6, section 6.2.4.

8.2.5 Calculation of the Hardy-Weinberg equilibrium

The karyotype frequencies in each sample were tested for deviation from the Hardy-Weinberg law using Wright's F statistic (Bryan *et al.* 1982). i.e. $[F = 4a_1a_3 - a_2^2 / (2a_1 + a_2)(2a_3 + a_2)]$ a_1 and a_3 are the frequencies of the two homozygous classes and a_2 the frequency of the heterozygote. There are significant ($P < 0.05$) departures from the Hardy-Weinberg expectations if $F > 1.96 / N$. $F > 0$ indicates a deficiency of

heterozygotes and $F < 0$ an excess of heterozygotes. It's bias is negligible for samples above 20.

8.3 RESULTS

A total of 87 *An. arabiensis* were identified by polytene chromosomes and 65 were identified by both polytene chromosomes and enzyme electrophoresis (Table XXXIII). The remaining specimens included in this study were identified by enzyme electrophoresis. *An. quadriannulatus* was found to occur sympatrically with *An. arabiensis* at three of the four study sites, namely: Mamfene, Pelindaba and Chubu.

Polytene chromosome investigation of the *An. arabiensis* specimens collected at Mamfene and Dondota showed both populations to be polymorphic for the 2b inversion system. The only other polymorphic inversion was a 2bc heterozygote from Mamfene and only one was seen in 68 preparations. The other chromosomal arms scored showed the configurations 3a, 4, 5 and xbcd using the chromosomal arm designation of Green & Hunt (1980).

A total of 68 *An. arabiensis* chromosomes from specimens collected at Mamfene were scored for the 2b inversion karyotypes and subjected to the Hardy Weinberg equilibrium. Thirty-nine *An. arabiensis* collected by exit trap, of which 68% had fed on man, were further treated separately, as were 28 specimens that had fed on man and 25 that had fed on cattle. The total sample showed a significant

Table XXXIII. Number of polytene chromosome and paired polytene chromosome and enzyme electrophoresis identifications of *An. arabiensis* from two localities.

Locality	Trap	Polytene chromosome	Polytene chromosome and enzyme electrophoresis
Mamfene	Exit trap	65	53
Mamfene	Indoor resting	14	12
Dondota	Indoor resting	8	0

deficiency of heterozygotes as did the exit trap collected specimens and the sample that had fed on bovine. There was, however, no departure from equilibrium for those samples that had fed on man (Table XXXIV). The carriers of different 2b inversion karyotypes showed uniform distribution within human and bovine fed specimens ($\chi^2 = 1.2729$; $P > 0.5$).

The human blood index of indoor resting *An. arabiensis* from the three non-DDT sprayed areas of Pelindaba, Dondota and Chubu (Table XXXV), showed no statistically significant difference using Fisher's exact test and the Bonferroni adjusted level of significance. At all three localities, more than 90% of feeding was on man (Table XXXV).

In the DDT sprayed area (Mamfene), only 31.7% of those *An. arabiensis* caught resting indoors had fed on man as opposed to 68.3% on bovine. This difference in host preference of indoor resting *An. arabiensis* in the DDT and non-DDT sprayed areas is statistically significant ($\chi^2 = 109.59$; $P < 0.001$). 66.8% of the bloodfed *An. arabiensis* caught leaving huts (exit trap) at Mamfene had fed on man, which is significantly different ($\chi^2 = 37.85$; $P < 0.001$) from the 31% that were human fed and caught resting indoors.

Ninety five percent of the *An. gambiae s.l.* caught in the exit traps and 91% *An. gambiae s.l.* which were caught resting indoors in huts were from huts in close proximity (<30 m) to a cattle kraal. Despite this factor, exit trap catches showed a significantly higher H.B.I. than indoor resting catches, (Table XXXVI) ($\chi^2 = 39.75$; $P < 0.001$).

Table XXXIV. The calculation of the Hardy Weinberg equilibrium using Wright's F statistic and the frequency of 2b inversion karyotypes of *Anopheles arabiensis* from Mamfene.

2b karyotypes						
	n	2b/b	2b+b	2+ / +	1.96 / n	F
Total sample	68	0.5588	0.2794	0.1617	0.2376	0.3365
Exit trap	39	0.4871	0.3076	0.2051	0.3138	0.3314
Bovine fed	25	0.52	0.24	0.24	0.392	0.4791
Human fed	28	0.5714	0.2857	0.1428	0.3704	0.3

Table XXXV. The bloodmeal analyses of *An. arabiensis* caught at four localities in exit traps (E.T.) from huts and resting indoors (I.R.) in huts.

Locality	Trap	Bovine		Human	
		n	%	n	%
Mamfene	E.T.	51	33.1	103	66.9
Mamfene	I.R.	97	68.3	45	31.7
Pelindaba	I.R.	0	0.0	44	100
Dondota	I.R.	5	9.6	47	90.4
Chubu	I.R.	0	0.0	14	100

Table XXXVI. The bloodmeal analyses of *An. arabiensis* caught in exit traps (E.T.) from huts and resting indoors (I.R.) in huts at homesteads with a cattle kraal at Mamfene.

Trap	Bovine		Human	
	n	%	n	%
E.T.	44	35.8	79	64.2
I.R.	73	76.0	23	24.0

This trend of a higher H.B.I. in exit trap catches than in indoor resting catches was unaffected by whether the hut had been sprayed with DDT or not (Table XXXVII). A χ^2 test shows no significant heterogeneity between the H.B.I. in the presence or absence of DDT for either the indoor resting or exit trap catches ($P>0,05$).

8.4 DISCUSSION

There was a marked difference in the human blood index (HBI) of the indoor resting *An. arabiensis* in the unsprayed and the DDT sprayed areas, and between the indoor resting and exit trap catches in the DDT sprayed area. This variation in the HBI is consistent with the wide range in behaviour described for this species in Africa (White 1974). Should the behaviour of *An. arabiensis* in the non-sprayed area be predominantly to feed on man and rest indoors, then it would appear to be ideally suited to control by residual insecticides in houses. The fact that in the sprayed area the majority of the *A. arabiensis* entering houses to feed on man leave after feeding, has implications with regard to the efficacy of control of this component (Chapter 7, Sharp *et al.* 1990). The occurrence of the same trend in control huts, suggests that the leaving behaviour is not solely a result of DDT irritation. If the calculation of the HBI is based solely on those *A. arabiensis* caught resting indoors in the sprayed area, the anthropophilic biting behaviour of the population would be severely underestimated. This finding may have implications for the calculation of the

Table XXXVII. The bloodmeal analyses of *An. arabiensis* caught at Mamfene in exit traps (E.T.) from huts and resting indoors (I.R.) in huts that were (D.D.T.) sprayed and unsprayed.

Trap	Bloodmeal	DDT		No DDT	
		n	%	n	%
E.T.	Bovine	22	29.7	22	40.0
E.T.	Human	52	70.3	33	60.0
I.R.	Bovine	71	70.0	20	69.0
I.R.	Human	29	29.0	9	31.0

sporozoite rate in *A. arabiensis* populations in sprayed areas if based solely on one mosquito catching method.

Table XXXVIII shows the precipitin data from indoor resting samples of *A. arabiensis* populations studied in Africa. For all populations studied when there was no house spraying programme, the majority of specimens (>60%) had fed on man. In nine of the ten data sets from unsprayed areas more than 82% of feeding was on man. The data collected by White et al. (1972) are in contrast to other data sets in that they showed a lower overall HBI. They found the HBI varied between catching stations, ranging from 14 to 100%, and showed an inverse relationship to the number of cattle present.

The results for the four populations subjected to a spray programme are closely in agreement but differ markedly from the data from the unsprayed areas (table XXXVIII). In the sprayed areas the majority of the mosquitoes caught resting indoors had fed on bovine blood. The data of Highton et al. (1979), appear to be intermediate between those from the sprayed and unsprayed areas. However, their study in the Kisumu area, Kenya, followed three years of field trials in the area with OMS 543 (fenitrothion) (Service et al. 1978).

Coluzzi et al. (1977,1979) and Petrarca et al. (1987) give evidence for genetic mediation of behaviour in *A. arabiensis* and relate behavioural traits to chromosomal arrangement. Coluzzi et al. (1977, 1979) reported statistically significant differences in inversion frequency between

Table XXXVIII. The bloodmeal analysis of *An. arabiensis* caught resting indoors (I.R.) in huts in the present study and 10 other studies in relation to the presence of an intra-domiciliary residual insecticide control programme.

* Data quoted from Garrett-Jones et al. 1980.

Study	Insecticide	Total	%	
	Sprayed	Bloods	Human	Bovine
Mamfene	DDT	157	31.2	68.8
Service et al. 1978. Kenya.	Fenitrothion	76	27.2	71.1
White & Rosen. 1973. Nigeria.	OMS 1028 Carbamate	39	39.0	61.0
Highton et al. 1979. Kenya.	Fenitrothion	1309	44.9	55.1
Pelindaba	None	44	100	0.0
Dondota	None	52	90.4	9.6
Chubu	None	14	100	0.0
* 1974-1975. Nigeria.	None	164	93.3	3.7
Shelley, 1973. Zambia.	None	91	92.3	1.1
Service et al. 1978. Kenya.	None	220	85.9	13.6
Joshi et al. 1975. Kenya.	None	450	92.4	7.3
Coluzzi et al. *1972. Nigeria.	None	199	82.9	17.1
Service (1970). Nigeria.	None	103	82.5	1.0
White et al., 1972. Tanzania.	None	1277	60.9	38.1

individual *A. arabiensis* caught resting indoors and those caught outdoors. Mosquitoes caught leaving after feeding had the same inversion frequency as those caught outdoors. Petrarca et al. (1987) demonstrated a statistically significant non-uniform distribution of human and bovine fed specimens among the carriers of different 2b inversion karyotypes in indoor-resting *A. arabiensis*. The present study shows uniform distribution of human and bovine fed specimens among the carriers of different 2b inversion karyotypes. The polymorphic behaviour of *A. arabiensis* in the Natal Province does not appear to be related to polytene chromosome polymorphism as is considered to be the case in West Africa. The populations of *A. arabiensis* investigated chromosomally in this study were found to be polymorphic for the 2b inversion system. The only other polymorphic inversion found was a 2bc heterozygote and only one was found in 68 preparations. These findings are in contrast to that found for this species in West Africa, where no fewer than eight floating inversions are commonly detected (Coluzzi et al. 1979). Mosha & Subra (1982), however, found no significant differences in the frequencies of 2b inversions between outdoor and indoor habitats, suggesting a random distribution of this inversion.

Studies on the chromosomal polymorphism of this species in West Africa show that it constitutes a single panmictic entity in all the localities studied, thus *A. arabiensis* is a single genetic population characterised by a remarkable degree of polymorphism (Toure' 1989). Sharp et al. (1990) (Chapter 3) found a correlated difference in the size

distribution of the diagnostic leg bandings (Coetzee 1986) between *A. arabiensis* from the DDT sprayed (Mamfene) and un-sprayed (Dondota) areas.

The basis of these behavioural and morphological differences in *A. arabiensis* populations in sprayed and un-sprayed areas requires further investigation. Only then may conclusions be drawn as to whether it reflects genetically determined variation or a species difference which is highlighted in the presence of DDT.

CHAPTER 9

DISCUSSION AND CONCLUSIONS OF THE STUDY

9.1 THE TRANSMISSION AND CONTROL OF MALARIA

Malaria vector control in the province of Natal started in the 1930's and the basis of these measures was largely the result of the extensive investigations by De Meillon, Ingram and Swellengrebel in the Republic of South Africa as a whole (Appendix 1). These measures were initially based on the concept of species sanitation as outlined by Swellengrebel (1931), measures aimed almost exclusively at the control of the larvae of the malaria vector mosquitoes. The effective control of malaria on a large scale, both globally and locally, started in the late 1940's and 1950's with the advent of residual insecticides e.g. DDT, BHC and dieldrin, and their intra-domiciliary application for the control of mosquito vector species. These measures, coupled with the availability of new, potent, inexpensive and relatively well tolerated drugs, which enabled control measures to be directed at the parasite as well, formed the basis of vertical malaria control programmes (Wernsdorfer 1980, Davidson 1982). These measures, aimed at the control of both the parasite and the mosquito vectors of the disease still remain as the primary measures for the control of the disease in the Republic of South Africa. This study

highlights the fact that since the extensive malaria research of the 1930's, which led to the later worldwide use of intra-domiciliary residual insecticides for the control of vector mosquitoes, and the entrenchment of the current control programme, there has been little research conducted on the dynamics of malaria transmission and its control in the Republic of South Africa.

Malaria transmission still occurs within the borders of Natal province, irrespective of the current measures aimed at the control of the disease. During the eight year period of 1976-1983 an average of 644 cases of malaria were reported annually in Natal province (range = 208-1324 cases per annum). During the following five years (1984-1988) this averaged figure increased to 3846 cases per annum (range = 2193-7530 cases per annum). The geographic spread and endemicity of the disease has changed somewhat in comparison to the situation prior to the introduction of the malaria control programme. Prior to the introduction of this programme, the three northern districts of Ingwavuma, Ubombo and Hlabisa were classified as endemic malaria areas, with seasonal epidemics occurring to the south and inland of these districts. This study indicates that very little transmission of the disease occurs outside these three northern districts which lie in the KwaZulu area of the Natal province. Data pertaining to local transmission of the disease, suggest that local transmission only occurred at two localities in the Natal areas during the study period, namely at Mtubatuba in 1987 and at Tongaat in 1988. Only 12.1% of the malaria cases detected in the Natal area of the

Natal province were classified as indigenous (1985-1988), with the majority of cases having been classified as imported (82-97% of the total number of cases), mainly from Mozambique.

The disease transmission pattern in the three northern districts of the KwaZulu area has changed from the historic classification of an endemic malaria area to an epidemic malaria area. The situation in 1930 was as expected for an endemic malaria area, with the highest percentage of infected carriers being infants and toddlers and with the percentage infection decreasing with age. In fact the severity of the disease was such, that toddlers were infected without exception (Swellengrebel et al. 1931). The current findings show the percentage infection in age categories to closely follow the population structure and is more representative of a population subjected to epidemic malaria. This change is considered to be a direct result of the longstanding control programme. As a result of the control programme the endemic malaria area is now considered to lie to the north, in Mozambique. The latter country has not had any formal malaria control programme in the region north of Natal Province for more than 10 years.

The distribution of monthly totals for those persons reporting to hospitals, clinics and general practitioners with malaria, is considered to reflect the malaria transmission season. These patients would mainly be non-immunes who as a result of infection developed clinical symptoms. From these

data the disease showed a distinct seasonal transmission cycle, starting in early summer and reaching a peak during the months of April and May with the detection of passive cases virtually ceasing in July. A finding in keeping with what is expected from an area that encompasses the most southern distribution in Africa of a mainly tropical disease.

The importance of active detection in the control of malaria in the Republic of South Africa is highlighted by the finding that the actively detected cases harbour a statistically significant, higher number of gametocytes than do the passively detected patients. The necessity of treating both the asexual and sexual components of a malaria infection are highlighted by these data. In the Natal and KwaZulu areas 87.9 and 68.2% of cases were respectively detected by active surveillance. The reduction of this reservoir of infection for the vector mosquitoes is central to the containment of the disease in this area. Prior to 1988 all actively detected malaria cases were treated by the respective health authorities in Natal Province with a combination of chloroquine + pyrimethamine (Daraclor). The chloroquine component of the treatment would have been effective against the asexual parasites of *Plasmodium falciparum* and the pyrimethamine which has a sporontocidal effect (Teklehaimanat et al. 1985), against the sexual stages in their development in the mosquito. Both drugs are, however, only effective against strains that have not developed resistance. Resistance of *P. falciparum* to chloroquine was first detected in the Natal Province in 1985

(Herbst et al. 1985) and studies completed by Freese et al. (1988) found 94% of the isolates tested to be resistant, with 74% only being inhibited at 32 pmol of chloroquine or greater, indicating RIII resistance (World Health Organisation 1984). An *in vitro* investigation by Freese & Miles (personal communication) of the sensitivity of 20 southern African isolates of *P. falciparum* to pyrimethamine, indicated that these strains were not fully susceptible. In February 1988 the authorities responsible for malaria control in the Natal Province, changed to the use of pyrimethamine-sulphadoxine (Fansidar) for the treatment of the asexual stages of *P. falciparum* infection, coupled with the use of primaquine as a gametocytocide (M. Short, personal communication).

Entomological investigations were carried out at three different localities during or shortly after the detection of indigenous malaria cases in these areas, and eight species or complexes of anopheline mosquitoes were caught. Based on the results of this study and on the scientific literature, the majority of these species could be classified as mainly zoophilic in their biting habits and were as a result of no direct medical importance. *An. arabiensis*, a primary vector of malaria at numerous localities in Africa, was, however, caught at all three localities and by implication it is considered that this species is the primary vector of malaria in the Natal Province. An investigation of the distribution of the *An. gambiae* s.l. in the Natal Province found three of the six

member species of the complex to occur commonly, namely; *An. arabiensis*, *An. merus* and *An. quadriannulatus*. Sympatric distribution of these three species was further found to be common. This study further found *An. arabiensis* and *An. quadriannulatus* larvae to occur in the same larval sites, a finding in keeping with that of le Sueur & Sharp (1988). The sympatric distribution and the contrasting importance of these cryptic species in malaria transmission (White 1974) highlights the necessity of specific species identification of the *An. gambiae* s.l. in the Natal Province.

An. merus has been implicated in the transmission of human malaria elsewhere in Africa (Chapter 3, Section 3.1.1.1) and its possible role in this regard in Natal Province could not be disregarded. Investigations were thus undertaken in all the areas where this species was known to occur and where indigenous cases of malaria had been reported. *An. arabiensis* was found to occur sympatrically with *An. merus* at these localities and therefore *An. merus* could not be implicated in the transmission of the disease in these areas. Behavioural studies of this species found it to be predominantly zoophilic in its host choice, a finding in keeping with studies completed elsewhere in Africa (Iyengar 1962, Gillies 1966). The majority of the homesteads in the rural malarious areas of Northern KwaZulu have cattle kraals in close proximity to the homestead and it is considered that these could serve as a form of zoophylaxis against malaria transmission by *An. merus*.

9.2 SPECIES IDENTIFICATION OF THE *ANOPHELES GAMBIAE* s.l. AND

THEIR CAPTURE.

In the *An. gambiae s.l.* species criteria are currently differences in chromosomal rearrangements as seen in the polytene chromosomes (Green 1981). Correlated identifications by polytene chromosome analysis and electrophoresis of the three species of the *An. gambiae s.l.* found in Natal Province verified the use of the biochemical key of Miles (1978, 1979) for the identification of these species.

Identification of the *An. gambiae s.l.* species using hind leg banding patterns was a potentially useful technique developed by Coetzee et al. (1982) and Coetzee (1986) for use by the field entomologist. This technique further forms the basis of a key for the identification of the *An. gambiae s.l.* species based on morphological characters and multivariate discriminant function analysis (Coetzee 1989). The finding of this study that only 56% of the *An. arabiensis* could be correctly identified on the basis of their hind leg banding patterns, seriously detracts from the validity of this technique. Thus detracting from the key of Coetzee (1989) for the identification of the *An. gambiae s.l.* species in Natal province.

The investigation of the relative capture efficiency of the *An. gambiae s.l.* species by seven trapping techniques found no trapping technique to be species specific. The experimental tent and exit traps, however, had several

advantages over the other techniques investigated in the capture of *An. arabiensis* in a DDT sprayed area.

9.3 THE BITING BEHAVIOUR AND CONTROL OF *ANOPHELES ARABIENSIS*.

Investigations of the biting and resting behaviour of malaria vector mosquitoes species combined with an appreciation of the parasitology of the disease has always been the foundation from which logical (and effective) intervention measures are formulated. The present control measures utilised in the Natal province were essentially formulated on the basis of research findings from the first half of this century. Due to the inter-specific and intra-specific variation in the biting and resting behaviour of the *An. gambiae s.l.* species (White 1974, Toure' 1989) it is essential that local studies are conducted into the dynamics of malaria transmission and the efficiency of the control strategies used. This study further investigated the man biting behaviour of *An. arabiensis* and the efficiency of control of this species by the intra-domiciliary DDT spraying programme.

There was a marked difference between the human blood index (HBI) of indoor resting *An. arabiensis* in the non-DDT and the DDT sprayed areas and between those caught resting indoors and those leaving the huts in the latter area. This variation in the HBI is consistent with the findings from studies elsewhere in Africa, that the species shows a wide range in behaviour between localities (White 1974). Studies

on the chromosomal polymorphism of this species in West Africa show that it constitutes a single panmictic entity in all the localities studied. Thus *An. arabiensis* is a single genetic population characterised by a remarkable degree of polymorphism (Toure' 1989). Evidence for genetic mediation of behaviour in *An. arabiensis* is provided by Coluzzi et al. (1977,1979) and Petrarca et al. (1987). In their studies behavioural traits were correlated to chromosomal arrangements. The polymorphic behaviour of *An. arabiensis* in the Natal Province does not appear to be related to polytene chromosome polymorphism as is considered to be the case in West Africa. All three populations of *An. arabiensis* investigated chromsomally in this study were found to be polymorphic for the 2b inversion system. The only other polymorphic inversion found was a 2bc heterozygote and only one was found in 68 preparations. These findings are in contrast to that found for this species in West Africa, where no fewer than eight floating inversions are commonly detected (Coluzzi et al. 1979). Coluzzi et al. (1977) found statistically significant correlations to exist between chromosomal inversion karyotype and behaviour. In contrast, in this study the majority of the inversions were fixed and no correlation between the 2b inversion karyotype and behaviour was detected.

Further studies on the relationship between biting behaviour and polytene chromosome polymorphism are obviously necessary to conclusively ascertain whether or not any direct relationship exists in southern Africa.

Should the behaviour of *An. arabiensis* in the unsprayed area be predominantly to feed on man and rest indoors, then it would appear to be ideally suited to control by intra-domiciliary residual insecticides. The finding at Mamfene, that a significantly higher percentage of the *An. arabiensis* caught leaving huts in comparison to those caught resting indoors, had fed on man, has serious implications in respect to the calculation of the HBI. If the HBI was based solely on those *An. arabiensis* caught resting indoors, the anthropophilic biting behaviour of the population would be grossly underestimated. If some individuals are consistently anthropophilic and others zoophilic, the calculation of the sporozoite rate could also be misleading if based solely on one mosquito catching method. It is now clear that in the DDT sprayed area (Mamfene) most of the man-biting *An. arabiensis* which enter houses, leave after feeding. This finding has important implications in regard to the efficacy of control of this component of the *An. arabiensis* population. Furthermore the observation of this trend in non-DDT sprayed huts, suggests that this leaving behaviour might not solely be a result of DDT irritation.

The overall survival of mosquitoes caught leaving huts 1-3 months after DDT spraying was not markedly different to that of those caught leaving huts 8-12 months after application. The survival of mosquitoes varied according to the fed status of the mosquito and depending on whether the hut was not sprayed, replastered or fully DDT sprayed. The percentage of bloodfed mosquitoes that were caught leaving

showed an inverse relationship to the amount of DDT in the hut and suggests that the presence of DDT hampered the taking of a bloodmeal. The percentage survival of bloodfed mosquitoes caught in exit traps fitted to DDT sprayed huts (72.9% from huts 1-3 months after application and 88.2% from huts 8-12 months after spraying) compares well with similarly treated data collected by Haridi (1972) who found an 88% survival.

Bioassays performed in DDT sprayed huts indicated minimal loss of effectiveness of the DDT deposits over time in killing *An. arabiensis*. In fact, 8-12 months after application an exposure time of 15 minutes was sufficient to effect 100% mortality. Dose mortality curves further showed no marked increased vigour tolerance or physiological resistance to DDT in wild *An. arabiensis*, with 100% mortality occurring at a discriminating dosage of 4% DDT. Overall, a higher percentage of *An. arabiensis* were leaving huts than were resting inside. The mean number caught leaving replastered huts compared to control huts was reduced by 19% and in DDT sprayed huts by 52%. Assuming random entry of huts, this reduction in exit trap catches is considered to represent the numbers killed indoors.

These findings have implications for the malaria control programme. The inverse relationship between the percentage of mosquitoes bloodfed and presence of DDT in a hut clearly indicates behavioural avoidance. The replastering of the inner walls of huts by the inhabitants, for social and

practical reasons, is a further impediment to control of *An. arabiensis* by house spraying with DDT; the percentage of bloodfed *An. arabiensis* caught leaving these huts and surviving was high. Surveys in the area subjected to the DDT vector control programme showed that the percentage of huts replastered varied from 1.7 to 72% at different localities and times (K. Newberry personal communication). The exit trap survival data indicate that there was a 20 to 24% greater survival of mosquitoes leaving replastered huts than from DDT sprayed huts. A reduction in the replastering of huts should therefore result in more efficient vector control.

The HBI of indoor resting caught *An. arabiensis* from both the DDT sprayed area (Mamfene) and the unsprayed area (Dondota) is in close agreement to the data from studies done elsewhere in Africa (Chapter 8, section 8.4, Table 38). In the majority of the populations studied in Africa (nine out of ten studies) in areas where there was no intra-domiciliary insecticide spraying programme, in excess of 82% of feeding was on man. The results from the four studies performed at localities subjected to a spray programme are markedly different to the data from the unsprayed localities. The majority of the indoor resting caught specimens in sprayed localities had fed on cattle.

The morphological difference (leg banding) and behavioural differences (HBI) found between *An. arabiensis* populations from both the sprayed and unsprayed areas may be due to *An. arabiensis* being more than one species, that do not exchange

genes under natural conditions, or due to different selection pressures occurring in insecticide sprayed and non-sprayed areas acting on morphs of a behavioural polymorphism within one species. From both studies completed elsewhere in Africa and those reported here, it seems that these behavioural differences in *An. arabiensis* are not confined to the Natal region. Due to the importance of *An. arabiensis* in malaria transmission in this continent it is essential that further studies be undertaken to elucidate these apparent anomalies in the behaviour of this species.

The data presented strongly suggest that optimal control of *An. arabiensis* in Natal Province will not be achieved using the current control strategy of the annual intra-domiciliary application of DDT. To increase the effect of vector control in the study area would require an integrated approach utilising additional measures. In this respect the use of alternative insecticides which might have a reduced irritant effect on the vector species warrants investigation.

Further research is, however, essential to ascertain whether the pronounced hut leaving behaviour of *An. arabiensis* is the result of an irritational effect of DDT on the mosquito or due to genetic selection or a combination of both. The evaluation of alternative insecticides in the control of *An. arabiensis* should indicate whether the hut leaving behaviour shown by the species is due to an irritational effect of the DDT, or due to genetic selection for a particular

behavioural polymorphism.

This study also reveals that *An. arabiensis* does bite man outdoors. This has obvious implications for the control of the species and the interruption of malaria transmission. However, further investigations are, however, necessary to ascertain the amount of outdoor biting of man by this species and the contribution made by this outdoor biting in comparison to indoor biting, to malaria transmission in the region.

APPENDIX 1

SUMMARY OF STUDIES COMPLETED ON MALARIA (1900-1989)

The effectiveness of a malaria control programme is largely reliant on local knowledge of the disease and the factors effecting it's transmission. Therefore a brief summary is included of relevant studies published in refereed scientific journals on malaria case rate, mosquito vectors, transmission and control of the disease within the borders of the present day Republic of South Africa (including the Transkei, Bophutetswana, Venda and Ciskei).

Hill & Haydon (1905) reported on the malaria epidemic of 1905 in Durban and the surrounding area. Entomological investigations showed two species of anopheline to be present and salivary gland dissections found *Pyretophorous costalis* (*Anopheles gambiae* s.l.) to be sporozoite infected.

Pratt-Johnson (1918) reported on the distribution of malaria in South Africa. Compiled mainly from the reports of government medical officers, health officials and his own experience in military hospitals, the study included a mosquito survey of military hospital areas.

Ingram & De Meillon (1927): A comprehensive survey of mosquitoes was carried out in both the Transvaal and Natal with special reference to the carriers of malaria and their control.

Swellengrebel, Annecke & De Meillon (1931): A comprehensive study on the larval sites, adult biting behaviour and vectorial importance of *An. gambiae* s.l. and *An. funestus* was completed in the Transvaal and Natal. On the basis of this data the concept of species sanitation was introduced. An epidemiological study of the clinical severity of malaria in different race groups was carried out in both a highly endemic and an epidemic malaria area.

De Meillon (1930a) reported on the collection of *An. funestus* s.l. found resting in smoke filled rural huts.

De Meillon (1931) published notes on the larvae of some anophelines caught in the Transvaal.

De Meillon (1931) published keys to the fourth instar larvae and adults of South African anophelines.

De Meillon (1933) reported on collections made of *An. funestus* in the Transvaal.

De Meillon (1934): The eggs and pupae of some South African anophelines from the Transvaal are described.

De Meillon (1934a): The malaria vectors *An. gambiae* s.l. and *An. funestus* s.l. were investigated in the Transvaal. This investigation related the severity and distribution of malaria in the Transvaal to that of the insect vector, and

showed how the distribution of the latter was in turn a function of the climate.

De Meillon (1936a): Indoor resting by *An. funestus* s.l. and *An. lesoni* was investigated in the Transvaal. The latter species was rarely found indoors in contrast to *An. funestus* s.l.. *An. funestus* was also found to have a 12% sporozoite rate.

De Meillon (1936) summarised his experiments completed in Natal, which showed that anti-adult measures were a more effective form of mosquito control than anti-larval measures and further only cost about a third as much.

Park-Ross (1936) reviewed the malaria control measures in Natal and their organisation for the six year period 1929-1930 to 1934-1935.

De Meillon (1937) reported on the reaction of *An. gambiae* s.l. and *An. funestus* s.l. to certain environmental factors. Experiments were conducted to assess the effects of light, humidity and temperature on these species in the fed and unfed state.

De Meillon & Gear (1939) reported on the detection of several cases of malaria from the Witwatersrand. The potential for transmission of the disease in the area and the factors affecting it were discussed.

De Meillon (1947) published the first comprehensive volume

on the systematics and biology of the Anophelinae of Africa south of the Sahara (Ethiopian Zoogeographic Region).

W.H.O. (1955): This mimeographed document, very briefly, documents information on the malaria control programme in the Transvaal for the period 1950-1954.

Morsbach (1960) investigated the efficiency of both DDT and BHC treated huts for vector control as a result of continued malaria transmission in northern Natal. He concluded that DDT was less efficient than BHC for intra-domiciliary vector control as it was irritating *An. gambiae s.l.* and that a study utilising exit traps was necessary to confirm this finding.

Paterson et al (1964): Specimens of *An. gambiae s.l.* were collected in Mozambique, Swaziland and Natal. They documented saline breeding records for *An. merus* at distances up to 75 miles from the sea and found sympatric distribution between this species, *An. gambiae s.s.* and *An. quadriannulatus*.

Gilliland (1974) discussed administrative and financial aspects of malaria control in the Republic of South Africa and the role of the Anneck Institute at Tzaneen.

Nethercott (1974) reviewed the prevalence of malaria and the history of its control in the Natal coastal belt from the 1930's.

De Meillon et al (1977) reported on taxonomic and behavioural observations that were made on *An. aruni*. Artificial infection with *P. falciparum* found the species to be receptive to infection. It's role as an incidental vector of *P. falciparum* was discussed.

Smith et al. (1977) reported on the current situation in regards to the prevalence of malaria in the Transvaal, it's transmission and control.

Miles (1978) as part of a study on enzyme variation in the *An. gambiae* group of species, chromosomally identified *An. arabiensis*, *An. quadriannulatus* and *An. merus* from the Republic of South Africa.

Theron (1978) observed swarming by *An. merus* at Lake St. Lucia, Natal.

Coetzee & Cross (1983): Cross mating studies between *An. merus* from Northern Natal and the Transvaal coupled with chromosomal investigation showed the two allopatric populations to be conspecific.

Green & Hunt (1980): Produced a photomap of polytene chromosomes from ovarian nurse cells of *An. funestus* and suggested a new system of arm designation. Comparisons in terms of fixed and floating inversions were made between *An. funestus*, *An. parensis* and *An. aruni*. The relationship of fixed inversion differences and speciation events was

discussed in the light of homosequential species.

Coetzee, Newberry & Durand (1982) reported on the use of the banding patterns on the hind legs to separate *An. gambiae s.s./An. arabiensis* from *An. merus/An. quadriannulatus*.

Cross & Theron (1983) recorded the occurrence of *An. merus* breeding at a locality in the Transvaal, 300km from the sea.

Miles, Green & Hunt (1983) published a photomap of the ovarian polytene chromosomes of *An. pharoensis*. Based on polytene chromosome arrangements they found that *An. pharoensis* from most parts of Africa, had different X-chromosome arrangements to populations from Zululand.

Muspratt & Henning (1983) reported on the breeding of *An. merus* in a salt marsh in the Hans Merensky Nature Reserve, Transvaal. A locality approximately 350 km from the coast and approximately 600m above sea level.

Sharp (1983a): The biting cycle of *An. merus* was investigated under optimal conditions of wind, rain and temperature and compared well with that found for the *An. gambiae s.l.* elsewhere in Africa. Wind, rain and temperature were shown to effect the biting cycle.

Sharp, Quicke & Jansen (1984) reported on a mosquito outbreak due to an irrigational overflow in the endemic malaria area of Natal and recorded the presence of five

anopheline species and aspects of their behaviour.

Spracklen (1984a & b): a/ Guidelines were presented to assist the prescriber in the chemoprophylaxis of malaria. b/ Guidelines for the diagnosis and treatment of chloroquine-resistant malaria were outlined.

Herbst, Taylor & Joubert (1985) collected blood samples from 77 patients with *Plasmodium falciparum* malaria resident in the Natal/KwaZulu area. Six of these patients were found to have chloroquine resistant malaria using the *in vitro* microtechnique.

Hunt & Coetzee (1986) described techniques for: a/ the cryopreservation in liquid nitrogen of anopheline mosquitoes for the later removal and preparation of ovarian polytene chromosomes b/ the preservation of the wings, legs and palps of these mosquitoes for taxonomic studies.

De Meillon (1986) reviewed malaria and its control in the Republic of South Africa with special reference to the contribution made by the staff of the South African Institute Of Medical Research.

Sharp *et. al.* (1987) as part of a study on anthropophilic mosquitoes and arboviruses documented the capture of *An. tenebrosus* and *An. merus* in a man-baited net at Richards Bay, Natal.

Herbst, Taylor & Joubert (1987) collected blood from 110

patients infected with *Plasmodium falciparum* in Natal. Eighteen were found to be resistant to chloroquine by *in vitro* tests.

Herbst, Taylor & Joubert (1987a) reported on the reappearance of *Plasmodium ovale* which is responsible for one of the relapsing forms of malaria, in Natal. They reported the prevalence to vary between 2.63% and 6.56% during the period 1984-1987.

Sharp *et. al.* (1988): Data on the prevalence and distribution of malaria cases from the Kwazulu areas of Natal for the 10 year period 1976-1985 were analysed.

Strebel *et. al.* (1988) reported on the geographic distribution of malaria in South Africa in 1986.

Coetzee & Le Sueur (1988) reported on the effects of salinity on the larvae of five Afrotropical anopheline species.

Freese *et. al.* (1988a) described a technique successful in the *in vitro* establishment and maintenance of South African isolates of *Plasmodium falciparum*.

Freese *et. al.* (1988): In 1987 and 1988 the chloroquine sensitivity of 39 isolates from the Ubombo and Ingwavuma districts of KwaZulu were determined using an *in vitro* test. Of these isolates 95% were found to be resistant to

chloroquine.

Le Sueur & Sharp (1988): The distribution and certain chemical and physical characteristics of the breeding sites of three species of the *An. gambiae* complex were investigated in Northern Natal.

Sharp et. al. (1989) investigated the value of hind leg banding patterns in the identification of *An. gambiae* complex species. The study found a significant difference between the size distribution of this character in two populations of *An. arabiensis* from Natal.

Coetzee (1989) published a key using morphology and multivariate analysis for the discrimination of four members of the *Anopheles gambiae* group in southern Africa.

APPENDIX 2

ANOPHELES GAMBIAE COMPLEX SPECIES DISTRIBUTION RECORDS FOR NATAL PROVINCE, REPUBLIC OF SOUTH AFRICA.

Locality	Species	Reference
Chatsworth 30°57'E, 30°48'S	<i>An. quadriannulatus</i>	Sharp
Buwensi 32°11'E, 27°12'S	<i>An. quadriannulatus</i>	le Sueur & Sharp 1988
Dondotha 31°58'E, 28°34'S	<i>An. arabiensis</i>	Sharp
Fanies Island 32°24'E, 28°06'S	<i>An. merus</i>	Theron 1978, Sharp 1983
Guyoni 31°28'E, 29°05'S	<i>An. merus</i>	Sharp 1983
Kirkwood Farm 32°09'E, 28°26'S	<i>An. quadriannulatus</i> <i>An. arabiensis</i>	Sharp & Eckard Sharp & Eckard
Kosi Bay 32°52'E, 26°54'S	<i>An. merus</i>	Sharp 1983
Kwamsame 32°10'E, 28°24'S	<i>An. arabiensis</i> <i>An. quadriannulatus</i>	Sharp & Eckard Sharp & Eckard
Lumbongwenya 32°12'E, 27°51'S	<i>An. quadriannulatus</i>	le Sueur & Sharp 1988
Makanies Drift 32°15'E, 27°00'S	<i>An. merus</i>	Sharp
Mamfene 32°15'E, 27°22'S	<i>An. arabiensis</i> <i>An. quadriannulatus</i>	Sharp, le Sueur & Sharp 1988 Sharp et al. 1984
Mandlankunzi 32°23'E, 26°54'S	<i>An. quadriannulatus</i>	Sharp
Mfekayi 32°15'E, 28°11'S	<i>An. arabiensis</i> <i>An. quadriannulatus</i>	Sharp Sharp
Mtubatuba 32°09'E, 28°24'S	<i>An. arabiensis</i> <i>An. quadriannulatus</i>	Sharp Sharp
Ndumu 32°19'E, 26°54'S	<i>An. quadriannulatus</i>	Sharp
Nibela 32°27'E, 27°50'S	<i>An. quadriannulatus</i>	Sharp

Locality	Species	Reference
Nkunduse 32°20'E, 28°05'S	<i>An. arabiensis</i> <i>An. merus</i>	Sharp Sharp 1983
Numaneni 32°16'E, 26°58'S	<i>An. arabiensis</i> <i>An. quadriannulatus</i>	le Sueur & Sharp 1988 Sharp le Sueur & Sharp 1988
Nyamithi Pan 32°18'E, 26°48'S	<i>An. merus</i>	Paterson 1964 Sharp 1983
Ophansi 32°18'E, 27°34'S	<i>An. merus</i> <i>An. arabiensis</i> <i>An. quadriannulatus</i>	Sharp, le Sueur & Sharp 1988 Sharp Sharp
Qhubu 31°58'E, 28°51'S Sharp	<i>An. arabiensis</i> <i>An. quadriannulatus</i> <i>An. merus</i>	Sharp Sharp Sharp 1983,
Reservoir Hills 30°57'E, 30°48'S	<i>An. quadriannulatus</i>	Sharp
Richards Bay 1988; 32°02'E, 28°47'S	<i>An. merus</i>	Sharp 1983, Eckard et al. 1988
Sihangwane 32°33'E, 27°03'S	<i>An. arabiensis</i> <i>An. quadriannulatus</i> <i>An. merus</i>	Sharp Sharp Sharp 1983
Tongaat 31°06'E, 29°34'S	<i>An. arabiensis</i>	Sharp & Eckard
Tugela River Mouth 31°30'E, 29°13'S	<i>An. merus</i>	Sharp 1983

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