A STRATEGY FOR PERSONAL AND COMMUNITY PROTECTION AGAINST THE VECTORS OF

MALARIA IN PAPUA NEW GUINEA WITH EMPHASIS ON THE EVALUATION OF BEDNETS

IMPREGNATED WITH PERMETHRIN

by

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ABSTRACT

Malaria is a serious disease in Papua New Guinea and causes significant morbidity and mortality. The objective of this study was to identify, implement and evaluate a strategy for protection against vectors of malaria in a lowland village in Papua New Guinea. The emphasis of the study was on the evaluation of bed nets treated with the synthetic pyrethroid, permethrin (3-phenoxy benzyl ($\pm$) cis, trans, 2-dimethyl-3-(dichlorovinyl) cyclopropane carboxylate).

Entomological evaluation of the strategy was made by comparing densities, delayed oocyst rates and parous rates of malaria vectors collected in two geographically isolated villages, both using mosquito nets, the one set impregnated, the other not. The results did not demonstrate any effect of bed net impregnation on the vector population.

After bed nets were impregnated in August 1984, two malariometric surveys were conducted in November 1984 and April 1985. Parasitological and clinical evaluation was made by comparing malariological indices from the experimental and control populations. Indices were lower in the population with impregnated bed nets, and the age-specific prevalence of *Plasmodium falciparum* indicated that the effect was greatest in the 1-4 years age group. Gametocyte rates of *P. falciparum* for all ages combined were lower in the experimental population after bed net impregnation and the difference was significant in survey 3. The percentage of enlarged spleens in the 2-9 years age group were significantly different in the November survey but not in the April survey. Bioassays to test the duration of effectiveness of permethrin indicated that excellent residual activity can be expected for at least six months. The activity of permethrin on the vectors is probably through non-lethal effects such as repellency or biting inhibition.

Bed nets impregnated with permethrin offer increased protection as people sleep under them. Because young children spend most of the night under bed nets, they receive the greatest benefit from their use. The importance of these results in an area of intense malaria transmission is discussed.

ACKNOWLEDGEMENTS

DEDICATION

To my parents, for their support and encouragement throughout my studies.

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INTRODUCTION

1.1 Global Malaria Control

Human malaria is caused by one or more of four species of protozoa[1]
belonging to the genus *Plasmodium*. The parasites are transmitted to man by
female mosquitoes of the genus *Anopheles*. (Mosquitoes acquire the parasites from
infected humans and the life cycle is thus characterized by an alternation between
the intermediate host, man, and the final host, mosquito. A diagramatic
representation with a generalized description of the life cycle of *Plasmodium* is
given in Figure 1.)

In a community, the existence of malaria depends on the presence of three
epidemiological factors; the **parasite**, the **vector** and the **susceptible human being**.
Through effective action on any of the three factors, malaria can theoretically be
decreased or eliminated. On an individual basis, a **susceptible human being** can be
prevented from acquiring an infection and the **parasite** can be eliminated from the
human being through drug prophylaxis or drug treatment. Action on these two
factors resulting in reduced prevalence or elimination of the disease becomes much
more complex and difficult, however, when dealing with large populations of human
beings. Total elimination of the parasite from large communities by the
administration of drugs has repeatedly been shown to be impossible, and the
prevention of infection in susceptible human beings through the mass administration
of drugs has proven to be impractical (Gabaldon 1978). Since the interdependence
of the three factors became known at the turn of the century, much of the malaria
contol effort has been directed at the third factor, the **vector**. Anti-vector measures
of exclusion, larviciding, and source reduction proved to be of benefit in
semi-tropical and temperate areas where malaria was unstable,[2] but the same
benefits were not realized in tropical areas of stable malaria. With the discovery of

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[1]The systematics of malaria is a matter of opinion and the parasite is often
classified as belonging to the Phylum *Sporozoa.*

[2]Malariological terms are defined in Appendix A.

Figure 1: The generalized life cycle of human malaria
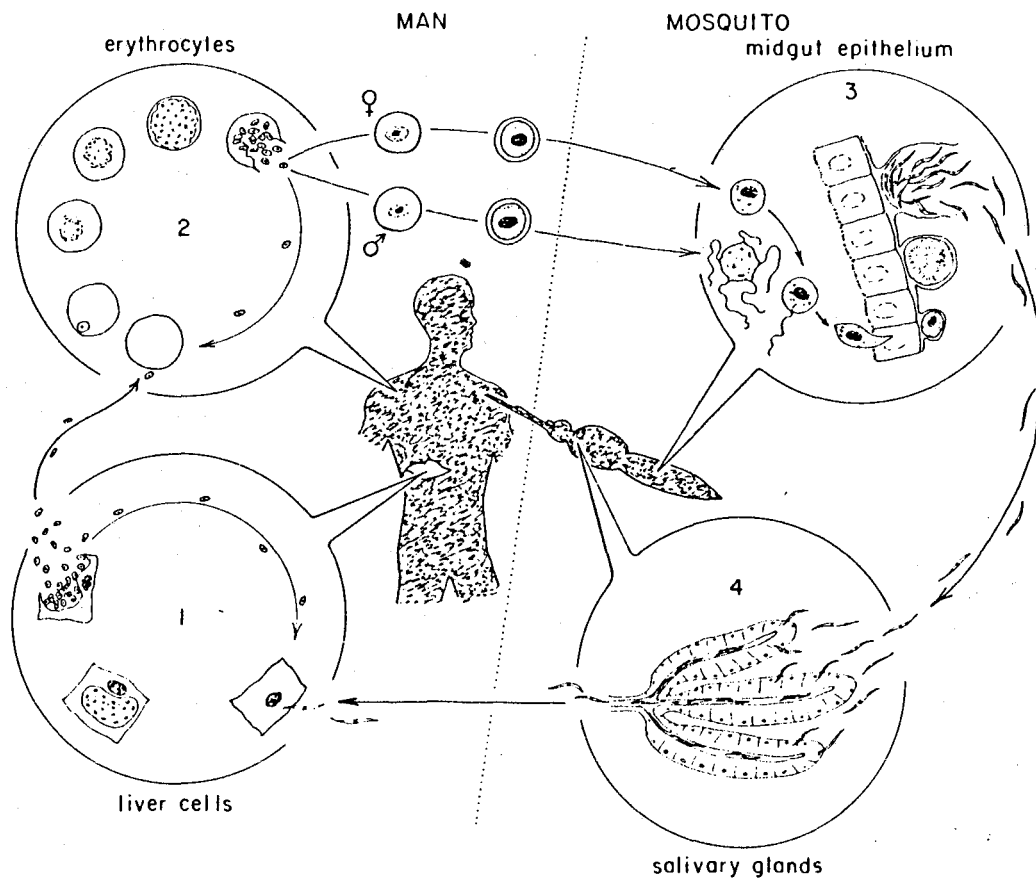
Life cycle of human malaria.

Man Cycle

1. Sporozoites are introduced into man from salivary glands of mosquito in act of biting (arrow from *4* to *1*) and, *1*, sporozoites enter parenchyma cells of liver to establish primary exerythrocytic schizogony. In man there is only this single cycle in *falciparum* malaria; in malaria and various Haemosporidia of man and other animals secondary cycles are characteristic.

2. Merozoites are formed and invade (arrow from *1* to *2*) erythrocytes (red blood cells) where the developing stage is called a trophozoite. The trophozoite grows, destroys its erythrocyte to release merozoites which enter more erythrocytes to continue erythrocytic cycles. Micro (male) and macro (female) gametocytes are also periodically released from erythrocytes and, when ingested by an *Anopheles* mosquito (arrows from *2* to *3*), the microgametocyte divides to form motile microgametes.

Mosquito Cycle

3. A microgamete enters a macrogamete to form a zygote that becomes a motile ookinete. Ookinetes pass through the midgut epithelium to form oocysts that grow, burst, and release sporozoites into the hemocoel (arrow from *3* to *4*).

4. Sporozoites are active, pass freely throughout body cavity, and concentrate in salivary glands where they can be introduced into blood of vertebrate in act of feeding.

the residual insecticidal properties of DDT, a new and very effective strategy, referred to by Gabaldon (1978) as *vector interception*, was adopted. The strategy of killing infected mosquitoes in the days following an infected meal, and before they became able to transmit the parasite, was so effective that the worldwide eradication of malaria seemed a realistic goal. During the 1950's and 1960's considerable advances were made against malaria, however, the goal of eradication proved to be unrealistic and during the 1970's there was a serious global resurgence of malaria (Farid 1978). Among the many problems which led to the deterioration of the malaria situation around the world, the ability of the malaria parasites and the mosquito vectors to acquire resistance to the chemicals used against them, was, and remains the most serious problem.

1.2 Malaria Control in Papua New Guinea

The first person to record the prevalence of malaria among the indigenous peoples in Papua New Guinea was the Russian scientist and humanitarian Nikolai Miklouho-Maclay (Webster 1984). His recently translated diaries (Sentinella 1975) give a fascinating account of the years he spent during the 1870's and 1880's among the the people of the Rai coast (Figure 3), and of the struggle with his most formidable enemy, malaria.

During the colonization period from the early 1900's to the second world war, there were few control measures directed against the vectors of malaria in Papua New Guinea (PNG). There was some reported success at controlling mosquitoes in towns by source reduction and through the introduction of *Gambusia* fish (Parkinson and Tavil 1973), but, it is apparently not known whether this had any effect on the transmission of malaria. Vector control designed to protect the rural indigenous population from malaria began in the 1950's following the apparent success of a pilot project using residual DDT in 1957 at Maprik (Peters and Standfast 1960) (Figure 2, lower). Indoor residual spraying with DDT was then adopted as the

primary method of malaria control. From the late 1950's to the 1970's, often referred to as the malaria or DDT eradication era, the history of malaria control in PNG paralleled that of the global history of the disease. Farid (1978) describes the latter as a period of "euphoria to anarchy", but the description is not quite appropriate for PNG because the achievements during the 1960's were never so dramatic. In Papua New Guinea as in many areas of tropical Africa, it has always been recognized that although residual spraying could be of benefit, the interruption of malaria transmission was not a realistic objective.

A chronology follows, outlining some of the important events from the initial experiments with DDT to the suspension of the indoor residual DDT spray program, a recommendation recently made to cover most parts of PNG (Liang and Thevasagayam 1985).

1945   Populations of anophelines were reduced by using a DDT and kerosene mixture as a residual spray, but there was only a small reduction in the amount of malaria (Bang et al. 1945). Following this trial, it was generally believed that because the vectors did not rest indoors, a DDT residual spray program would not be successful (Gunther 1974).

1956   On subsequent observations that some vectors did rest indoors, a pilot project was started to test the effectiveness of residual spraying in a holoendemic area. Transmission of malaria was not interrupted but it was suggested that in most of Papua and New Guinea, residual spraying alone would accomplish control. (Peters and Standfast 1960)

1969   The residual spray program expanded so that by 1969 50% of the population at risk was benefiting from some degree of protection. It was known at this time that the program was becoming unpopular with the villagers (Parkinson and Tavil 1973).

1973   Following a period of stabilization and in consideration of difficulties within the program, it was recognized that in spite of the drawbacks, residual spraying of DDT was the most effective, economical and safe method of malaria control. Control of the disease was the objective of the program with the ultimate objective being eradication, as part of the World Health Organization's global strategy of malaria eradication.

1977   Due to financial constraints the spraying area was reduced by half. Results from a study in Madang Province suggested that transmission was, to some extent, seasonal, and that the two-cycle residual spray should be abandoned in favour of a one-cycle residual spray in certain areas of the country (Afifi et al. 1980).

1979   From 1977 to 1979 there was a further deterioration of the malaria control program at all levels. In an evaluative report of the control program in specific areas of the country, Liang and Thevasagayam (1985) suggested that some of the problems were: inadequate spray coverage, the construction of the houses and the outdoor activity of the people

associated with the outdoor biting behavior of the vector. It appeared that residual DDT had little or no effect on malaria prevalence in sprayed areas when compared with non-sprayed areas.

1984   Following a review of the malaria control program in 1983, Liang and Thevasagayam (1985) recommended suspension of DDT residual house spraying which had been the only control measure. This applied to areas of high transmission (holo- and hyperendemic areas) and exceptions were made for areas of unstable malaria and of economic importance.

Reasons for failure of the program in PNG are often cited and well known. As with many malaria control programs around the tropical world based on residual DDT, there was initially much enthusiasm, a good funding base and well-trained malariologists. This situation began to deteriorate worldwide, concurrent with the emergence of the very serious technical problem of vector resistance to DDT. In PNG, however, physiological resistance to DDT has never been demonstrated (Liang and Thevasagayam 1985). Although there was always controversy over what could be expected from DDT residual spraying (Gunther 1973), it was well known that high standards of DDT coverage would be necessary if any degree of control was to be expected. The high standards of spray coverage required, were probably rarely met with any degree of regularity. Along with the inability to deliver a program of high standard because of financial and logistical difficulties, there arose a growing resentment to residual spraying by the people. Complaints that DDT caused rapid deterioration of roof thatch were apparently true. Bourke (1973), found that DDT on thatch killed the parasites and predators of the pyralid moth, *Herculia nigrivitta* Walk., while the moth larvae avoided the residues because of their feeding behavior. There were other complaints of cats being killed, and increases in bedbugs, *Cimex hemipterus* F., body lice, *Pediculus humanus humanus L.*, and mealybugs, *Planococcus discoreae* Williams, on yams in storage houses. Although these side effects were not substantiated (Bourke 1973) it was clear that whether true or not, the association was being made with DDT. The combination of high cost, apparent ineffectiveness, and unpopularity amongst villagers made it inevitable that a new strategy be adopted with much less reliance on DDT residual spray.

In Papua New Guinea as in the rest of the malarious world, the response to these problems has been a reorientation of strategy from one relying on individual

methods of control through vertically administered programs, to one of integrated control through local, decentralized programs. With the transfer of health care and vector control activities to the village level, the participation of the community is recognized as being indispensible to the approach (WHO 1978). In collaboration with the PNG Malaria Control Program, the World Health Organization and the Papua New Guinea Institute of Medical Research (PNG IMR) this pilot project was undertaken to study alternative methods for individual and community protection against the vectors of malaria.

THE STUDY AREA

2.1 Features of the Study Site

2.1.1 Geography and Vegetation

Papua New Guinea, the eastern half of the island of New Guinea in the southwestern Pacific, is situated north of Australia between $1°$ and $12°$ S latitude (Figure 2). The country was previously the Australian Territory of Papua and the United Nations Trust Territory of New Guinea, until it gained its independence in 1975. The national census of 1980 reported a population of 3.7 million. Of these, 87% are considered rural, with roughly one half living in the highlands and one half in lowland areas.

Madang town is located centrally on the north coast (Figure 2) at $5°$ 15' S latitude and is the administrative center of the Madang province. In 1979, the Papua New Guinea Institute of Medical Research (PNG IMR) established a multi-disciplinary malaria research program based in an area of approximately 400 km^2 around Madang town. The PNG IMR study area includes a population of about 16,500 from 72 rural villages within a 22 km radius of Madang (Figure 3). The investigation reported here, was conducted in a group of eight dispersed hamlets collectively known as Gonoa which are outside and to the immediate S of the PNG IMR study area (Figure 4).

Gonoa is 25 km SW of Madang and 7 km inland from the Pacific ocean. The area borders on the Bagasin foothills, an extension of the Finisterre Range of mountains, and is characterized as sub-coastal alluvial plain. A major geomorphological feature of the area is the Gogol river valley which rises from the coastal plain to 150 m. The valley supports a well drained alluvial rainforest but as it merges with the coastal plain, the low relief results in frequent flooding during the wet season. In the Gonoa area, drainage is generally SW to NE, from the higher relief to the sago-palm swamps surrounding the hamlets. From the swamps, many small streams dissect the area feeding into larger rivers which eventually discharge into the Gogol flood plain.

Figure 2:   Map of Papua New Guinea and its location relative
             to other countries of the south west Pacific

Phillipines

Malaysia

Papua New Guinea

Indonesia

Australia

Pacific Ocean

Madang Provincial
Boundary

Indonesia

Sepik River

Madang

Fly River

Port
Moresby

0   100   200
Km

Coral Sea

Australia

8b
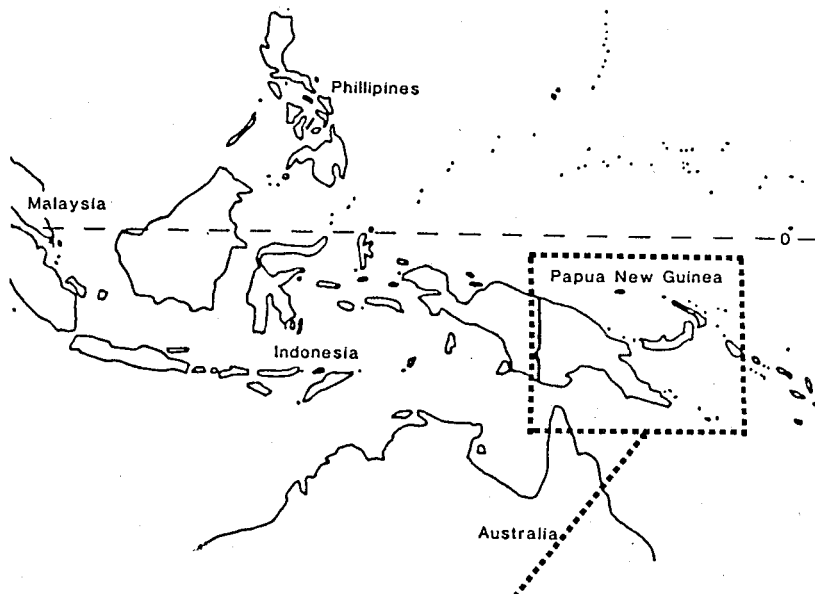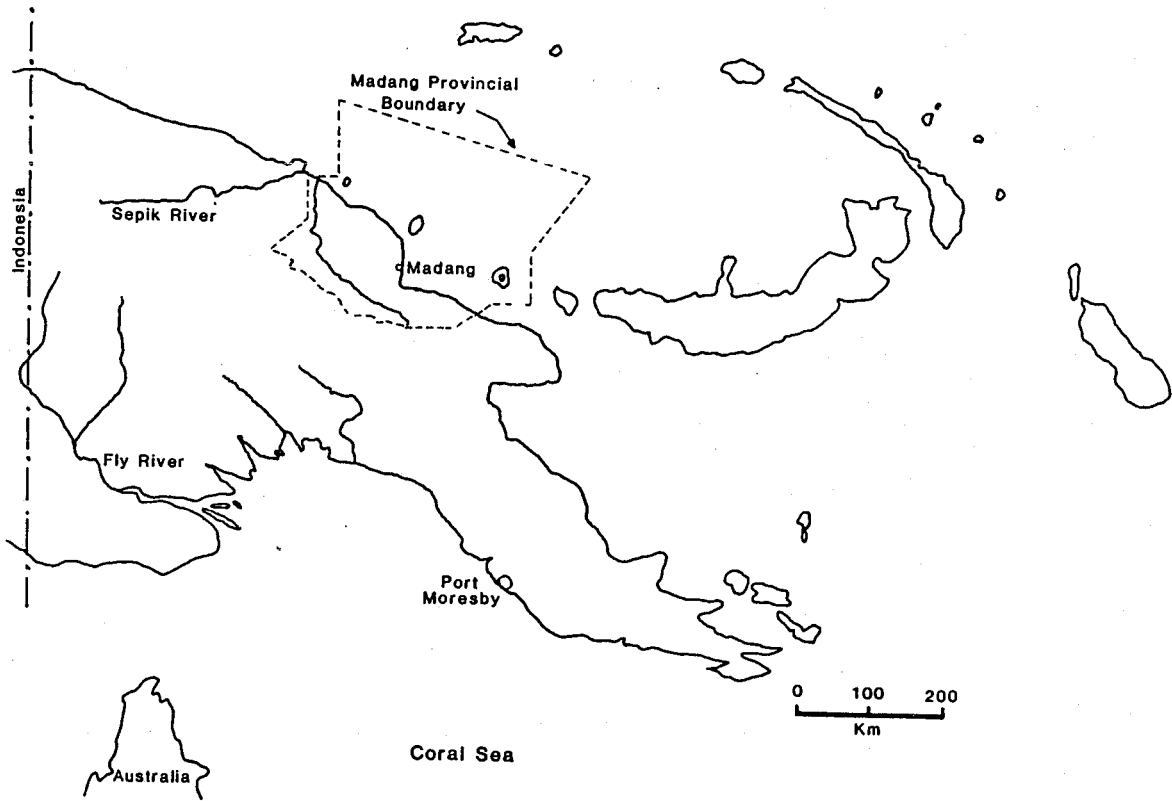
Figure 3:   Map of the area surrounding Madang town

PNG IMR Study Area

Madang

Gogol River

N

Tadup

GONOA

Maraga

0   km   10

Naru River

Rai Coast

Finisterre Range

9b
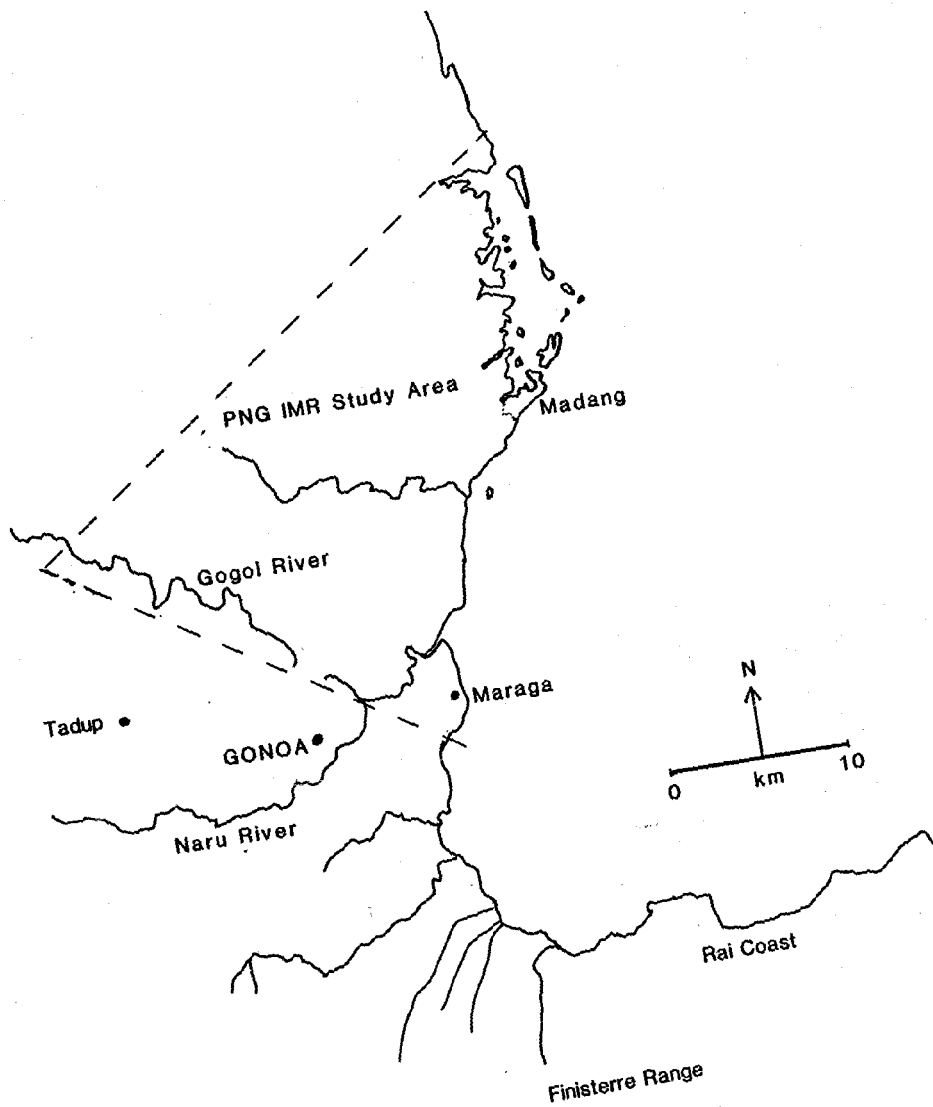
Figure 4:   Map of Gonoa showing the location of the different hamlets in the study area

LEGEND

rainforest

secondary growth

hamlets ■

hills ▲

aid post ☆

Naru River

Goro River

1

2 ☆

4

5

3

9

7

8 ☆

0 ▬▬▬▬▬▬ 1
Km

10b
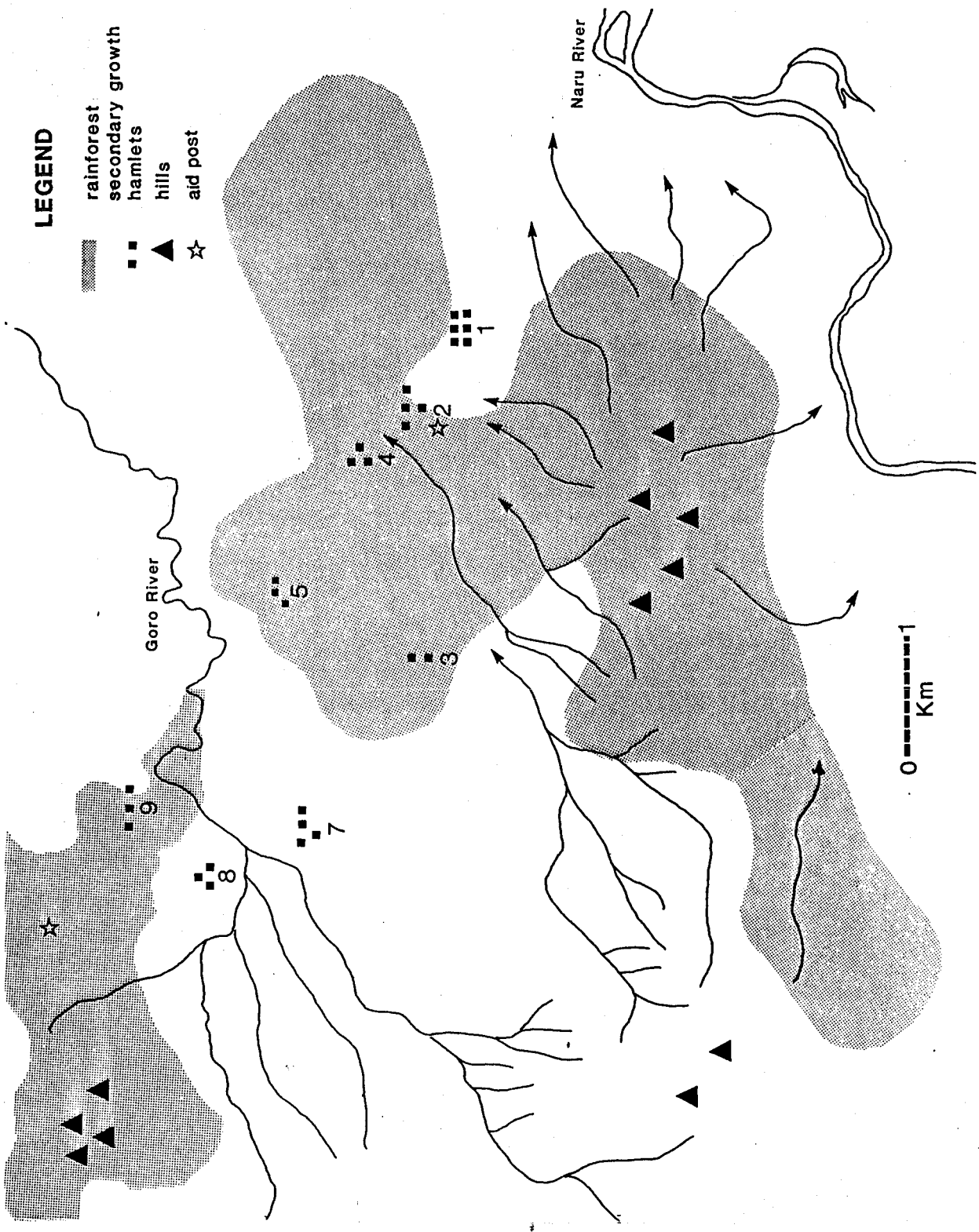
The two vegetation zones characteristic of the Gogol valley, i.e. the swamp forest and the mixed lowland forest, are both represented in the immediate area of Gonoa. Johns (1977) descibes these vegetation zones as follows:

Tropical Swamp Forests include a mosaic of communities varying from tall, well-drained mixed forest of alluvial deposits which are occasionally flooded to extensive areas of seasonally inundated *Metoxylon sagu*, the sago palm, and *Pandanus* swamps. It has a high canopy at 35m with usually no one species dominating. As the site becomes wetter, sago palms may form a distinctive sub-canopy in the forests...

Mixed Lowland Tropical Rainforest remains the most complex and least understood of the earth's vegetation types. The forests are very diverse in composition and various species can attain local dominance for ecological reasons not yet fully understood. Throughout the lowlands, the forests have been extensively destroyed or modified by shifting agriculture and many of the present communities represent seral stages developed on old garden sites or on sites where the forest has been destroyed by natural disaster. The forests are composed of trees mainly with large buttresses which reach a canopy height of 45m. The natural canopy is often dense and allows little penetration of light resulting in surprisingly sparse undergrowth despite the over-all luxuriance of the forest. The canopy and sub-canopy of these forests contain more than eighty genera which include probably more than 1,200 tree species.

2.1.2 Climate

The Madang climate is classified as tropical wet. Temperatures vary little throughout the year with the, monthly mean maximum varying between 30.2° and 29.6°, and the minimum between 22.8° and 23.3°. Monthly figures for the average RH range between 85% and 89%. Rainfall is seasonal; the wet season normally occurs fron October to May and the dry season from June to September. Records of 42 years for Madang (McAlpine 1979) show that about 20% of the rain falls during the four-month dry season. The annual mean rainfall is 3533 mm; the maximum mean monthly rainfall is 439 mm, occurring in April, and the minimum monthly is 123 mm occurring in August.

2.1.3 The Study Population

During the study period there were approximately 620 persons living in nine hamlets, which are scattered over an area of about 30 km². The geographic distribution of the hamlets is shown in Figure 4. The villagers of this area usually refer to themselves as the Gonoa people, however, each hamlet and groups of hamlets have different names. In this investigation, the area comprising all hamlets is referred to as Gonoa or the study area; frequent reference is also made to the PNG IMR study area which is delimited in Figure 3.

Primarily, the people lead a subsistence lifestyle based on gardening. A bush-swidden-fallow farming practice is employed with new gardens being prepared annually. Much effort is spent preparing and maintaining gardens, and family groups typically spend the entire day in various garden plots. Income supplements to the subsistence economy come from a variety of sources including cash crops of cocoa and coconuts, timber royalties, sale of pigs, trade stores and selling garden produce in Madang town. During the study period, four local men were employed full-time by the Japanese and New Guinea Timber Co. (JANT) and several as casual laborers.

The influence of missions in the community is considerable and basic literacy, the result of both mission and public education, is common in most age groups. Although few children were attending school in 1984, the situation has now changed with the recent opening of a community school in Gonoa.

A household is typically made up of parents and children with the grandparents and members of the extended family usually living nearby. Females stay with the family until marriage but adolescent males leave the household and reside in bachelor houses which are usually located away from the major hamlets. The mean number of occupants per dwelling in January 1985 was 6.2.

2.1.4 Housing

Houses are of similar construction throughout the study area (Figure 5). They are timber framed and elevated 1 to 2 m above the ground. The floors are made of long strips of palm wood and the roofs of woven sago palm leaves, set high to allow for ventilation. Exterior and interior walls are made either from woven bamboo strips or sago stalks erected in palisade fashion. Each house has several rooms for sleeping, usually without windows. These rooms have doors, walls 2 to 2.5 m high, but are without ceilings, therefore, allowing easy entry of mosquitoes into rooms. In most houses, there would be further access into sleeping rooms through spaces in the walls and floor.

The father usually has his own room and sleeps on a platform raised above the floor. The mother, infants, and young children share a room and sleep on the floor, while older children and adolescents occupy the remaining rooms. All houses have an open verandah where the people spend most of their non-sleeping hours while in the village. Although the size of rooms and the number of individuals sleeping in each room are varied, the design of the houses is similar throughout the area.

The common domesticated animals are dogs, pigs and chickens. Pig husbandry varies considerably amongst the villagers. Some householders allow their pigs to forage freely around the village whereas others prefer to keep their pigs in an enclosure either near their houses or in the bush.

2.1.5 Health

Information on age-specific mortality rates is not available for the PNG IMR study area. Although it is known that lower respiratory tract infections and malaria are the leading causes of death in children under 5 (Moir 1983), overall, mortality directly related to malaria is low in contrast to that reported for parts of Africa and Thailand (Cattani et al. 1985). In a less-developed lowland population of 3,500

Figure 5:   A typical house in Gonoa village

14b

in the Sepik area (Figure 2), Sturt (1972) recorded an infant mortality rate of 104/1000 from all causes and a toddler mortality rate of 19.8/1000.

The epidemiology of malaria in the area surrounding Madang is well documented (Cattani *et al.* 1985) and pertinent to this study. Results show that although levels of endemicity vary geographically, the Madang area in general is characterized as having intense year-round transmission, indicated by parasite prevalence and splenic enlargement that decrease with age, and low-density asymptomatic parasitemias (Cattani *et al.* 1985).

Relative to most rural areas in PNG, health services in the Gonoa area are good. An Aid Post to provide basic outpatient care was established by the PNG IMR in the village in 1980 and a government Aid Post at Tadup, about 8 km from hamlet 1, serves people living in the outlying hamlets. Common health complaints are of upper and lower respiratory tract infections, malaria, infected sores, scabies from *Sarcoptes* mites, head lice and skin infections, particularly of the fungus, *Tinea imbricata.* Chloroquine is available at no cost and fever cases are treated presumptively as malaria. The endemicity of malaria in the PNG IMR study area has been referred to as hyperendemic (Jenkins *et al.* 1984). A further description of the area and characteristics of the population relating to the level of endemicity is given by Cattani *et al.* (1985) as follows:

> Malaria was found to be highly endemic throughout the Madang area, and the study population exhibited traits of acquired immunity consistent with intense exposure and year-round transmission. Rates of parasitemia and enlarged spleens were significantly higher in children (<15 years) than adults, high density parasitemias ($>1440/mm^3$) were observed most frequently in young children, and rates of seropositivity increased with age to approximately 14 years following which virtually 100% of the population was positive for antimalarial antibodies.

In Gonoa, as in most of Madang Province, there have not been active control measures taken against the malaria vectors since 1979. The presumptive treatment for malaria during a fever episode is common, however, and the people do use mosquito nets, primarily for protection from bites. Cattani *et al.* (1985) reported that the overall utilization rate of mosquito nets for all age groups in the PNG IMR study area to be 81.9%. As utilization was reported by individuals and not verified, they suggested that this value might be overinflated.

2.2 The Anopheline Fauna

The three important malaria vectors in PNG are found in the study area. They are *Anopheles punctulatus* Donitz, *A. koliensis* Owen, and *A. farauti* Laveran (Belkin 1962). All are members of the *Anopheles punctulatus* Donitz complex of Australasian mosquitoes, and belong to the series *Neomyzomyia* of the subgenus *Cellia*. Studies have shown *A. farauti* to be made up of at least three sibling species called *A. farauti* Nos. 1 and 2 by Bryan and Coluzzi (1971), and *A. farauti* No. 3 by Mahon and Miethke (1982). Only sibling sp. No. 1 of the *A. farauti* group has been identified from PNG.

The three members of the *A. punctulatus* complex found in PNG are considered highly anthropophilic. The human blood index (HBI) of vector populations is defined as the proportion of bites on man, and values greater than 0.5 indicate a high feeding preference for man (Bruce-Chwatt *et al.* 1966). In a study on biting prevalence and malaria transmission in Madang Province, Afifi *et al.* (1980) cited HBI values from their own and several other studies carried out in New Guinea. Values ranged from 0.68 to 0.96. After considering all the data, they assigned an arbitrary value of 0.75 to each of the three species of the complex.

The survival rate of adult female mosquitoes is an important factor in the transmission of malaria (MacDonald 1957, Garret-Jones 1964). For a mosquito to become infective, it must acquire gametocytes from a carrier and the parasite must develop through the sporogonic cycle, the process which follows the sexual union of gametes and ends with the formation of sporozoites. It is also referred to as the extrinsic cycle and the length of the cycle depends upon temperature and the species of malaria. For the coastal Madang area the cycle probably varies between about 9 days for *P. vivax* and 19 days for *P. malariae*; *P. falciparum* requires about 11 days (MacKerras and Ercole 1948, Peters and Standfast 1960). Long-lived vectors thus have a much greater potential for transmitting malaria and those methods aimed at decreasing the longevity of the adult female mosquito, such as the spraying of a house with residual insecticide, have been most successful in controlling malaria (MacDonald 1957). Because of the importance of the survival of

the mosquito in assessing the potential for transmission, considerable attention has been given to determination of survival rates in members of the *A. punctulatus* complex (Peters and Standfast 1960, Spencer 1970, Spencer 1979, Afifi *et al.* 1980, Charlwood 1985, Charlwood *et al.* 1985).

The probability of a mosquito surviving through one day is the most important parameter in the mathematical approach used to assess the vectorial capacity of a population. Vectorial capacity is the daily rate of potentially infective contact between persons through the vectors; potentially infective refers to the proportion of mosquitoes which survive through the extrinsic period of the malaria parasite and can transmit infective sporozoites (Molineaux and Gramiccia 1980). Vectorial capacity (C) is described by the formula (Garret–Jones 1964):

$$C = \frac{ma^2 p^n}{-\log_e p}$$

where,

m = manbiting rate
a = human blood index
p = probability of a mosquito surviving through one day
n = duration of extrinsic cycle of development of the malaria parasite in the mosquito

In a study to estimate monthly variations in vectorial capacity from two coastal and two inland villages in Madang Province, Afifi *et al.* (1980) suggested that malaria transmission can be perennial but is characterized by periods of intense and low transmission as mosquito survival rates change during the year. The same authors concluded (Afifi *et al.* unpublished report) that differences in endemicity between inland and coastal areas could be explained by lowered humidity inland, a major factor responsible for the increased daily mortality rate. In a study comparing several different methods of estimating daily survival rates, Charlwood *et al.* (1985a) found the survival rates of *A. farauti* No. 1 in a coastal village near Madang town to be the same at the end of both the wet and dry seasons. The results of capture–recapture studies (Charlwood *et al.* unpublished

data) indicated that survival rates were not significantly different between members of the *A. punctulatus* complex nor did the rates seem to vary much between villages. Although there are differences in vector survival rates and this may partially explain differences in malaria endemicity, it is clear that the longevity of members of the *A. punctulatus* complex is sufficient to account for the intense transmission of malaria in the Madang area which persists throughout the year.

Members of the *A. punctulatus* complex spend the greater part of their gonotrophic cycle out of doors, but there are well recognized differences in the resting and feeding behavior amongst the three species. *A. punctulatus* is regarded as more strongly endophilic and endophagic than the other two species and field observations have shown it to respond well to residual DDT, whereas, the response of *A. koliensis* has been variable. *A. farauti*, being more exophilic and feeding earlier in the night, responded the most negatively to the spray program (Spencer *et al.* 1974, Van Dijk and Parkinson 1974). Although the HBI may vary considerably between villages depending on the availablity of host groups, observations and studies on feeding behavior of *A. farauti* give no evidence of separate or exclusive endo- and exophagous populations, nor for the existence of populations preferentially feeding on non-human hosts (Spencer *et al.* 1974, Charlwood *et al.* 1985b, Charlwood *et al.* unpublished data).

The major difference between the members of the *A. punctulatus* complex is in their choice of breeding sites. The general distribution of the three species and a description of the larval habitats of *A. farauti* and *A. punctulatus* are given by Spencer *et al.* (1974). It has been suggested that the choice of larval habitat of each species may explain observed differences in oviposition interval, biting time, flight range, and population density (D. Charlwood, pers. comm.).

Tropical anophelines generally have a shorter flight range than mosquitoes in temperate climates and are usually not found more than 2-3 km from their breeding places (Bruce-Chwatt 1980). The flight range of *A. farauti* based mainly on anecdotal observations of biting activity in relation to distance from breeding sites, has been estimated at 1.2 to 1.6 km (Daggy 1945, Horsfall and Porter 1946). Results

of recent capture–recapture experiments (Charlwood unpublished data, Millen and Charlwood unpublished data) gave recapture rates of released bloodfeds, consistently greater than 3% and as high as 17.6%. It has been suggested that the vector populations are often isolated and in close association with a particular community, because the distance between hamlets is often greater than the flight range, and because of a lack of alternative hosts in the area between villages (Spencer *et al.* 1974).

2.3 The Malaria Parasites

The epidemiology of malaria in Papua New Guinea has been documented by Peters and Standfast (1960) and Van Dijk and Parkinson (1974). The two most significant developments over the past thirty years have been the change in status of *Plasmodium falciparum* from being less predominant than *P. vivax* to being highly predominant, and the emergence of resistance of *P. falciparum* to antimalarial drugs (Cattani *et al.* 1985).

In 1976, a village-based chemoprophylaxis program for children under the age of 6 years was started in villages in the Madang area, using voluntary village collaborators to dispense Amodiaquine (infant Camoquin) in weekly doses of 5 mg/kg. The project, which by 1980 covered 2,500 children in 66 villages was initially successful in reducing parasite rates in children to 4%, compared with 48% in a non-prophylaxis group (Stace and Pariwa 1982). A survey in 1980, however, showed that parasite rates in children taking prophylaxis had returned to pre-treatment levels. It was speculated that *P. falciparum* resistance to amodioquine had increased enough to render weekly prophylaxis ineffective at the standard dose (Moir 1983). Resistance to chloroquine in *P. falciparum* malaria was first reported from PNG in 1976 and has since been documented in many of the malarious provinces (Grimmond and Riley 1976, Yung and Bennet 1976, Darlow and Vrbova 1981). Resistance to Fansidar (pyrimethamine 25mg plus sulfadoxine 500mg) has also been reported (Darlow *et al.* 1982).

From the recent study in the Madang area, Cattani *et al.* (1985) reported *P. falciparum*, *P. vivax* and *P. malariae* to have a parasite ratio in the wet season of 76:20:4 respectively. The parasite rate, defined as the proportion of blood samples with blood films positive for any species of malaria parasite, was 40.5% in the dry season (1982) and 42.7% in the wet season (1983); these rates were for all age groups combined. Because of the proximity of Gonoa to the PNG IMR study area, this investigation is of particular importance to this study, and this paper should be referred to for a comprehensive description of the epidemiology of malaria in the Madang area.

THE STUDY DESIGN

The malaria control program based on residual spraying with DDT has not been successful in the rural areas of PNG and malaria continues to cause significant mortality and morbidity. In view of the importance of this disease as a public health problem, the goal of this project was to identify and evaluate a community- and rural-based strategy for personal and community protection against the vectors of malaria. In consultation with the villagers, various measures that had potential use as part of an integrated approach were assessed. Several of the measures were not considered to be appropriate and reasons for rejecting them are given in the discussion. The emphasis of this investigation was placed on the evaluation of mosquito nets impregnated with the synthetic pyrethroid permethrin (3-phenoxy benzyl ($\pm$) cis, trans, 2-dimethyl--3-(dichlorovinyl) cyclopropane carboxylate). Research on the impregnation of bed nets with pyrethroid insecticides is currently being recommended by the World Health Organization (WHO 1983). The specific objective of this part of the project was thus stated as follows: To evaluate the efficacy of permethrin-treated bed nets as a method for increasing protection against the vectors of malaria.

The study population was divided into two geographically isolated groups: villagers from hamlets 1, 2 and 4, and 3, 5, 7, 8 and 9 comprised the experimental and control groups respectively (Figure 4). They are referred to in this paper as the experimental and control villages. The people from hamlet 6 were not included in the study. Information on bed net use, mosquito breeding sites and baseline data on entomological and parasitological parameters were collected from January 1984 to June 1984. It was decided that before mosquito nets were impregnated, it would be necessary to:

1. Increase the number of mosquito nets in all hamlets, because in some households people were not using them, and in other households there was obvious overcrowding as people slept under them.

2. Reduce the community prevalence of malaria in both experimental and control villages.

To increase the number of bed nets in both the experimental and control villages, bed nets were bought in Madang town and resold to the villagers at a reduced price.

To reduce the community prevalence of malaria, all people in the study area who were positive for malaria in survey 1 (mid–August 1984), were treated with a combination of chloroquine and sulfadoxine–pyrimethamine (Fansidar®). Concurrent with the drug administration, the new and existing bed nets in the experimental village were impregnated with permethrin.

If the impregnation of bed nets was to be successful at reducing the transmission of malaria, it was hypothesized that the effectiveness could result from:

1. A non–lethal effect such as the inhibition of feeding which would result in a reduction of man/mosquito contact as people slept under the bed nets.

2. The toxic action of permethrin resulting in a reduction in the density and longevity of the vectors.

3. A combination of the above.

Previous studies had shown that treatment of fabric with permethrin can be an effective method for preventing insect bites because of toxic action of the insecticide (Shreck et al. 1978a). Using lower treatment levels, Carter (1983) measured such responses as biting inhibition and percentage knockdown from exposure of mosquitoes to treated fabrics. The expected response of the vector population to impregnation of mosquito nets would therefore determine the methods for evaluation of efficacy. Because it was not clear what response to expect under field conditions several entomological and parasitological parameters were measured. Entomological parameters of manbiting, oocyst, sporozoite and parous rates were determined throughout the study; malariometric indices were measured on the entire study population before impregnation and at 3 and 7 months after.

LITERATURE REVIEW

4.1 <u>Clothing Impregnated With Synthetic Pyrethroids As A Method For Providing</u>
<u>Personal Protection Against Biting Insects</u>

The impregnation of fabric with synthetic pyrethroids apparently arose from
the desire to find a measure for personal protection from biting arthropods, that
did not rely on conventional repellents. When applied to fabric or skin, these lose
their effectiveness in a relatively short time, have an unpleasant oily feeling and
must be applied, frequently, at high doses. Synthetic pyrethroids were considered as
clothing impregnants because of their residual effectiveness, ability to cause a rapid
knockdown and mortality at low concentrations, and very low mammalian toxicity.

In 1977, Schreck *et al.* conducted a series of field, cage and olfactometer
tests using the synthetic pyrethroid resmethrin. They found that people wearing
impregnated jackets could, apparently, reduce the number of biting mosquitoes in an
area. In cage tests, the daily survival rates of biting flies could be decreased by
placing impregnated fabric over mammalian hosts, thus allowing contact with the
toxicant as the flies attempted to feed. It was postulated that the observed effects
were due to contact with the fabric because results of olfactometer tests with
Aedes aegypti (L.) and *Anopheles quadrimaculatus* Say. gave no evidence to suggest
that resmethrin repelled mosquitoes. Further studies with resmethrin demonstrated
that cotton military uniforms treated at 1.25 g/m^2 gave greater protection from
bites on exposed skin than did repellent treated clothing (Schreck *et al.* 1978b). The
results of these experiments indicated that repellency may occur and they suggested
that the activity of the toxicant on the biting population could:
1.   Result from spatial repellency.
2.   Affect the behavior through intoxication and death
3.   Influence the approach of the insect.
4.   Affect the population through a combination of these factors.

In subsequent experiments with several species of biting arthropods, Schreck
et al. (1978a) determined the minimum effective dose that caused 100% mortality at

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15 and 60 min after short exposure periods to permethrin impregnated fabric. The dosages ranged from 0.08–1.25 g/m^2 on four species of mosquitoes, including *Anopheles albimanus* Wiedemann and *A. quadrimaculatus*, and on two species of flies, after only 10 s exposure. The 100% mortality response was not observed with the bedbug, *Cimex lectularius* L., at a dosage of 10 g/m^2 and 30 s exposure. It was also determined that the permethrin impregnant was resistant to heat, light, rinsing and weathering. After rinsing treated fabric 10 times in cold water, the observed response of *Aedes aegypti* exposed for 30 s, remained at 100% mortality within 1 h, and after exposure to weathering for 28 days, fabric treated at 1.25 g/m^2 killed 100% of *Aedes aegypti* and *Anopheles quadrimaculatus* after 30 s exposure.

Mesh jackets treated with permethrin at 0.25 g/g of netting gave good protection in field trials against both mosquitoes and blackflies (Lindsay and McAndless 1978). It was observed that protection was afforded about 10 minutes after exposure, due apparently, to the overall reduction of the population in the area. Whether the effect on the population was due to mortality through brief contact with the toxicant, or from some other response, was not known.

To determine the effect of wear and aging on military clothing, permethrin–treated fabric was subjected to both biological and chemical assays (Schreck *et al.* 1980a). These results showed that little permethrin was lost from unworn fabric but with the worn fabric, there was loss in both the amount of permethrin and its biological effectiveness when tested for 30 days. Gas chromatographic (GC) analysis determined that there were no major degradation products from the worn fabric and that the loss of the two permethrin isomers occurred at nearly the same rate indicating that the loss was due to abrasion and rubbing and not to chemical degradation.

Permethrin has also been reported to give effective protection against bodylice (Nassif *et al.* 1980), ticks (Schreck *et al.* 1980) and chigger mites (Breeden *et al.* 1982). In the first study, people were dusted with 50 g of powder containing 2.5 or 5.0 g/kg of permethrin. Analysis of urine samples for metabolites of permethrin showed that an insignificant amount of permethrin was absorbed during the trial.

They stated that there was a very substantial margin of safety for the application of permethrin to man as a dusting powder for bodylouse control.

4.2 Bed Nets Impregnated With Permethrin As A Method For Providing Personal Protection Against Vectors of Malaria

Recently, there have been studies on the efficacy of bed nets impregnated with permethrin as a method of personal and community protection against biting flies. The results of a trial in west Africa using bed nets impregnated with permethrin at 0.08 g/m² indicate that the insecticide has a repellent effect, and that non-lethal effects on the vector population were important (Darriet *et al.* 1985). In this trial, huts with impregnated bed nets (0.08 g/m²) were compared to huts with control bed nets. Several entomological parameters were measured, including; density of mosquitoes in the huts; entry, exit and engorgement rates; and mortality rates of mosquitoes collected in the huts. The results showed that in huts with impregnated nets the numbers of the two major vectors of malaria collected were each reduced by about 70%. They attributed this to the spread of volatile substances that are an irritant to the mosquitoes, and to the overall repellent effect of the hut caused by the permethrin treated bed nets. The exit rate, as measured by the numbers of mosquitoes found in verandah traps, was greatly increased. In the control huts, about 30% of the mosquitoes were caught in verandah traps, 10-15% under mosquito nets and 50-60% on the hut; in the experimental huts, 97% of the mosquitoes were caught in the verandah traps and less than 1% in the mosquito nets. Of the mosquitoes that were collected in the huts with impregnated nets, the overall mortality was about 15-20%, one-third being immediate and two-thirds being deferred *ie.* mortality during the 24 hr observation period. In the control huts, the mortality rate was <1%. They suggested that because the number of bloodfed mosquitoes remained relatively high (engorgement rates of 80- 90%), permethrin did not have a particularly significant knock-down effect. They did not, however, speculate as to how much man/mosquito contact resulted from the improper use of mosquito nets. Although the mortality rate of the reduced numbers of mosquitoes that entered the huts was about 17%, of significant importance were

the non-lethal effects which resulted in decreased entry rates and increased exit rates.

The results of a recent investigation in Tanzania suggest that bed nets impregnated with permethrin may be a useful method of malaria control (Lines et al. 1985). In this investigation, several indices were measured comparing a control hut with no bed net, to experimental huts with bed nets intact[1] & treated; intact & untreated; with holes & treated; and with holes & untreated. In contrast to the west African study, however, results of the Tanzanian study did not demonstrate that permethrin was particularly effective at decreasing the number of mosquitoes entering a hut i.e. it did not appear to have a strong repellent effect. There was little difference in the numbers of mosquitoes entering huts with treated intact nets when compared to huts with untreated intact nets. Compared to the unprotected control, however, impregnated bed nets, both intact and with holes, and untreated intact bed nets, all decreased the the number of bites from mosquitoes, and the results were highly significant. The most obvious effect of permethrin was its effect on the survival of bloodfed mosquitoes. Impregnated bed nets, both intact and with holes, caused about 50% mortality among females which fed.

Also investigated in this trial, was the effect on the vector population of permethrin-impregnated curtains. Compared to control huts without curtains, and with untreated curtains, impregnated curtains greatly reduced the numbers of mosquitoes entering the huts, and caused many to leave after feeding. The results with impregnated curtains in this trial were similar to the results with impregnated bed nets in the west African trial (Darriet et al. 1985), in that non-lethal effects such as repellency were the most important effects.

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[1] Investigators included bed nets with holes in their experiments because it has been observed that many bed nets in Africa are in poor condition with holes.

METHODS

5.1 Entomology

5.1.1 Man-biting Rates

Human bait catches, the most useful method for collecting anthropophilic mosquito populations (Service 1976), were used to estimate the man-biting rate (MBR), defined as the number of mosquito bites received by one man per night. Two collectors were stationed in each of the control and experimental villages for a four-night collection period each month. The two six-hour collection shifts in each village, began at 1800 and 2400. Mosquitoes were collected by aspirator, and each hour's collection was put into paper cup cages, which were kept in a humidified container. Information on cloud cover, rainfall, wind and human activity were recorded on site by the collectors. During the four-night collection period, two man-night collections were taken indoors and two were taken outdoors in both control and experimental villages. One of the primary objectives of the collections was to enable a comparison of the man-biting rates in the two villages. Because of manpower limitations, only one type of collection (*i.e.* indoor or outdoor) was made during a night. This allowed for a comparison of populations in the two villages on the same night, but was less appropriate for comparing the indoor and outdoor feeding behavior because large variations in mosquito density may occur from one night to the next due various factors such as daily weather conditions. As requested by the villagers, indoor collections were made in rooms where no one was sleeping. The collectors were rotated between villages and shifts so that each person collected indoor and outdoor in each village. From the man-night collections, samples were kept to determine the delayed oocyst rates, the parous rates and the sporozoite rates. All mosquitoes were eventually identified and summated, giving the total night's contribution to the man-biting rate for each species.

5.1.2 Delayed Oocyst Rates

Since decreased contact with man could result in lower oocyst rates but not necessarily lower densities or decreased longevity, the delayed oocyst rates were measured to provide an index of man/mosquito contact that would show a response to bed net impregnation where other entomological parameters might not. To determine the delayed oocyst rate, approximately 10 mosquitoes from each hour's collection were aspirated into separate cups, fed on sugar solution and kept in an insectary for 4 or 5 days. The guts were extracted in an aqueous solution of mercurochrome, (disodium salt of 2,7–dibrom–4–hydroxymercurifluorescein). Guts treated in this manner showed oocysts stained red without marked staining of the mosquito tissue. The mosquitoes were recorded as being either infected with oocysts, or not. A distinction was made between mature oocysts that had collapsed during preparation and oocysts that had ruptured due to maturation.

5.1.3 Sporozoite Rates

For determination of the sporozoite rate, mosquitoes were taken from each hour of the night–biting collection; the sample size depended on the availability of mosquitoes and varied as to the season. Mosquitoes were knocked–down, identified, pooled as to species and times collected, then frozen. For detection of sporozoite–infected mosquitoes and identification of sporozoites, the double antibody ELISA, (enzyme–linked–immunosorbent–assay), test was used (Burkot *et al.* 1984). It was suggested that a sporozoite rate of 0.5% could be expected in an area with a known oocyst rate of 1–5% (Thevasagayam, pers. comm.). The mosquitoes in a sample were therefore pooled by species and time collected, in groups of 5, and ground in plastic vials for the ELISA test.

5.1.4 Age Composition

The proportion of mosquitoes parous was determined, to enable comparison of the age composition of the vectors from the two villages, and for the estimation of longevity. Mosquitoes were taken from each hour of the night-biting collections, the sample size depending on the availablility of mosquitoes from that night's collection. The mosquitoes were anesthetized and the ovaries removed, transferred to a slide in a drop of water and allowed to dry. Once dried, the ovaries were examined for the presence or absence of coiled tracheolar skeins.

5.1.5 Estimation of the Human Blood Index

The source of bloodmeals was investigated to determine feeding preferences and to enable estimation of the Human Blood Index, HBI. Bloodfed mosquitoes were collected from both indoor and outdoor biotopes. A search of the outdoor biotope was made during each morning after the collection period, between 0700 and 0800. Indoor bloodfeds were collected by CDC traps which were placed in bedrooms and allowed to run for the entire night. Bloodfeds were taken to the laboratory as soon as possible, knocked-down and identified. Their abdomens were squashed with a glass rod on a sheet of 11 cm dia filter paper, sixteen to a sheet, and each spot was assigned a reference number. Completed sheets were sent to the Bloodmeal Identification Unit of Imperial College, University of London. Identification of the bloodmeal source was made by the precipitin test using antisera from the common host groups found in the village. A description of the test and its application was given by Tempelis (1975).

At various times during the investigation, anopheline larvae were collected so that breeding sites of the three vector species in the study area could be located and mapped. Larvae were collected with a ladle, returned to the laboratory and reared to the adult stage for positive identification.

5.1.6 Contact Bioassays to Test for the Duration of Effectiveness of Impregnated Bed Nets

To investigate the duration of effectiveness of permethrin impregnated bed nets under field conditions, bioassay tests were conducted on bed nets from the village at various time intervals after treatment. Untreated and treated nets were set-up in a village house where nets were not being used. Groups of about 30 mosquitoes were released into the nets for 30 min. This was repeated several times depending on the availability of mosquitoes. Following exposure, they were returned to the insectary and held for 24 hours. The mosquitoes initially used in the bioassays were unfed females of *A. farauti* (Rabaul x Rabaul self-mating strain) from the colony at the PNG IMR. As these became unavailable, wild members of the *A. punctulatus* complex were used.

5.1.7 Treatment of Mosquito Nets With Permethrin

Treatment of mosquito nets with permethrin began in mid-August 1984. Several weeks before, the villagers were asked to wash their nets. During the morning of the day specified for treatment, a station was set up in the hamlet. The people were requested to bring their nets to the station where the following information was recorded:

1.   Net size.
2.   Condition of net (with or without holes)
3.   Cleanliness of net recorded as 1, 2 or 3 (dirty - clean).
4.   Presence or absence of bedbugs *Cimex hemipterus*.
5.   House number

All nets were marked with an identity number and the house number prior to treatment. The method used to treat the nets was similar to that described by Self

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(1983). The procedure was as follows:

1. Nets were marked with an identification and house number.

2. The total surface area of the net was calculated.

3. The amount of active ingredient was determined for treatment at 0.2 g/m^2 of netting.

4. The quantity of water needed to saturate the net without runoff, was determined.

5. The emulsifiable concentrate and water were mixed and poured on the net in a plastic tub.

6. The net was squeezed to ensure that all of the net became wetted with the solution.

7. The net was then placed on a black plastic film and allowed to dry without runoff.

Proper safety precautions were followed to ensure protection from any hazards of exposure to the insecticide.

5.1.8 The Prevalence of Head Lice

Several weeks after impregnating the nets, a village mother reported to me that she and her children no longer had head lice. She thought that the head lice had been killed by the "medicine" used to treat the nets. The belief that head lice had been controlled as a result of net impregnation with permethrin was common in the village at that time. As a result, a retrospective investigation was conducted to determine if there was a difference in the prevalence of head lice, *Pediculus humanus capitis* De Geer, between the experimental and control villages. It had to be assumed that there was no difference in the prevalence of head lice in the villages before impregnation of the nets, and there was no reason to suspect otherwise.

It is known that the prevalence of head lice is somewhat dependent on age and sex with the prevalence in children being higher than adults and the prevalence in females being higher than in males (Anonymous 1985). This is due, in part, to the people's habits; children are often in close contact with each other and sleep in groups, and girls typically have longer hair than boys. In this investigation, the prevalence rates were compared in the greater than 14 years age group, and the 14 years and under age group. This age grouping roughly separates children and young adolescents from adults.

The survey was conducted 20 weeks after net impregnation. Approximately 100 people were chosen at random from each of the two villages. A visual search for eggs (nits), nymphs and adults was made for 30 seconds by a mother from one of the villages. On people positive for lice, a count was made of all stages visible in a field which was defined as the area exposed in the lower—right quadrant of the head, behind the ear, as the hair was parted by the hands of the person inspecting for lice. The following information was recorded on the survey forms: name, date of birth, sex, house number, length of hair, presence or absence of any stage per 30 s search, nits per field and adults/immatures per field. A person's hair was classified as being either "shaved", "short" (hair present but scalp showing) or "long". Two medical students from the PNG IMR assisted with the survey to ensure that procedures were conducted properly as described.

5.2 Malariology

The three malariometric surveys were held in August 1984 (before bed net impregnation), November 1984, and April 1985. During each survey, spleens were examined and blood samples were taken and examined for the presence of parasites. Each survey was conducted over a three-day period with between 150 and 200 people examined each day. As people arrived during the morning of the survey, they reported to a station where demographic information was recorded on a malaria survey form (Appendix B). To assess mosquito net utilization, people were asked if they had slept under a net during the previous night. They then went to other stations where spleen and blood examinations were made.

To determine the number of people with enlarged spleens and to approximate the degree of splenomegaly, the Hackett Grading Method (WHO 1963) was used with subjects recumbent. All spleens were examined by the same investigator.

Blood examination was made on thick and thin blood films which were stained with 4% Giemsa and examined for 100 microscope fields (thick film) under oil immersion prior to being declared negative. Parasite species were identified in positive films, and densities were recorded as the number of parasites/200 white blood cells. For scanty infections, the number of parasites/100 thick film fields were reported. Sexual and asexual densities were calculated separately for *P. falciparum* only. A routine quality control procedure assured reexamination of a random sample of blood films by the microscopy supervisor, without knowledge of the previously recorded result.

5.2.1 Administration of Chloroquine and Sulphadoxine-pyrimethamine

To decrease the prevalence of malaria in the community (both experimental and control villages), all people surveyed in August 1984, and found to have positive blood slides, were treated with a combination of chloroquine or amodiaqine, and sulfadoxine-pyrimethamine (Fansidar®, Roche). Drugs were

administered under supervision, in tablet form, with doses given according to the PNG standard treatmant manual. Chloroquine (amodioquine)[1] was given daily for three days and sulfadoxine-pyrimethamine[1] was given as a single dose on the first day of treatment. A follow-up survey to monitor the clearance of parasitaemia was conducted 14 days after drug administration on 120 people chosen at random from the study population.

5.2.2 Data Analysis

Data from the survey forms were checked for errors or omissions before entry into the computer. All data were entered twice and files were crossed-checked against each other for mistakes. Frequency tables were compiled using BMDP statistical software (Dixon 1980) and the results (entomological and parasitological) were analysed using the chi-squared test to determine the degree of association between indices measured in the experimental and control villages.

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[1]Chloroquine for adults and amodioquine for young chidren and infants given daily for 3 days, was the standard treatment for malaria in 1984. Sulfadoxine-pyrimethamine (Fansidar) is a restricted drug in PNG and is given as a single-dose treatment for chloroquine-resistant malaria. Resistance to both chloroquine and Fansidar is documented in the Madang area (Darlow and Vrbova 1981) (Darlow *et al.* 1981).

RESULTS

6.1 Entomology

6.1.1 Vector Density

The number of bites/man/night, measured by the mean of indoor and outdoor whole-night-biting collections, was used to estimate the man-biting rates. The man-biting rates and rainfall for the months January 1984 to April 1985 are shown in Figure 6. Seasonal variation in vector density was greatest for *A. koliensis*; man-biting rates in excess of 600 were recorded in the wet season but as low as 2 in the dry season. The man-biting rates of both *A. farauti* and *A. punctulatus* were in general, an order of magnitude lower than those for *A. koliensis*, except during the four-month dry season. The direct relationship between rainfall and biting density of *A. koliensis* was obvious (Figure 6), but less so for *A. farauti* and *A. punctulatus*.

Larvae of *A. punctulatus* were frequently found in the many small ponds that are continuously being formed as a result of activities of man and animals. During the wet season the number of pools increased, but, the productivity of many of these pools as breeding sites decreased. It was observed that a layer of algae often formed on the surface of the pools when water remained in them for extended periods, making them unsuitable for breeding. Many of these pools seemed most productive as breeding sites before and after the periods of continuous rainfall.

Larvae of *A. koliensis* were found mostly along the edges of small streams and in the outer sun-exposed margins of sago swamps. Although the biting density of *A. koliensis* was very high in the wet season, the larvae of this species were difficult to find at this time. It is assumed that the previously cleared areas of secondary growth around the hamlets, which become flooded during the wet season, provide an extensive breeding habitat for *A. koliensis*.

Larvae of *A. farauti* were collected along the margins of the many small streams in the study area. Fewer streams dried up in the control village area which probably explains the higher biting densities recorded there from July to October. Although the number of *A. farauti* collected in the Gonoa area was the smallest percentage of the total number of anophelines collected during the study period (*A. farauti* 7%, *A. koliensis* 77%, *A. punctulatus* 16%), in Maraga, a village just 10 km from Gonoa, the highest biting densities in the PNG IMR study area are recorded. The predominant species in Maraga is *A. farauti* and biting densities remain high throughout the year. At the end of the dry season, the man-biting rate often exceeds 1000 bites/man/night. The breeding sites in Maraga are brackish lagoons that remain filled with water throughout the year. Studies by Charlwood *et al.* (1985a) revealed that the survival rate of *A. farauti* in this area does not vary much throughout the year. This indicates that a lack of suitable breeding sites is important in regulating population density of this species in Gonoa, and that factors such as changing humidity are probably less important. It has been suggested that different larval habitats of the three species may explain differences in oviposition interval, biting times, flight range and population density (Charlwood, pers. comm.).

As described by others (Spencer *et al.* 1974), and observed in this study, the biting cycle of the three vector species (Figure 7) shows *A. koliensis* and *A. punctulatus* to be most active in the second half of the night; *A. farauti* has the same tendency but to a lesser degree. The indoor man-biting rates and outdoor man-biting rates as measured by whole-night biting collections were similar for *A. koliensis* but not for *A. farauti* and *A. punctulatus* (Figure 7). The ratio between the total numbers collected indoor:outdoor was 0.96 for *A. koliensis*, 0.77 for *A. farauti* and 0.42 for *A. punctulatus*. The last-named vector is usually considered more endophagic and endophilic than either *A. koliensis* or *A. farauti* (Spencer *et al.* 1974) and results indicating a preference for outdoor feeding are not easy to explain since this was observed both before and after the impregnation of bed nets. Because the objective of the whole-night biting collections in this study was to compare populations in the villages and not to investigate indoor and outdoor feeding behavior, this result must be interpreted with caution.

Figure 6: The monthly rainfall and man-biting rates of *A. farauti*,
A. koliensis and *A. punctulatus* in the experimental and
control villages as estimated from the average of indoor and
outdoor whole-night biting collections (bed nets impregnated
in August 1984)

37b
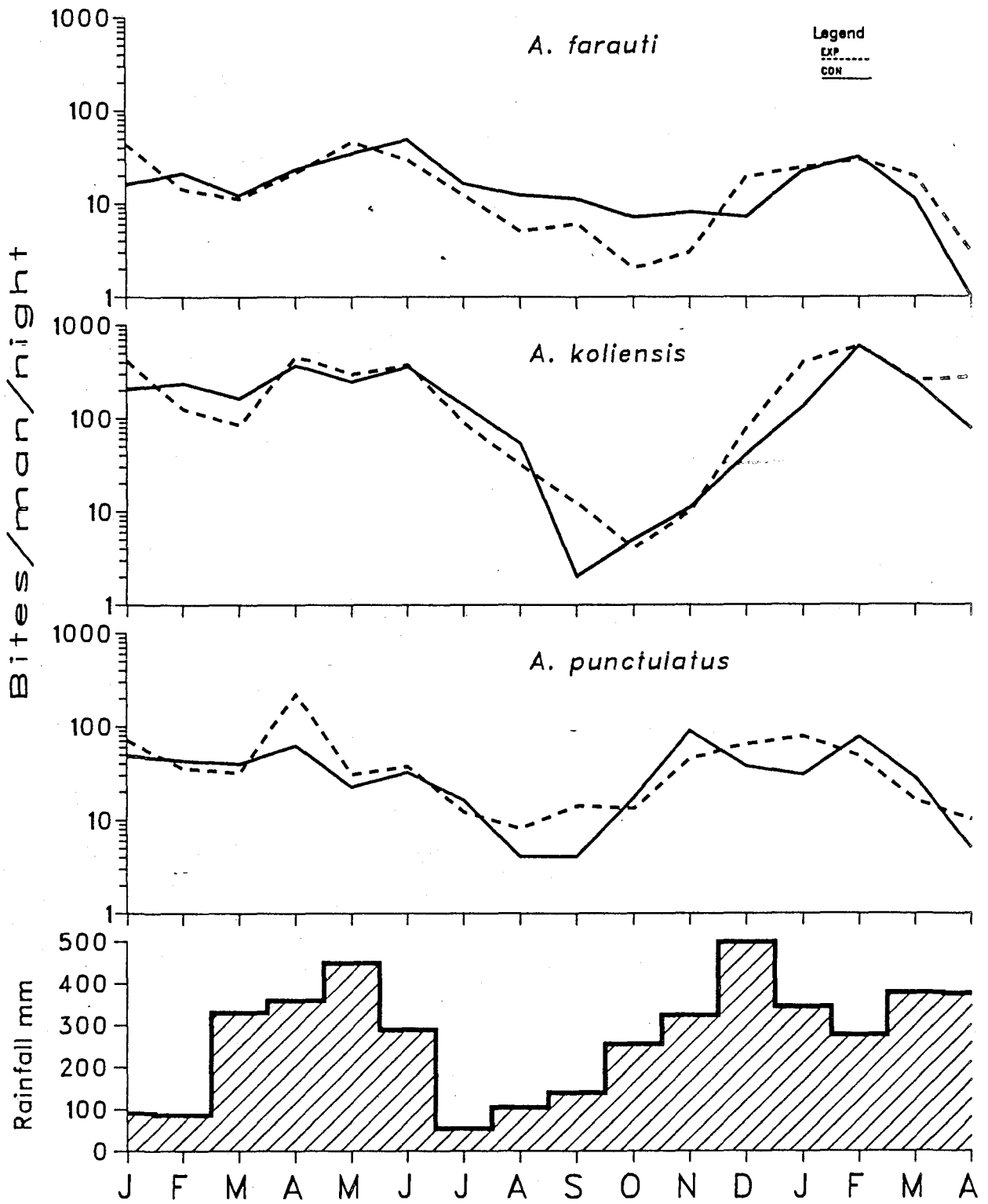
Figure 7: The biting cycle of *A. farauti*, *A. koliensis* and *A. punctulatus*,
           indoors and outdoors from whole-night biting collections,
           January 1984 to March 1985

A. farauti

A. koliensis

A. punctulatus

Legend
⬚ indoor
■ outdoor

Time of day

% of TOTAL BITES 1800 − 0600

38b
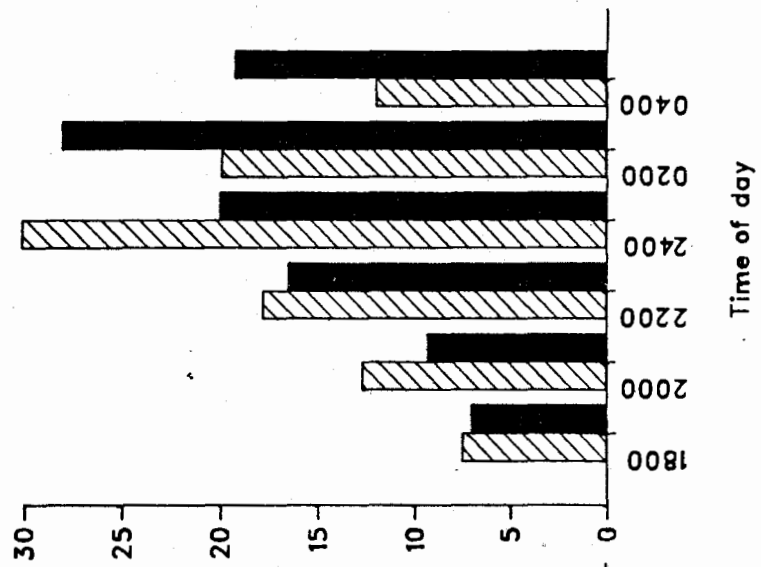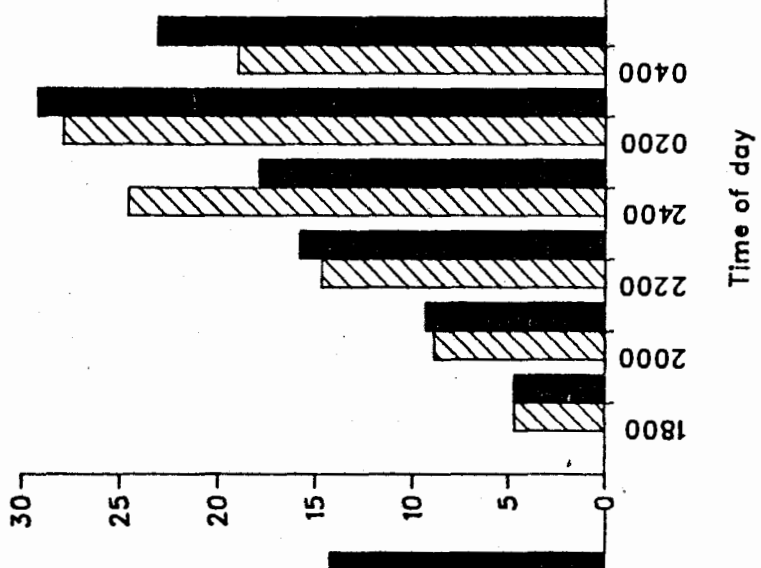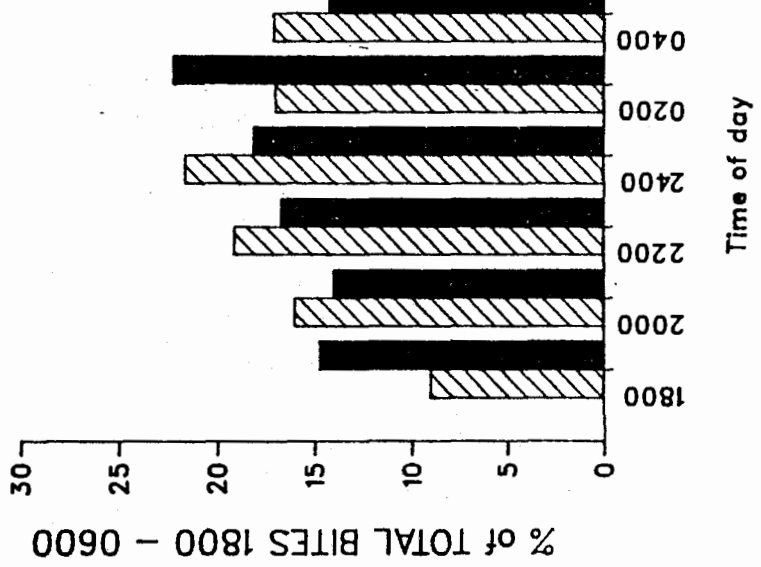
6.1.2 Bloodmeal Analysis

To estimate the proportion of mosquitoes feeding on man, *i.e.* the Human Blood Index (HBI), bloodmeals of resting mosquitoes were analyzed. The most serious difficulty in estimating the HBI is in getting an overall unbiased sample of resting bloodfeds from various biotopes. In this investigation, the outdoor and indoor biotopes were sampled and it was found that most of the bloodfeds collected from the indoor biotope had fed on humans. The percentage feeding on man for each species, as collected from CDC traps placed in sleeping rooms were as follows: *A. farauti* 86% (n = 13); *A. koliensis* 89% (n = 112); *A. punctulatus* 90% (n = 20). Since members of the *A. punctulatus* complex spend most of their gonotrophic cycle outdoors, the results of the bloodmeal analysis from mosquitoes collected from the outdoor biotope were used for estimation of the HBI (Table 1). The collection sites for *A. koliensis* and *A. punctulatus* were varied and representative of the outdoor biotope, however the number of outdoor resting *A. punctulatus* was small. Difficulties in finding outdoor resting *A. punctulatus* and *A. koliensis* have been reported by others (Charlwood *et al.* 1985a). The HBI for *A. koliensis* is lower than that reported by others (Afifi *et al.* 1980), although it is still suggestive of a highly anthropophilic species. It is possible that the use of mosquito nets contributes to the lower HBI values recorded in this study. Vectors unable to feed on humans in the second half of the night would feed on the nearest available host, which in many households are dogs. The percentages of *A. koliensis* and *A. punctulatus* which fed on dogs were 34% and 44% respectively. These are high values considering the relatively few numbers of dogs in the village.

The mosquitoes collected from the outdoor resting sites were usually found within 50 m of the village houses. Typical resting sites were dark moist areas under a canopy of secondary growth, often at the base of a tree or near rotting logs. Whereas no one site seemed favoured by *A. koliensis* or *A. punctulatus*, bloodfeds of *A. farauti* were very abundant in an area near the edge of a sago swamp in hamlet 1. Villagers kept several pigs at this end of the hamlet, and bloodmeal analysis showed that 97% (n = 152) of the blood meals were from pigs. There were very few outdoor resting *A. farauti* found in other sites.

Table 1: Bloodmeal Analysis for Outdoor Resting *A. farauti*, *A. koliensis*, and *A. punctulatus*

	Human	Pig	Dog	Total	HBI[1] %	Negative
A. farauti	3	68	7	78	3.8	7
A. farauti No. 9	5	119	16	140	3.6	9
A. koliensis	50	22	37	109	45.9	31
A. punctulatus	7	2	7	16	43.7	2

[1]Human Blood Index (HBI) is the percentage of bloodmeals from human hosts

Identification of members of the *A. punctulatus* complex is made primarily by inspection of the colour pattern on the probosces of females. Bryan (1974) described three basic types of probosces and other markings, that are used to identify females of the three species found in PNG. On examination of the bloodfeds from the resting site previously descibed in hamlet 1, it was observed that the proboscis pattern of many of the mosquitoes collected did not resemble any of the three type patterns as descibed by Bryan (1974). The appearance of these mosquitoes was similar to *A. farauti* No. 1 except for the proboscis markings. Whereas 99.5% of the *A. farauti* No. 1 described by Bryan (1974) had all black labium or labium with a small white patch on the apical end, these mosquitoes had an irregular pattern of white scales on the dorsal or ventral, apical half of the labium. The pattern was not confused with that of *A. koliensis* which has a small white patch of scales that may be variable in size but is well defined. With a few specimens, the irregular pattern of scales was so extensive on the apical half of the proboscis that it was confused with *A. punctulatus*. I arbitrarily called these specimens *A. farauti* No. 9 because of their obvious similarities in feeding (Table 1) and resting behavior to *A. farauti* No. 1.

6.1.3 Age Composition

The parous rates of *A. koliensis* and *A. punctulatus* from the experimental village did not show any significant changes after net impregnation when compared to the parous rates of the same species in the control village (Table 2). Because the nets were treated in the dry season, the populations were under-sampled during the months after net treatment when any effect should have been greatest. However, the results of the bed net bioassays indicated that during the period from August 1984 to February 1985, permethrin had excellent residual activity (Table 8). As a result, the post-impregnation values, as shown in Table 2, were combined to give the proportion parous from August 1984 to February 1985. Further studies are needed to determine if the difference in parous rates between *A. koliensis* and *A. punctulatus* reflects a difference in longevity or gonotrophic cycle.

6.1.4 Delayed Oocyst Rates

The number of mosquitoes dissected each month and the number positive for any number of oocysts, is given in Tables 3, 4 and 5 for *A. farauti*, *A. koliensis* and *A. punctulatus* respectively. The oocyst rates for all three species combined are presented in Table 6. The delayed oocyst rates from mosquitoes collected in the experimental village, did not demonstrate any effect of bed net impregnation when compared to similar rates of mosquitoes in the control village. The rates in the study area were observed to change throughout the year and in general, varied inversely to the population densities. This reflected the changing age status of the populations so that following periods of little rain, the rates increased as the relative numbers of parous mosquitoes increased and the population in general decreased. The delayed oocyst rates from the total number of dissections for the three species were as follows: *A. farauti* 2.2% (n=541); *A. koliensis* 2.8 (n=5332); *A. punctulatus* 2.7% (n=1065). The lower value for *A. farauti*, although not significantly different from the other species, possibly reflects the greater host preference for pigs. There was no significant difference in oocyst rates from

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Table 2: The proportion parous of *A. koliensis* and *A. punctulatus* from whole-night biting collections

	Collection period	Collection site	Proportion parous	N
A. koliensis	Pre-impregnation[1]	Hamlets 1, 2	0.59	857
	Post-impregnation[2]	Experimental	0.52	305
	Post-impregnation[2]	Control	0.53	249
A. punctulatus	Pre-impregnation[1]	Hamlets 1, 2	0.61	236
	Post-impregnation[2]	Experimental	0.62	180
	Post-impregnation[2]	Control	0.58	293

[1] collections from February'84 to July'84
[2] collections from August'84 to February'85

mosquitoes collected indoors (2.9%, n=4330) and outdoors (2.4%, n=3409).

6.1.5 Sporozoite and Inoculation Rates

The sporozoite rate is defined as the proportion of mosquitoes with sporozoites of any species of malaria. In this study, sporozoite rate was determined from the number of mosquitoes positive for sporozoites of *P. falciparum* and *P. vivax* as determined from the **ELISA** assay. The rates were not used to evaluate the effectiveness of net impregnation because the sample sizes were small, particularly from the drier months just after net impregnation. Furthermore, there was no evidence to suggest that the longevity of the vectors had decreased as a result of net impregnation or that there was a decrease in oocyst rates. Nevertheless, the data can be used to describe the source of infection in the study area through estimation of the sporozoite and entomological inoculation rates. From June 1984 to February 1985, 2,541 *A. koliensis* mosquitoes from the experimental and control villages were tested for sporozoites. The overall

Table 3: The number of *A. farauti* with oocysts, from whole-night biting collections before and after treatment of nets

| Date | Experimental village | | Control village | |
	No. dissected	No. with oocysts	No. dissected	No. with oocysts
February	12	0	12	0
March	10	0	32	0
April	59	1	25	0
May	10	0	37	0
June	28	0	22	0
July	15	0	38	2
August	19	0	34	0
September[1]	7	0	7	0
October	8	0	14	0
November	23	1	17	1
December	19	0	30	0
January	19	0	6	1
February	26	0	8	0
March	4	0	0	0

[1]post-impregnation September to March

Table 4: The number of *A. koliensis* with oocysts, from whole-night biting collections before and after treatment of nets

| Date | Experimental village | | Control village | |
	No. dissected	No. with oocysts	No. dissected	No. with oocysts
February	127	4	204	7
March	254	7	264	5
April	227	6	251	6
May	227	5	233	4
June	248	8	319	9
July	68	4	179	7
August	39	1	5	0
September[1]	11	0	9	0
October	25	1	29	0
November	147	7	83	3
December	313	5	272	5
January	322	3	306	1
February	374	11	261	10
March	339	10	196	2

[1]post-impregnation September to March

Table 5:   The number of *A. punctulatus* with oocysts, from whole-night biting collections before and after treatment of nets

| Date | Experimental village | | Control village | |
------	No. dissected	No. with oocysts	No. dissected	No. with oocysts
February	35	0	40	0
March	48	0	50	0
April	40	0	12	0
May	21	0	18	1
June	28	2	31	1
July	16	2	13	1
August	33	0	12	0
September[1]	41	3	41	1
October	127	4	77	1
November	84	3	84	0
December	39	0	57	0
January	20	0	40	1
February	11	1	17	1
March	18	3	12	2

[1]post-impregnation September to March

Table 6:   Oocyst rates of *A. farauti*, *A. koliensis* and *A. punctulatus* combined, from whole-night biting collections before and after treatment of nets

| Date | Experimental village | | Control village | |
------	No. dissected	Oocyst rate %	No. dissected	Oocyst rate %
February	174	2.3	256	2.7
March	312	2.2	346	1.4
April	326	2.1	288	2.1
May	258	1.9	288	1.7
June	304	3.3	372	2.7
July	99	6.0	230	4.3
August	91	1.1	51	0
September[1]	59	5.1	57	1.7
October	160	2.5	120	1.3
November	254	4.3	184	2.2
December	371	1.3	359	1.4
January	361	0.8	352	0.8
February	411	2.4	286	3.8
March	361	3.6	208	1.9
TOTAL[1]	1977	2.6	1566	1.8

[1]post-impregnation September to March

sporozoite rate was 0.48%; eight mosquitoes were positive for sporozoites of *P. falciparum* and four mosquitoes were positive for sporozoites of *P. vivax*. The highest monthly sporozoite rate of 1.1% was recorded during a period of low rainfall in early 1985. As observed with the oocyst rates, the sporozoite rates were generally low following periods of heavy rain, and increased as the population aged during the drier months.

The entomological inoculation rate is defined as the number of bites/man/night from mosquitoes with sporozoites. The rate is defined in this study as the number of *P. falciparum* or *P. vivax* sporozoite-positive bites received by one person in one night and is computed from the man-biting rate times the sporozoite rate for the months June 1984 to February 1985 during which samples were taken. The entomological inoculation rate was calculated to be 1.2 infected bites/man/night, from a sporozoite rate and man-biting rate of 0.47% and 257 respectively for *A. koliensis*. The total entomological inoculation rate is the sum of inoculation rates from each vector species. Assuming a similar sporozoite rate of 0.47% for *A. punctulatus*, the contribution of this species to the total inoculation rate over the same period would have been 0.18. The contribution of *A. koliensis* and *A. punctulatus* to the total entomological inoculation rate was therefore 1.38 sporozoite-positive bites/man/night. This rate is exaggerated because sampling man-mosquito contact by whole night-biting collections is considered to give an over-estimation of the true man-biting rate. This is particularily true in Gonoa where for the second half of the night, most people in the village are sleeping under mosquito nets.

Although the entomological inoculation rate can be used to describe a particular malaria situation, intrinsically it is of little value in estimating the number of inoculations which result in successful transmission of malaria. This is known as the parasitological inoculation rate and is determined from malariometric surveys on large samples of infants. In the extreme, the parasitological inoculation rate, which is a measure of the number of successful inoculations, may be 100 times below that predicted by the entomological inoculation rate because of factors such as low viability of sporozoites, low numbers of sporozoites inoculated, the immune status

of the host (Macdonald 1957) and over-estimation of the man-biting rate.

6.1.6 Head Lice

A retrospective survey was conducted to determine if there was a difference in the prevalence of head lice in the control and experimental villages. It is known that the prevalence of head lice is somewhat dependent on age and sex with the prevalence on children being higher than on adults, and the prevalence on females being higher than on males. This is due, in part, to the habits of people; children are often in close contact with each other and sleep in groups, and girls typically have longer hair than boys. In this investigation, the prevalence rates were compared in the greater than 14 years age group, and the 14 years and under age group. This roughly separates children and young adolescents who tend to share bed nets, from adults. The prevalence of head lice on males and females for all ages combined was similar, 48.9% and 53.6% respectively, and therefore the sexes were combined for comparison within age groups in the two populations. Three children with shaven heads, two from the control village, were not included in the study although it is probable that their heads were shaved because of lice.

The results of the survey show that the prevalence rates were significantly lower in the experimental village in all groups except the >14 years age group (Table 7). The results indicate, as the people suggested, that bed nets impregnated with permethrin reduced the prevalence of head lice. A follow-up of the people with nits or lice in the experimental village revealed that at least four were from non-compliant households and were not using impregnated bed nets. One female with lice reported that her head lice had been controlled, but she was reinfested while staying with relatives in another village.

Table 7: The prevalence of head lice, *Pediculus humanis capitis*, 20 weeks after impregnating bed nets with permethrin

	Percentage with Nits and Lice (n)		
Age group (years)	Experimental village	Control village	P values
<15	6.1 (66)	62.8 (48)	<0.001
15+	26.1 (38)	43.7 (48)	>0.05
All ages	13.6 (104)	52.7 (91)	<0.001
	Percentage With Lice Only (n)		
All ages	6.7 (104)	25.3 (91)	<0.001

6.1.7 Bioassay Tests

The objective of the bioassay test was to determine the duration of residual effect of permethrin applied to bed nets. The nets used throughout the testing period were the cotton type used by all the villagers (Figure 10). The results show that up to 195 days after impregnation, mortality remained greater than 90% following 30 minutes exposure (Table 8). Similar results have been reported elsewhere; laboratory tests in Malaysia with *A. maculatus* released into cotton and nylon nets ten months after impregnation with permethrin, gave 100% kill at both 30 and 10 minute exposure periods (Thevasagayam pers. comm.). In west Africa, bed nets impregnated with permethrin at 0.08 g/m² gave excellent residual effect for up to 5 months (Darriet *et al.* 1984).

A survey of the number of mosquito nets in the village was conducted in January 1984, to enable an estimate of the people:bed net ratio. The results showed that the average number of people per bed net was about 3.6:1; a large net was considered the equivalent of two small nets. To decrease the ratio in both experimental and control villages, mosquito nets were bought at wholesale price in Madang for K3.50 (1 Kina = 1 $US) and resold through village tradestores for K1.20. This was a considerable saving over the retail price of K4.50–K6.50 and proved very popular with the villagers. The ratio of people:bednet was decreased to about 1.9:1, at the time nets were impregnated in late August 1984, and to about 1.6:1 by December 1984.

To estimate the number of villagers using mosquito nets, the people were asked during the malaria surveys if they had slept under a net during the previous night. The self-reported use of nets for the November 1984 and April 1985 surveys combined, was 96.1% and 94.3% for the experimental and control villages respectively. The reported use was highest in the 0–10 year age group (98.7%) and lowest in the 21 years and greater age group (93.5%). In family households, it was observed that mothers always shared their bed nets with infants and young children. Most of the bed nets were small, and under such crowded conditions, the effectiveness of bed nets was undoubtedly diminished.

In the experimental village, a total of 196 bed nets were treated with permethrin at 0.2 g/m^2, on thirteen different days. There were 174 nets impregnated by September 7; the remaining were new nets brought into the village and treated after that date, or were bed nets brought to me by persons who were initially non-compliant to having their nets impregnated. If a family household did not bring their nets on any of the thirteen days during which nets were treated, the household was considered non-compliant. Five of the 43 family households and several adolescent males living in the bachelor houses were non-compliant.

All bed nets were 100% cotton, most made of the fabric type shown in Figure 8 (sample B). A sleeping room with the standard size mosquito nets is shown in

Figure 10. Of the total number impregnated, 158 (80.6%) were small nets 1.8m x 0.9m x 0.9m (6ft x 3ft x 3ft); 30 nets (15.3%) were large nets of about 1.8x x1.8m x1.2m (6ft x 6ft x4 ft); the remaining 4% were of various sizes and fabric weights. Only eight (4.1%) of the total number of nets treated had holes that were noticeably large; many nets with holes had been repaired and were in excellent condition. There were no difficulties associated with bed net impregnation and no complaints or symptoms of intoxication or skin irritation. Most people questioned stated that nuisance bites from bed bugs had decreased while they slept under the bed nets, but no investigation was undertaken to verify this. Bed bugs were seen on many of the mosquito nets when the nets were being impregnated.

Although overall compliance was good, initially there was an attitude of indifference toward bed net impregnation, and much time was spent getting some of the villagers to bring their bed nets to be impregnated. This was in contrast to the attitude at the end of the project. As part of the agreement made with the people in the control village, their bed nets were impregnated when evaluation of the project was completed. On the day specified for impregnation, virtually all bed nets from the hamlets were brought to be impregnated, and all had been recently washed. The excellent response was undoubtedly because of the effect permethrin had on head lice and bed bugs in the experimental village. It is well documented that the initial acceptance of malaria control programs by villagers from many areas around the world, was because of the effect residual insecticides had on secondary target pests such as head lice, bed bugs and cockroaches (Gramiccia 1980). It is also known that later rejection of the programs was often associated with population increases of the same pests once they developed resistance to the insecticides.

In cooperation with a small clothing manufacturing company in Madang, an investigation was carried out to determine if mosquito nets could be made in Madang at a cost-effective price. Although it is not possible to estimate accurately the cost per bed net, we determined that they could be made for about the same as the wholesale price for imported nets in Madang if either material A or B (Table 9, Figure 8) was used. The estimate was based on urban minimum wage costs. It

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Table 8: Bioassay tests with mosquitoes of the *A. punctulatus* complex, exposed for 30 mintues to bed nets impregnated with permethrin

Date	Days after treatment	Impregnated nets			Control nets		RH	°C	Species
		N	% Kd 30 min	% mort 24h	N	% mort 24h			
19-9-84[1]	5	61	100	**100**	23	**13.0**	74	28	A. farauti
6-10-84[1]	22	111	100	**100**	38	**5.3**	70	28.5	A. farauti
28-10-84[1]	44	107	100	**100**	44	**6.8**	30.5	-	A. farauti
28-10-84[2]	44	113	100	**99.2**					A. farauti
16-12-84[1]	93	70	97.1	**96.9**	51	**2.0**	79	30.0	A. koliensis
16-12-84[2]	93	73	100	**98.6**					A. koliensis
14-3-85[1]	165	50	96.0	**90.0**	57	**13.0**	-	29.5	A. koliensis
14-3-85[2]	165	40	100	**97.5**					A. koliensis
12-4-85[1]	195	47	95.7	**93.6**	26	**7.6**	-	28	A. koliensis

[1]bed net from house #2090
[2]bed net from house #1160

was suggested that if rural wages were applied to labor costs and if machinery were purchased to facilitate manufacturing the nets, the cost of production could be substantially lowered. One of the mosquito nets made at the clothing company and used in Gonoa village is shown in Figure 9. The bed net was made of polyethylene material, similar to that shown in Figure 8, sample A; the lower 50 cm was made from a cotton-synthetic fabric. Although the nets were liked by the people, the material was unsuitable for village conditions; within 8 weeks the nets had many holes and the people were again using their cotton nets. Sample C (Figure 8) is the same type of material but with a tighter stitch and twice as many filaments. It is much stronger and would probably be appropriate for village conditions. Of all the samples, I consider the cotton fabric (Figure 8, sample B) to be most appropriate. The fabric weight is considerably lighter than that of most of the bed nets in the village, and allows for adequate ventilation. The material is inexpensive and seems to be very durable under village conditions. Since only one material would be used for making each mosquito net from this fabric, labor costs would be less than if the bed nets were made from a light synthetic material which would necessitate having a lower border made of a heavier material.

As a method for reducing bites from mosquitoes, bed nets are appropriate, highly valued and used by virtually all people in Gonoa as well as by most people in the the PNG IMR study area. The 0-10 years age group, and particularly infants and children less than 5 years, have the most potential benefit from mosquito net use because they spend most of the night under bed nets in an area where the vectors are primarily late-night biters. Futhermore, in an area hyper- or holoendemic for malaria, infants and children are the highest-risk age group because their immunity is not well developed. Unfortunately, the effectiveness of bed nets was diminished in this age group, because of crowded conditions. Although most people in the study area report using mosquito nets, I consider the small size of most of the bed nets as being a very important factor in limiting their effectiveness (Figure 11).

Table 9: The cost of various fabric types used to manufacture mosquito nets

Sample	Material	Cost/metre^2 $US	Width of bale (feet)	Manufacturer
A[3]	polyethylene	0.113 CIF[1]	6	Lokumal Co. Taiwan
B[3]	cotton	0.185 CIF	6	Yat Hing Co. Hong Kong
C[3]	polyethylene	0.236 FOB[2]	7	Kevin Impex Sinapore
D[3]	cotton	0.286 FOB	7	Kevin Impex Singapore
E[3]	nylon	0.406 FOB	6	Chellaram Hong Kong
F[3]	nylon	0.618 FOB	7	Kevin Impex Singapore

[1]CIF—cost, insurance, freight
[2]FOB—free on board
[3]Fabric types shown in Figure 8.

Figure 8:   Fabric types used to manufacture mosquito nets
(described in Figure 9)
(0.8:1 reproduction)

A

B

C

D

E

F

53b
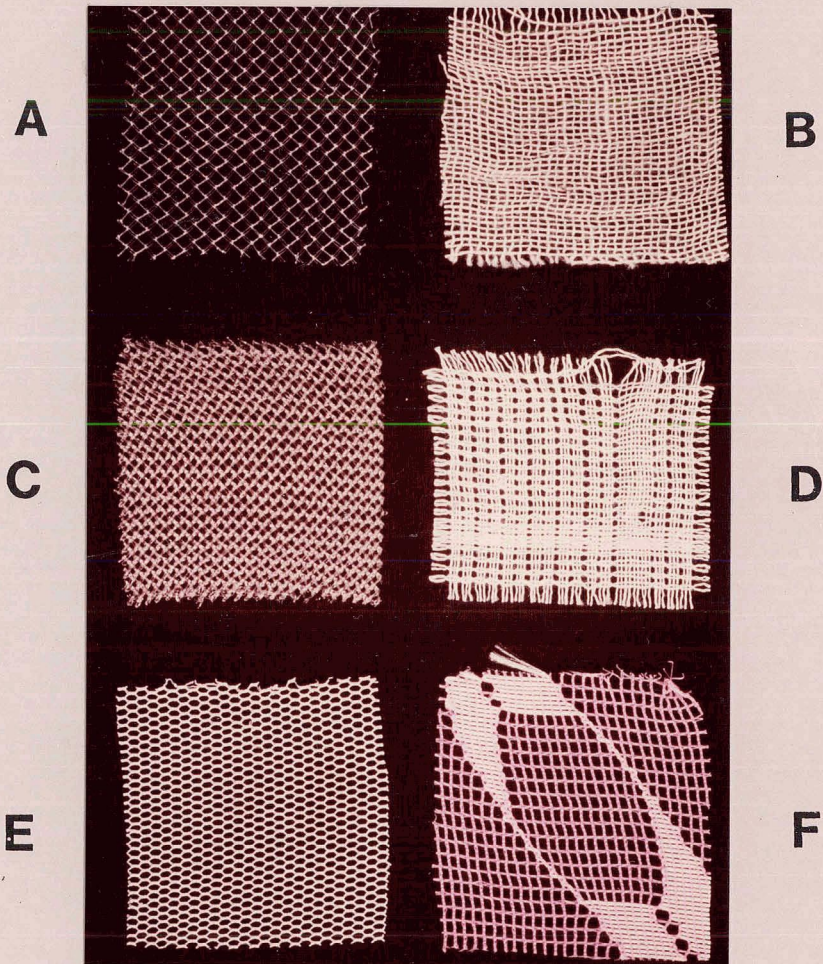
Figure 9:   A mosquito net manufactured in Madang and made of polyethylene netting with a cotton-synthetic fabric base

Figure 10:   A sleeping room in Gonoa with the standard
              small cotton mosquito nets

Figure 11:   One of two young girls sleeping, under a mosquito
              net but receiving little protection from it

6.2 Parasitology

6.2.1 Independent Re-examination of Slides by the Microscopy Supervisor

About 30% of the slides examined for parasites by the microscopists were re-examined by the microscopy supervisor without knowledge of the previous results. For detecting asexual stages of *P. malariae* and gametocytes of *P. falciparum*, the microscopists were less sensitive than the microscopy supervisor and thus the percentage positive as reported by the supervisor was slightly greater than that reported by the microscopists (Table 10). For the asexual stages of both *P. falciparum* and *P. vivax*, the percentage positive as reported by the microscopists and the supervisor were nearly the same. The number of slides reported positive by the microscopists only, was slightly higher for *P. falciparum* and about equal for *P. vivax*, to the percentages reported positive by the supervisor only. Most of the slides reported positive by the microscopists only were false positive recordings from slides of low parasite densities. Overall, the agreement between the microscopists and the supervisor was very good, and for the analysis of parasite rates in this study, the results as recorded by the microscopists were used.

Table 10:   Results of the re-examination of 531 slides by the microscopy supervisor

	% positive of re-examined slides as reported by		% positive of re-examined slides as reported by	
	microscopists	supervisor	microscopists only	supervisor only
P. falciparum[1]	62.9	63.6	2.4	1.3
Pf gametocytes	9.4	10.2	0.38	1.13
P. vivax[1]	24.9	24.3	1.5	1.7
P. malariae[1]	6.8	7.7	0.18	1.1

[1]asexual stages only

6.2.2 Administration of Chloroquine and Sulphadoxine-pyrimethamine

The number of people that were to be treated with a combination of chloroquine or amodiaquine and sulfadoxine-pyrimethamine was 271. This represented all the people positive for any species of malaria in survey 1. Seven people from the experimental village and nine from the control village were either away for an extended period or were non-compliant and did not receive treatment; all were adults. A further 2 people from the experimental village, both children, left the village before the treatment date and did not return for several months. As sulphadoxine-pyrimethamine was administered on the first day of treatment, all people received it, plus at least a single dose of chloroquine; 8.4% of the people did not complete the full three day treatment of chloroquine. Several complained of side effects from the administration of drugs; dizziness and vomiting were the most common complaints.

A follow-up survey to monitor the clearence of parasitaemia was conducted 14 days after drug administration on 120 people chosen at random from the study population. Parasites were cleared in 119 of 120 cases. The one positive case, an eight year old male who had received full treatment of both chloroquine and sulfadoxine-pyrimethamine, had a low density *P. falciparum* parasitaemia.

6.2.3 Parasitological Evaluation of the Control Strategy

Parasitological observations of the people from the experimental and control villages were made over a period of eight months, during which time there was a series of three surveys. Survey 1, the pre-control survey which provided the baseline data on the parasite prevalence, took place at the end of the dry season in August 1984. Surveys 2 and 3, the evaluative surveys, took place at the beginning and end of the wet season in November 1984 and April 1985 respectively.

The parasite rate is defined as the percentage of a sample of the population showing malaria parasites in the blood. The malaria parasite rates (for any species of malaria) in all age groups combined are shown in Table 11. Although the rates of the experimental and control populations were not significantly different in any of the surveys, the prevalence decreased from 54.2% to 41.6% in the experimental population from survey 1 to survey 2. During the same period, the crude parasite rate in the control population increased slightly. At the end of the wet season (survey 3), the parasite rate in the control population was the highest recorded for either population during the study, and in the experimental population was slightly below the rate recorded in survey 1.

The parasite formula indicates the relative prevalence (percentage) of the various species of malaria. From the malaria prevalence of both the experimental and control populations combined in survey 1, the formula was 75:18:7 for *P. falciparum P. vivax* and *P. malariae* respectively. In survey 2, the first survey after bed net impregnation and drug administration, there was a decrease in the predominance of *P. falciparum* and a relative increase of *P. vivax* infections in both villages; the prevalence of *P. malariae* was reduced to one infection in the experimental village, while in the control village there was a slight increase from six to seven infections. There were no *P. malariae* infections in the 0–4 age group in either population during the entire study and the results from surveys 2 and 3 show that none of the ten *P. malariae* infections recorded in the experimental population was from the 0–10 years age group. By April (survey 3), the parasite formula in both villages was again similar to that observed in survey 1.

The parasite rates of *P. falciparum* asexual stages for all age groups combined, are shown in Table 12. Survey 2 was conducted about three months after the people positive for malaria in both villages were successfully treated with anti-malarial drugs. The results show that the parasite rate of *P. falciparum* in the control village was just slightly below that recorded during the baseline survey. A return to pre-drug treatment prevalence rates within three months is not unexpected in an area of intense malaria transmission. In the experimental population, the prevalence of *P. falciparum* asexual stages decreased from 46.4% (survey 1) to

30.8% (survey 2), however, the difference between the rates of *P. falciparum* in the two populations (survey 2) was not significant. By the end of the wet season (survey 3), the prevalence of *P. falciparum* asexuals in the experimental population was slightly less than that recorded in survey 1, and in the control population the prevalence was the highest recorded for either population during the study.

The age-specific parasite rates for any species of malaria (crude), and for *P. falciparum* asexual stages only, are presented in Figures 12 and 13 respectively. Because most infections were of *P. falciparum*, the curves in the two figures are similar. The shape of the curves as shown by a pronounced increase in prevalence after 12 months, followed by a gradual decrease in older age groups, is typical for areas hyper- or holoendemic for malaria. The overall greater prevalence in the experimental population during survey 1 (Table 11), was observed in all age groups except for the 0-12 month (infant) age group (Figures 12 and 13). The infant parasite rate is an important epidemiological parameter because it provides an index of the liability to contract an infection in a given locality. However, because the number of infants in this study was small, and varied over the three surveys, the infant parasite rates do not provide an adequate basis for comparing malaria transmission in the two villages. The infant parasite rates from the experimental and control populations for surveys 2 and 3 combined, were 13.6% (n=22) and 17.5% (n=20) respectively. The rates are similar to those observed in the PNG IMR study area in that they were relatively low. Cattani *et al.* (1985) reported infant parasite rates of 21.2% in the dry season and 16.7% in the wet season. They suggested that these rates are lower than might be expected in an area where parasite rates in children of one to nine years are consistently greater than 50%.

Although the experimental population's prevalence of *P. falciparum* decreased in all age groups from survey 1 to survey 2, the experimental and control age specific prevalence of *P. falciparum*, as recorded in survey 2 and survey 3 (Figure 13), shows little difference in prevalence rates in most age groups except the younger age groups. In the younger children, particularly the 1-4 age group, the prevalence rates in the experimental population were lower than those recorded in the control population. The rates between villages in the 1-4 age group were not

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Table 11: The prevalence (%) for any of *P. falciparum*, *P. vivax*, *P. malariae* trophozoites or *P. falciparum* gametocytes in all age groups combined, before and after impregnating bed nets with permethrin

	Survey	Percentage of people positive (N)	
		Experimental	Control
1	Aug.'84	54.2 (295)	48.6 (207)
2	Nov.'84[1]	41.6 (286)	49.5 (218)
3	Apr.'85[1]	52.3 (281)	55.3 (206)

[1]post-impregnation

Table 12: The prevalence (%) of *P. falciparum* trophozoites in all age groups combined, before and after impregnating nets with permethrin

	Survey	Percentage of people positive (N)	
		Experimental	Control
1	Aug.'84	46.4 (295)	40.6 (207)
2	Nov.'84[1]	30.8 (286)	37.6 (218)
3	Apr.'85[1]	44.1 (281)	51.0 (206)

[1]post-impregnation

Figure 12:   The age specific prevalence (%) for trophozoites of
               P. falciparum, *P. vivax*, *P. malariae* or gametocytes
               of *P. falciparum* in the experimental and control
               villages, before (survey 1) and after
               (surveys 2 and 3) impregnating bed nets with
               permethrin

survey 1

survey 2

survey 3

Legend
+ EXP ----
+ CON
+ .

p<0.05

Percentage positive for *P. falciparum P. vivax* and *P. malariae*

Age Groups in Years

<1 1-4   5-9   10-14   15-20   21+ →

61b
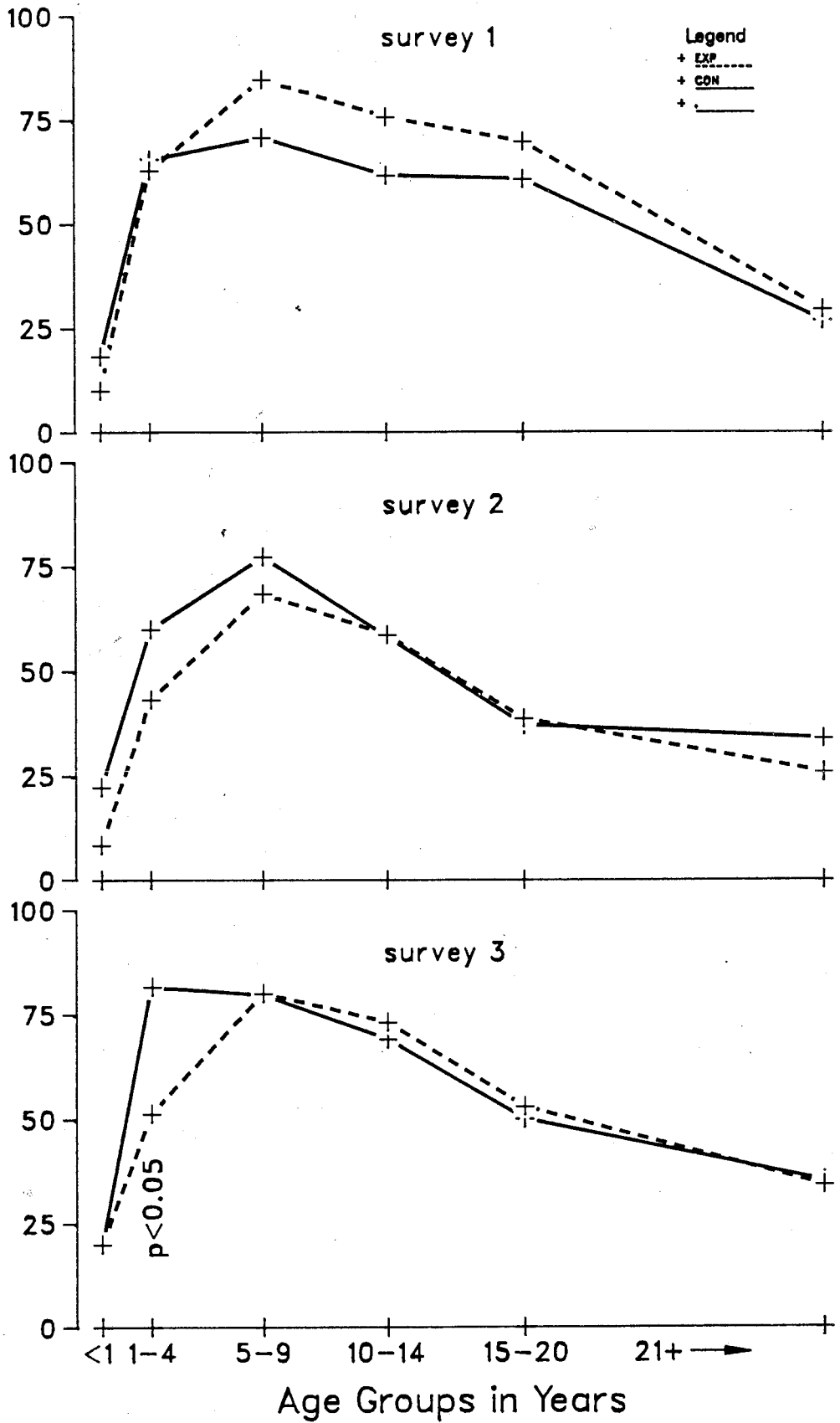
Figure 13:   The age specific prevalence rates of *P. falciparum* before (survey 1) and after (survey 2) impregnating bed nets with permethrin

Percentage positive for *P. falciparum*

survey 1

survey 2

survey 3

Legend
+ EXP ......
+ CON ——
+ . ——

p<0.05

Age Groups in Years

<1 1-4   5-9   10-14   15-20   21+ →

62b
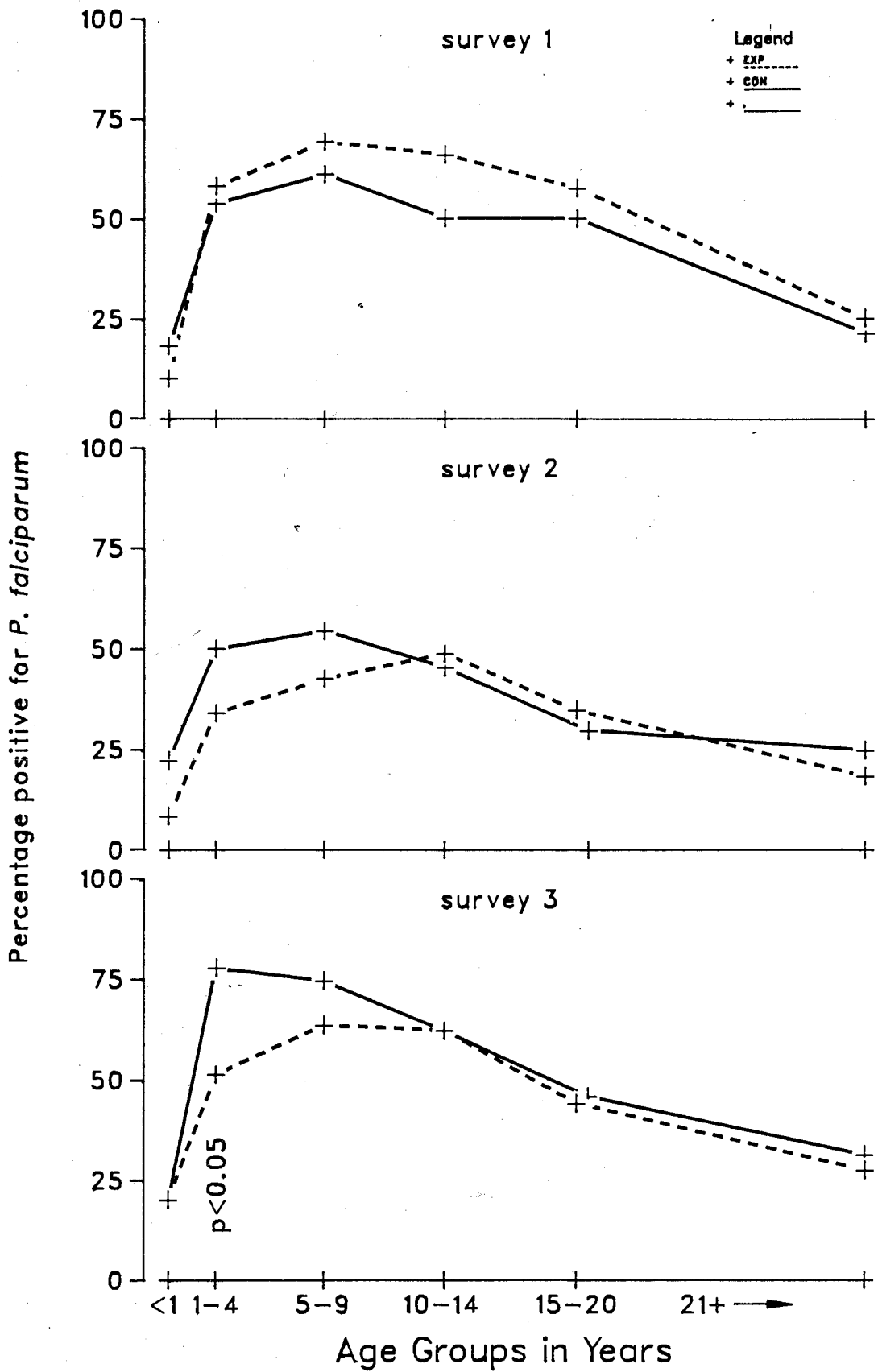
significantly different in survey 2, but were significantly different in survey 3 as indicated on Figure 13. At the end of the wet season (survey 3), the 1-4 age group from the control population had the highest prevalence of *P. falciparum* (77.7%) recorded during the study.

6.2.4 Gametocyte Rates

The gametocyte rate is defined here, as the proportion (%) of people with gametocytes of *P. falciparum*. The gametocyte rates for all age groups combined in the experimental and control populations are shown in Table 13. In August (survey 1), the gametocyte rate was slightly higher in the experimental population, and in both surveys after bed net impregnation the rates were lower in the experimental population; the difference was significant in survey 3.

As observed in both the PNG IMR study area (Cattani *et al.* 1985) and in this investigation, *P. falciparum* gametocytes occurred most frequently in the one to four years age group. Results of the combined populations from survey 1 show that 42.1% (8/19) of the gametocyte carriers were from the one to four years age group and they comprised about 14% of the study population at that time.

6.2.5 Parasite Density Index

The parasite densities were recorded as described in Methods and were graded from 1 to 8 according to the schema shown in Appendix B. The parasite density index was determined for trophozoites of *P. falciparum* only, and was computed as follows:

The prevalence (%) of *P. falciparum* gametocytes for all age groups combined, before and after impregnating bed nets with permethrin

	Percentage of people positive (N)		
Survey	Experimental	Control	P
1 Aug.'84	4.1 (295)	3.4 (207)	
2 Nov.'84[1]	1.7 (286)	4.6 (218)	<0.1
3 Apr.'85[1]	3.6 (281)	7.3 (206)	=0.01

[1]post-impregnation

Σ(the number of people in each density class) X (the class

number) ÷ number of people with *P. falciparum* positive slides

The *P. falciparum* density indices from the experimental and control populations are shown in Table 14. They were not tabulated for age specific groups and for an investigation of this type and a population of this size, the parasite density index is not an important parameter to measure. Analysis of the densities did reveal, however, that the highest density index from the experimental group was recorded during survey 1 when the prevalence of malaria was at the lowest level. From a review of the parasite densities recorded during survey 2, it was determined that there were an unusually large number (9) of high density grade 8 infections. It was found that the sources of some of these high density infections were a mother and her two children who were sharing the same mosquito net. The three were from a household that was non-compliant with the bed net impregnation program. Two other grade 8 parasitaemias were from two young sisters from another household. They were also sharing the same net, and a third child using the net had a grade 6 density parasitaemia. Although it is suspected that their net was not impregnated, it could not be determined for certain. All five of the children had previously been

Table 14:   The parasite density index of *P. falciparum*
             trophozoites before and after impregnating bed nets
             with permethrin

Survey	Experimental	Control
1 Aug.'84	2.99	3.14
2 Nov.'84[1]	3.25	2.85
3 Apr.'85[1]	3.18	3.35

[1]post-impregnation

treated with chloroquine and pyrimethamine-sulfadoxine in August.

6.3 Spleen Rates

The spleen rate is defined as the proportion of enlarged spleens in a sample population. The following description of the response of the spleen to malaria and the importance of spleen measurements is given by Black (1968).

> In a single infection and clinical attack of malaria, the spleen becomes enlarged but with effective treatment recedes fairly rapidly to its normal size, in which it is not palpable. Persisting untreated infections result in a more pronounced and longer lasting enlargement of the spleen. The spleen rate is therefore a valuable measure of endemic malaria since it is not so liable as the parasite rate to seasonal changes.

The spleen rate is usually determined in the 2-9 age group because when the immune response to malaria is building, enlargement of the spleen is greatest. The spleen rates of the age groups greater than 10 years are included in this study because people positive for malaria in all age groups were treated for malaria prior to the implementation of the control strategy. Furthermore, adult spleen rates in the PNG IMR study area appear to be relatively high. Cattani et al. (1985) reported adult spleen rates greater than 25%, and a measurable response to a control program might therefore be expected.

The spleen rates recorded during the three surveys are presented in Table 15. Compared to the rates recorded in the PNG IMR study area (Cattani et al. 1985), the rates in all age groups, particularly the adult age group, were very high. This was observed in both populations and in all three surveys. The spleens were measured by a staff member of the PNG IMR who had many years experience as a trained aid post orderly (APO). To ensure measurement was conducted properly, spleens were initially measured by the APO and a medical officer together. These results were then compared with measurements of spleens made by the APO alone on another day from the same population. Although the difference in all age groups from 2-20 years, was negligible, the adult spleen rates (>20 years) were over-estimated by 10 to 15%. Adult spleens are difficult to measure because of the greater musculature development. The main errors made are to mistake the outer end of the left rib or a hard faecal bolus in the large intestine for the enlarged spleen (Bruce-Chwatt 1980). Since the spleens were measured by the same person throughout this study, spleen rates are compared, even though the adult rates were

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probably over-estimated in both the experimental and control populations.

The spleen rates for the 2 years and older age groups combined were not significantly different in survey 1 but were significantly different in both surveys after implementation of the control strategy. Whereas the difference in survey 2 was highly significant, $p < 0.0001$, the difference in survey 3 was much less significant, $p < 0.025$. In the 2-9 age group, there was no significant difference in survey 1. In survey 2, the rates were significantly different, $p < 0.025$, however, at the end of the wet season in survey 3, the rates were again near 100% in both populations.

Table 15: Percentage with enlarged spleens from experimental and control populations before and after impregnating bed nets with permethrin

Age Groups	Survey 1		Survey 2[1]		Survey 3[1]	
	Exp % (N)	Con % (N)	Exp % (N)	Con % (N)	Exp % (N)	Con % (N)
2–9	100 (80)	100 (61)	81.5 (81)	96.8 (62)	97.5 (80)	98.3 (58)
10–20	94.3 (70)	96.2 (52)	83.7 (64)	95.4 (57)	92.3 (65)	100 (52)
20+	83.0 (100)	88.4 (69)	61.1 (90)	82.41 (67)	80.0 (90)	93.0 (71)

[1]post–impregnation

DISCUSSION

7.0.1 Other Methods For Personal Protection Against Mosquitoes

The goal of this project was to identify, implement and evaluate a method for protection against the vectors of malaria, that would be village-based, community-supported and would have potential application on a regional scale. Initially, a considerable amount of time was spent with the villagers, discussing various approaches that might be used. Mosquito-proofing rooms in houses was considered possible, but was not attempted because the people in Gonoa, as in most coastal areas in the country, spend virtually all non-sleeping hours in places that are difficult, if not impossible to mosquito-proof. For protection during sleeping hours, major repairs would be necessary to the floors and walls, in addition to constucting ceilings in every house, and this type of effort would have little application on a regional scale.

Also considered, was the use of mosquito coils as part of an overall strategy. The efficacy of mosquito coils (Zebra Brand, China, active ingredient not stated) in reducing man/mosquito contact was evaluated in Maraga, a village about 8 km from Gonoa (Charlwood and Jolley 1984). When used in sleeping rooms, the coils were observed to reduce the number of biting mosquitoes by a factor of about ten. Although they are readily available in the Madang area and relatively inexpensive for a wage-earner to purchase, I have not observed mosquito coil use in any rural households, and presumably, the people do not perceive the benefit from mosquito coils as being worth the cost.

Source reduction through community effort was not encouraged, although the people were aware that mosquitoes breed in water and that mosquitoes transmit malaria. Eliminating breeding sites was left to the discretion of the individual and there was some minor success at this level. One very prolific breeding site for anopheline and culicine mosquiotes was near a village water-supply tap. People with houses in the area began filling the pool with vegetative waste, and the breeding site was eventually eliminated. It is probable that a well-organized community effort to eliminate breeding sites could have measurably decreased the

density of *A. punctulatus*. Populations of other species would have been little affected and the resultant decrease in density would not have been perceptible to the people, nor would it have had any significant effect on the community prevalence of malaria. If an area can be identified as an important breeding site and if it can be permanently eliminated, then an effort should be made to so. But, to expect a community to become engaged in an effort that does not provide good results, and must be sustained over time, is counter-productive to the goals of malaria control as the credibility of the program will inevitably be questioned, and the success of future efforts will be jeopardized.

7.0.2 Impregnated Bed Nets As An Alternative Method For Protection Against the Vectors of Malaria

The emphasis of this investigation was on the implementation and evaluation of bed nets impregnated with permethrin. The method was chosen because research over the past decade has shown that fabric impregnated with permethrin is effective as a personal protection measure against biting arthropods. Furthermore, the technique was used to enhance a method of protection that was already accepted by the people and therefore had good possibilities for further application on a larger scale. The strategy was used to complement the existing program of drug distribution for the presumptive treatment of malaria, which is an essential part of the primary health care system in PNG.

Entomological evaluation of bed net impregnation with permethrin as measured by mosquito density, delayed oocyst rates and parous rates, did not demonstrate any effect on the vector population. These results must, however, be discussed in the context of the project's limitations, the bionomics and behavior of the vectors, the epidemiology of malaria in the study area and perhaps most important, what the expected response of the vector population to the strategy should be.

The first consideration will be given to the problem of immigration of mosquitoes into the experimental area from areas not under the control strategy.

Although it is difficult to assess what effect this had on both entomological and parasitological parameters, the results of capture-recapture experiments conducted in Gonoa and in several villages in the PNG IMR study area, indicate that vector populations do not disperse widely, and that they exist in close association with human populations. Service (1976) states, that in favorable conditions it is rare for as many as 5% of marked-released specimens to be recovered. From experiments in the Madang area, observed recapture rates were consistently above 5% and as high as 17.6% (Charlwood, unpublished results). In one experiment, 6.3% of the total *A. koliensis* mosquitoes that were released, were recaptured; 74 were recaptured in the village of release and 2 in a village that was 1.5 km away. A capture-recapture experiment was also conducted in hamlets 1 and 2 of Gonoa which are separted by 0.7 km of bush. Released mosquitoes were marked with different coloured dusts, one colour from each hamlet. Of the 42 mosquitoes recaptured (4% of the total released), 77% were were collected in the hamlet of release, and 24% in the opposite hamlet (Millen and Charlwood unpublished data). Except for the hamlets 4, 5 and to a lesser extent 3, the distance between the hamlets of experimental and control populations was probably greater than the normal flight range of the vectors, and consequently, the anopheline fauna surrounding those hamlets were probably well isolated geographically. Immigration of mosquitoes into the experimental area undoubtedly did occur, but in consideration of all factors which determined the effectiveness of impregnated nets, the immigration of mosquitoes was probably of lesser importance than other factors such as the vector's behavior.

Concerning the study area in general, it is characterized by intense transmission of malaria that is supported by a vector density that remains very high throughout most of the year. The three different members of the *A. punctulatus* complex that are found in the study area, are efficient vectors of malaria and although there is no evidence to suggest separate endo- or exophagous sub-populations (Charlwood 1985, Charlwood *et al.* 1985) their longevity can sustain intense transmission throughout the entire year. The vectors are highly anthropophilic but will feed both indoors and outdoors, generally following movements of the host populations. As people spend their non-sleeping hours

outdoors or in locations such as on verandahs which are considered outdoors, there is significant outdoor contact with man and this would tend to lessen the effect of impregnated bed nets. The exophagic behavior of mosquitoes limits the effectiveness of any vector control program based on the indoor application of a residual insecticide. This was cited as one of the reasons for the failure of DDT residual spray in PNG (Thevasagayam 1984) and it obviously imposed limitations on the effectiveness of impregnated bed nets in this study.

In addition to the exophagic behavior of the vectors, other behavioral characteristics, such as resting behavior, undoubtedly influenced the efficacy of impregnated bed nets. In Africa, experimental hut studies have shown that impregnated bed nets have two major effects on the vector population, namely, repellency and mortality (Darriet *et al.* 1984, Lines *et al.* 1985). I have further classified these effects as follows:

1. Mortality (immediate or deferred) caused by contact with impregnated bed nets after the mosquito has fed *i.e.* when it is resting.

2. Mortality (immediate or deferred) caused by contact with impregnated bed nets when the mosquito is attempting to feed.

3. Feeding inhibition caused by contact with the bed nets.

4. Repellency.

5. Sublethal effects.

I will not consider the last factor except to mention that responses such as inhibition of egg laying have been observed when insects are exposed to sublethal doses of synthetic pyrethroids (Carter 1983). The first effect listed above is related to resting behavior. It can be assumed that if bed nets were a favoured resting place for mosquitoes, and if they were not repelled, there would be significant contact with impregnated bed nets after the mosquitoes obtained a bloodmeal. In west Africa, Boreman and Port (1982) studied the distribution and movement of bloodfed *Anopheles gambiae* Giles s.s. and found that over 50% collected were resting on bed nets. This vector is known to spend much of its gonotrophic cycle indoors, and it would be expected that some of the mosquitoes in the bloodfed state would come in contact with impregnated bed nets. Members of the *A. punctulatus* complex, however, are primarily exophilic and spend most of their

gonotrophic cycle outdoors. They will often rest on walls before leaving a house at daybreak, but, bed nets are not preferred resting sites.

Although entomological evaluation showed no effect of bed net impregnation, the evidence from human parasitological indicies was consistent over both evaluative surveys in demonstrating that there was greater protection in the experimental population. In survey 2, the spleen rate for all age groups combined was significantly lower in the experimental population when compared to spleen rates in the control population, and the lower rates were observed in all age groups indicating that initially, there may have been some degree of protection in all age groups. The crude parasite rates after bed net impregnation were lower in the experimental village when compared to the control village. The asexual parasite rates for *P. falciparum* were lower in the experimental village after impregnation. It was observed in both evaluative surveys that younger age groups seemed to have had greater protection from transmission than older age groups (Figure 13). Furthermore, the prevalence of *P. falciparum* gametocytes was lower in the experimental population during both surveys after bed net impregnation and the difference in prevalence between the two populations was significant in survey 3.

With the evaluation of the project showing an effect in the human population but not in the mosquito population, and the results of the bioassays indicating that there was excellent residual activity of permethrin throughout the study, it is probable that the main effect of permethrin was not through the toxic action on the vector population, but rather through non-lethal effects, such as repellency or biting inhibition. In the recent west African study where permethrin-impregnated bed nets were used, the effect on the mosquito population was a 70% reduction in the numbers of mosquitoes entering the huts, an increase from 25% to 97% of the mosquitoes exiting the hut, and a reduced engorgement rate of *A. gambiae* by about 20%. These observations were attributed to the repellent and irritant effect of permethrin (Darriet *et al.* 1984). Results from the east African study indicate that repellency was less important than the overall mortality that was caused by the impregnated bed nets. With impregnated curtains, however, a strong repellent effect was observed (Lines *et al.* 1985).

One explanation for the observed results, and perhaps the most obvious, is that there was increased protection from mosquito bites as people slept under impregnated bed nets. Gonoa is an area where the major vectors of malaria are early morning biters. Because young children are under impregnated bed nets well before the most active feeding period of the vectors, they would receive fewer bites than other people and this would explain the age specific response that was observed. All people would be better protected as they slept under the impregnated bed nets, but, because of the intense transmission and the significant man/mosquito contact before retiring to sleep, sleeping under an impregnated bed net would decrease the number of infected bites, but would have very little impact on the prevalence of malaria in these age groups over time.

7.0.3 The Use of Chloroquine In The Study Area

It was beyond the scope of this project to either control the use of chloroquine, which is readily available at low or no cost from many sources, or to quantify its use by testing for the presence of 4-aminoquinoline drugs in urine samples. From the baseline survey before bed net impregnation, it is known that most malariological indices were greater in the experimental population than in the control population. After bed nets were impregnated, the indices were lower in the experimental population, many significantly, but not in the control population. The wide use of chloroquine in the Madang area complicates the evaluation of malaria control measures (Cattani, pers. comm.), however, there is no reason to suspect that there was a greater use of chloroquine in the experimental village only after bed nets had been impregnated.

7.0.4 The Use of Bed Nets as a Method of Personal Protection Against Mosquitoes

Any method of personal or community protection from malaria must fulfil two objectives; the method must be effective, and it must be appropriate *i.e.* suited for local conditions and accepted by the people so it can be sustained over time. How effective a certain strategy should be depends on many factors including the endemicity of malaria in that particular location. In areas hyper- or holoendemic for malaria, it is possible to reduce the prevalence of malaria to very low levels with residual insecticides and/or drugs, but, it is known that such efforts cannot be sustained over time. If a program is effective at greatly reducing or interrupting malaria transmission, the immunity acquired by the people as a result of prolonged exposure to malaria, is reduced. This may have serious consequences when the program fails, because in addition to the young children who are always at risk, the older people with reduced levels of immunity, will also be at risk. This is why, in areas of hyper- or holoendemicity where malaria eradication cannot be achieved or sustained, the main purpose of malaria control is to protect high risk groups, namely, young children and pregnant women. The protection of young children from malaria by weekly prophylaxis of amodiaquine was the objective of the Madang trial in the late 1970's. It was initially very successful, but later failed because of resistance of *P. falciparum* to 4-aminoquinoline drugs (Stace and Pariwa 1982, Moir 1983). The true effectiveness of any malaria control method is therefore not a measure of how well it reduces the incidence of malaria, but, how effective the measure is over time. Russel (1936) recognized this before the DDT era of malaria control and stated:

> constant striving for continuity of modest effect should be preferred to great effectiveness and speed when their maintenance cannot be ensured.

This objective was neglected during the eradication era of malaria control, but, it is now precisely what is being advocated through vector control activities today.

The distribution of antimalarial drugs for the presumptive treatment of malaria will continue to have the greatest impact on reducing morbidity and mortality from malaria in rural Papua New Guinea. To complement drug distribution and in consideration of available resources and methods of protection or control against

the vectors of malaria in PNG, I feel that emphasis should be place on encouraging greater use of mosquito nets because, they are effective, their effectiveness may be further enhanced by such methods as impregnation, and they are appropriate. Port and Boreham (1982) studied the effect of bed nets (non-impregnated) on feeding of *A. gambiae* and concluded that bed nets are a very effective means of reducing attack by this vector. In east Africa, Lines *et al.* (1985) observed that non-impregnated bed nets reduced the total number of bites by 83%, and were as effective as impregnated bed nets. In the Madang area, it is known that mosquito net utilization is high and that treatment for malaria in infants is common (Cattani and Wolstenholme 1983, Cattani *et al.* 1985) . During the recent study on the epidemiology of malaria (Cattani *et al.* 1985), it was observed that infant parasite rates were low, and that spleen and parasite rates peaked in the five to nine age group rather that the one to four age group, indicating delayed development of acquired immunity. They suggested that high utilization of bed nets and frequent therapeutic intervention, particularly in infants, may contribute to delayed development of immunity and at the same time to reduced morbidity and mortality from malaria.

Bed nets can have a very significant effect on reducing man/mosquito contact but as stated by Lines *et al.* (1985), in areas of holoendemicity for malaria there is no simple relationship between the reduction in mosquito biting and reduction of malaria incidence. In other words, an 80% reduction of mosquito bites does not confer an 80% reduction in the incidence of malaria, but, it does mean that people will receive far fewer infective mosquito bites. This may not be of importance to older people with considerable immunity, but to younger children with low immunity and chronic anemia, reducing superinfection undoubtedly improves the overall health status of the child.

Appropriateness is the second condition that must be met by all vector control activities and it is this factor which ultimately determines if the method can be sustained over time. The reasons why methods fail and are not appropriate at the community level have been described with lucidity by Gramiccia (1980). Perhaps the most important reason, is the fact that the disease is part of a

socioeconomic depression complex from which people have difficulty in singling out malaria for particular concern. This was evident in Gonoa where the majority of people would certainly not consider malaria to be their most serious health problem, which it is not. In describing some of the difficulties associated with disease prevention methods at the community level, and particularly where it concerns malaria, Brieger (1981) stated the following:

> A lack of understanding is not the main reason why people do not accept new kinds of health behavior. The principal reason is that the behavior being advocated is inconvenient, expensive, produces unwanted side effects or does not give visible results.

It is the appropriateness not just the effectiveness which determines the success of malaria control methods, and this is why I am suggesting that a greater emphasis be placed on increasing the effectiveness of a method that has already demonstrated its acceptance by the people.

SUMMARY AND CONCLUSIONS

The purpose of this study was to investigate alternative methods of protection against the vectors of malaria. Impregnating bed nets with permethrin was the method adopted because evidence has shown that impregnated fabrics can be an effective method of protection against biting insects, and because it was a way of further enhancing mosquito net use, a method of personal protection that was already being practiced by the people in the study area.

The investigation was conducted in two groups of geographically isolated hamlets comprising a population of about 500 people. All people found positive for malaria during the baseline survey were treated with anti-malarial drugs in order to reduce the community prevalence of malaria. A total of 196 bed nets were impregnated with permethrin in the experimental village at a dosage of 0.2 g/m² of netting. There were no problems associated with the application of permethrin, and no complaints from the villagers of side effects such as skin irritation or odor. Entomological evaluation did not give any evidence to indicate that vector density, as estimated by man–biting rates, oocyst or parous rates were affected by bed net impregnation. However, malariometric indices indicated that there was an effect of bed net impregnation. Spleen rates in the experimental and control populations for all ages combined, and for the 2–9 years age group, were not significantly different in the baseline survey but were in the November survey. Asexual and sexual parasite rates were lower in the experimental population when compared to the control population after bed net impregnation. The analysis of age specific parasite rates showed that the greatest effect was observed in the in the 1–4 age group and this was consistent over both evaluative surveys. Bioassays to test for the duration of effectiveness of permethrin indicate that it has excellent residual activity. It was also observed that head lice were controlled in the experimental village and this is undoubtedly why there was excellent community acceptance of the strategy.

During the past few years interest in mosquito nets as a method of personal protection against vectors of malaria has increased. Although the relationship

between bed net use and malaria prevalence in areas of varying endemicity is not well established, it is known that mosquito nets effectively reduce bites from mosquitoes. The impregnation of fabric with permethrin now offers a method to further enhance the effect of mosquito nets. As primary health care becomes better established and essential drugs such as chloroquine become available to more people, it will become even more important that any community-based method of malaria control be appropriate for local conditions. People are generally more interested in treatment rather than prevention of disease and with the increased availablity of drugs, it will become more difficult to motivate people to undertake and sustain self-help measures against malaria. I feel that a greater effort should be made to optimize this method for the following reasons:

1. Bed nets, whether impregnated or not, have the advantage over other methods in that they reduce the nuisance from mosquito bites and this is clearly why people use them.

2. Bed nets can be made locally, and as observed in Gonoa; if made with appropriate material can be expected to last for several years. Bed nets are relatively inexpensive although the cost is related to the distance from distribution centers. In the Madang area, cost of mosquito nets was 10-30% higher outside of town. Unfortunately, people living in remote areas where health services are poor, are least able to pay for mosquito nets.

3. When people use bed nets they become conditioned to sleeping without the nuisance from mosquitoes and continued use is much more likely.

4. In areas holoendemic for malaria, infants and children are the most likely to suffer from severe mortality and morbidity. They also benefit most from mosquito net use because they spend the greater part of the night under them.

5. The 1-4 years age group is known to have the highest gametocyte rate. If children of this age group are effectively using bed nets, not only will they

be protected from mosquito bites, but the vectors of malaria will be unable to feed on members of the age group who are most responsible for malaria transmission. This should complement the recently introduced attempt by the Health department to decrease malaria transmission by administering a single dose of primaquine with chloroquine, for the standard treatment of uncomplicated malaria in PNG (anonymous 1984). [1]

6. In parts of Papua New Guinea there is hyperendemic transmission of filariasis (Knight *et al.* 1979), in addition to intense malaria tramsmission. The vectors of both diseases are members of the *A. punctulatus* complex. Unlike malaria, filariasis is not efficiently transmitted and attempts to reduce man/mosquito contact may have a greater effect on transmission of this disease than with malaria, particularly in PNG where the vectors are late-night biters. In stating reasons for the apparent decline in *Wuchereria bancrofti* in The Gambia, Knight (1980) suggests that in addition to climatic changes, bed nets may have been important.

> There is a much greater use of anti-mosquito measures, in particular bed nets which are now used very extensively throughout The Gambia. Motivation for bed nets is mainly for protection against nuisance...The persistence of infection in poorer parts of the communities is perhaps caused as much by a low standard of housing and fewer bed nets, as by introduced infections.

7. As demonstrated in this and other studies, the effectiveness of mosquito nets can be further enhanced by methods such as bed net impregnation with permethrin, a technique that requires little equipment and training, and is safe. If there arise undesirable side effects to the insecticide such as resistance of the mosquito vectors, head lice or bed bugs, the program can be terminated but the people will receive continued benefit from the mosquito nets. Although mosquito net use is high in the Madang area, the effectiveness of bed nets is undoubtedly reduced because of their small size and the tendency

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[1]Primaquine in a sporontocidal drug and is being given to people with malaria to reduce transmission. Chloroquine is active against the asexual stages of malaria but not against the sexual stages. A person with *P. falciparum* malaria may therefore be relieved of the symptoms after taking chloroquine, but may still be infective and therefore a source of continuing malaria transmission.

of two or more people to sleep under them.

RECOMMENDATIONS

1. Action should be to taken to encourage greater use of mosquito nets.

2. Mosquito nets should be made in PNG. The objective of manufacturing mosquito nets locally should be to make them as inexpensive as possible. The most appropriate material should used and the optimum size to provide protection for a mother and two or three children should be determined.

3. It is clearly not the role of governments to give mosquito nets to the people. They can, however, support the local manufacturing of mosquito nets and help in their distribution to remote areas. A transportation subsidy could be given so that the price of bed nets remains the same throughout a province.

4. In remote and economically depressed areas of PNG, it is common for institutions such as schools and health centers to buy food from local farmers; mosquito nets could be bartered for food.

5. Research should be conducted on a larger scale involving both entomological and malariological evaluation of impregnated bed nets in areas of different malaria endemicity. Because of the behavior of *A. punctulatus*, it is expected that the strategy would be most effective against this vector. In some areas of the Sepik provinces, *A. punctulatus* is the vector of both malaria and filariasis. Areas such as these should be identified and emphasis placed on implementing alternative strategies such as bed net impregnation.

6. Experiments with impregnated bed nets should be conducted in experimental huts designed to allow assessment of lethality, repellency and inhibition of feeding.

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Glossary of Terms

anthropophilic. Showing a preference for feeding on man even when non-human host are available.

biting cycle. Regular variations in the amount of blood-feeding activity exhibited by populations of mosquito species during each 24-hour day-and-night period.

bloodmeal. Ingestion by a female mosquito of the blood obtained from a vertebrate host

brood. Parasites belonging to the same generation and about the same stage of development.

chemoprophylaxis. Protection from or prevention of disease by chemotherapeutic means.

endemic. Term applied to malaria when there is a constant measurable incidence both of cases and of natural transmission in an area over a succession of years.

endophagy. Tendency of mosquitoes to feed indoors.

endophily. Tendency of mosquitoes to rest indoors, whether by day or night.

epidemic. Term applied to malaria when the incidence of cases (other than seasonal rises) in an area rises rapidly and markedly above its usual level or when infection occurs in an area where it was not present previously.

exophagy. Tendency of mosquitoes to feed outdoors.

exophily. Tendency of mosquitoes to rest outdoors whether by day or night.

gonotrophic cycle. One complete round of ovarian development in the mosquito, often stated in reference to the period time required for its completion.

incidence. Number of cases of disease ocurring during a given time period in relation to the unit of population in which they occur (a dynamic measurement).

index. Human blood (HBI)-Figure indicating the proportion of freshly fed anopheles found to contain human blood.

interruption of transmission. Cessation of transference of malaria by mosquitoes from one person to another.

malaria, holoendemic. Degree of malaria endemicity in an area characterized by a spleen rate in children (2-9 years) constantly over 75% but a low adult spleen rate. Areas of high transmission where there is high tolerance of the adult population to malaria.

malaria, hyperendemic. Degree of malaria endemicity in an area characterized by a spleen rate in children (2-9 years) constantly over 50% and also a high adult spleen rate. Areas of high transmission and high tolerance of the adult population to malaria.

malaria, mesoendemic. Degree of malaria endemicity in an area with a spleen rate in children (2–9 years) between 11% and 50%.

malaria, hypoendemic. Degree of malaria endemicity in an area with a spleen rate in children (2–9 years) of 10% or less.

malaria, stable. Epidemiological type characterized by steady prevalence which does not show great change during one transmission season, or from one season to another, except as the result of extreme changes in environmental factors affecting transmission. Epidemics are unlikely: the affected population often shows a high degree of immunity. This condition is linked with the presence of vectors exhibiting pronounced man–biting habits and longevity.

malaria, unstable. Epidemiological type characterized by a variable prevalence, typically showing great changes from one part of the transmission season to another and from one year to another. Epidemics are common and often attributable to minor causes; the population shows little immunity. This condition is usually linked to the presence of vectors which do not exhibit pronounced man–biting habits or great longevity.

malariometric survey. Investigation conducted in selected age–group samples of a population in randomly selected localities in order to assess the degree of malarial endemicity. Such a survey is concerned with the measurement of the prevalence of malaria as indicated by spleen and/or parasite rates.

parasitaemia. Condition in which malaria parasites are present in the blood.

prevalence. Number of cases of disease or infection existing at a given time. Malaria prevalence can be established on a single malariometric survey, whereas incidence, which is a dynamic measure, requires a method of continuous search.

superinfection. Fresh infection brought about in a host while a previous infection with a parasite of the same species is still present.

trophozoite. Intracellular erythrocytic forms of malaria in their early stages of development.

PNG IMR MALARIA EPIDEMIOLOGY SURVEY

EPIDEMIOLOGY SURVEY NUMBER	ESN	☐☐☐
SUBJECT NUMBER	SUBJ	E ☐☐☐ ☐☐☐☐ Cluster No Serial No
VILLAGE NUMBER	VIL	☐☐
HOUSE NUMBER / CHANGE	HAUS	☐☐☐☐ ☐ 0=No 1=Yes
SUBJECT NAME	NAME	☐☐☐☐☐☐☐☐☐
FATHER'S NAME	F NAME	☐☐☐☐☐☐☐☐☐
MOTHER'S NAME	M NAME	☐☐☐☐☐☐☐☐☐
CENSUS ID NUMBER	ID NO	☐☐☐
DATE OF BIRTH / CHANGE	DOB	☐☐☐☐☐☐ ☐ 0=No 1=Yes
BIRTH WEIGHT (Gms)	BWT	☐☐☐☐ 9999 = not known
SEX	SEX	☐ 1=male 2=female
MOSQUITO NET	MQN	☐ 0=No Did you sleep under a 1=Yes mosquito net last night?
DATE(S) ABSENT _____	AIR	☐ 0=Absent or refused 1=Present and included
DATE INCLUDED	DATE	☐☐☐☐☐☐
WEIGHT (Kilograms)	WT	☐☐☐☐☐
HEIGHT (mm) / EXAMINER	HT	☐☐☐☐ ☐
SPLEEN GRADE /. EXAMINER	SPL	☐ 9=not possible ☐
PREGNANCY-FUNDAL HEIGHT	FHT	☐ 1 = 20-28 weeks 0 = other female 2 = >28 weeks 9 = male

BLOOD SLIDE: ☐

		GRADE DENSITY / 200WBC
P.falciparum-asexual	PFA	☐ 0 = NEGATIVE 1 - 2, any per 100F
P.falciparum-gametocytes	PFG	☐ 2 = 3 - 5 3 = 6 - 15
P.vivax	PV	☐ 4 = 16 - 35 5 = 36 - 85
P.malariae	PM	☐ 6 = 86 - 200 7 = 201 - 499
P.ovale	PO	☐ 8 = 500+ 9 = No result
Ovalocytosis	OVAL	☐ 0=No 1=Yes
HAEMOGLOBIN ☐	HB	☐☐☐ 999 = not done
SERUM: IFA result ☐	IFA	☐ 9 = not done
ELISA result	ELI	☐ 9 = not done
URINE ☐	CHLOR	☐ 0=absent 1=present