Malaria control trial using lambda cyhalothrin treated nets in
Yanomami communities in Amazonas State, Venezuela

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Abstract

A community randomized controlled trial was carried out in an area of moderate malaria transmission in the Amazon region, in South East Venezuela, where the indigenous Yanomami population live. The aim of the project was to assess the impact of lambdacyhalothrin treated hammock nets (ITHNs), compared to placebo treated hammock nets (PTHNs), on the malaria incidence rate and on the malaria vector population *Anopheles darlingi*.

In both arms of the study intensive early case detection was performed and prompt malaria treatment administered. Baseline data were collected one year before the intervention and a population of around 924 Yanomami was followed for two years.

Despite the recent introduction of nets in the Yanomami villages and the adverse natural conditions in the area, the majority of Yanomami showed high compliance and took good care of the nets.

Analysis performed by gas chromatography of samples taken from the nets dried in different ways, i.e. vertically or horizontally, in the sun or in the shade, showed that there were no significant differences between methods with the only exception of drying the nets horizontally and in the sun performing significantly worse then the others. In addition bioassays, with *Aedes aegypti*, showed that hammock nets treated at village level with lambdacyhalothrin (10 mg/m²) and
dried vertically in the sun were effectively killing mosquitoes (87%) after six months of regular use and the mortality rate was 83% after washing the nets twice.

The malaria incidence rate per thousand person years at risk was 114.6 in the IHTNs group and 186.8 in the PTHNs group. The adjusted rate ratios indicated that ITHNs prevent 55% (IRR: 0.44, 95% CI: 52-59%) of new malaria cases. ITHNs reduced the prevalence of parasitaemia by 87% (RR: 0.17, 95% CI: 0 – 45%) in the first cross-sectional survey carried out during the high transmission season, six months after the intervention. The prevalence of splenomegaly and of anaemia was low in both groups, and there was no evidence of reduction due to ITHNs.

There was little evidence of a mass killing effect on the density of the vector population, although significant differences between study arms were found when the analysis was carried out adjusting for baseline An. darlingi density. The density of An. darlingi was 62% less in villages with ITHNs than those with PTHNs (density ratio: 0.38, 95% CI 52-70%).

The main conclusion of the present study is that ITHNs can reduce malaria incidence in the area and it is the most feasible method of malaria control in a forested area where indigenous villages are scattered over a large territory.
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At this point I prefer to dedicate this thesis in Spanish because it is my mother-tongue and allows me to express my feelings better.

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LIST OF ABBREVIATIONS

AOHD - Alto Orinoco Health District
API - Annual Parasite Incidence
CAICET - Centro Amazónico de Investigación y Control de Enfermedades Tropicales
CSP - Circum-sporozoite protein
DDT - Dichloro-diphenyl-trichloroethane
ELISA - Enzyme-linked Immunosorbent Assay
GC-MS - Gas Chromatography-Mass Spectrometry
GLC - Gas Liquid Chromatography
GLM - Generalized Linear Model
HBC - Human Biting Catches
HMS - Hyperactive Malarious Splenomegaly
IMT-UCV - Instituto de Medicina Tropical-Universidad Central de Venezuela
IRR - Incidence Rate Ratio
IRS - Indoor Residual insecticide Spraying
ITHNs - Insecticide Treated Hammock Nets
ITN - Insecticide Treated Net
IVIC - Instituto Venezolano de Investigación Científica
LRT -Likelihood Ratio Test
LSHTM -London School of Hygiene and Tropical Medicine
MARN -Ministerio de Ambiente y Recursos Naturales
MSDS -Ministerio de Salud y Desarrollo Social
Mw -William’s mean
P - P-value
p -Proportion
PAHO -Pan American Health Organization
PHC -Primary Health Centres
PTHNs -Placebo Treated Hammock Nets
RBM -Roll Back Malaria
RR -Risk Ratio
SUYAO -Shabones Unidos Yanomami del Alto Orinoco
VMR -Variance mean ratio
WHO -World Health Organization
CHAPTER 1. Introduction

1.1 Malaria in America

More than one hundred years after the transmission cycle of malaria was revealed and malariologists began to look forward optimistically to the possibility of breaking it and, ultimately, eliminating the disease, malaria is still one of the most serious and challenging health problems facing humanity (Dobson, 1999). Malaria kills more people than any other infectious disease after tuberculosis and HIV. Although its main impact is in sub-Saharan African countries, where at least 90% of the malaria deaths occur, it remains an important health problem in some parts of Asia, Central and South America.

In the American continent, malaria is present in 21 countries and it has been estimated that 36% of the population live in areas at risk of transmission (Figure 1.1). During the last decade there has been an increase in the number of reported cases, from 982,000 in 1993 to 1.4 million in 2000 (WHO, 1996; PAHO/WHO, 2002). The predominant parasite is Plasmodium vivax, but malaria mortality is associated with P. falciparum. In 2000 there were 301 malaria-associated deaths. There are a small number of cases caused by P. malariae, the third most prevalent parasite in the continent. The principal anopheline vectors in Mexico and Central America are Anopheles albimanus and An. pseudopunctipennis, while in the countries belonging to the Amazon region, An. darlingi and An. albimanus are the most important.
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Figure 1.1 Map of endemic areas in America, 2002

Of the total number of malaria cases reported on the continent, 87% are located in the Amazon region, which includes nine countries: Brazil, Colombia, Ecuador, Peru, Bolivia, Venezuela, French Guiana, Guyana, and Surinam. Of all the
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*P. falciparum* cases reported in America during 2000, 80% occurred in the Amazon region (PAHO/WHO, 2002).

In the above mentioned countries, inequity in income and access to health, education, adequate environmental sanitation, and housing remain high. The indigenous ethnic groups, particularly those living in the Amazon region, are the most vulnerable and in fact the most affected by malaria (PAHO/WHO, 2002).

1.2 Malaria in Venezuela

Venezuela is located in the Northern part of South America (0° 38’ 53” and 12° 11’ 46” N; 58° 10’ 00’ and 73° 25’ 00’ W), with a surface area of 912,050 km². It encompasses a variety of ecological areas as it forms part of the Caribbean, the Andean and the Amazon regions. It has been estimated that one third of the population of the country was affected by malaria before 1935, when Dr. Arnóldo Gabaldón established activities for its control on a national basis and the Malaria Control Programme was founded. Human and economic development was seriously affected throughout the country. In the decade 1935-1945 the average annual mortality rate was 110 per 100,000 inhabitants. During this period the newly created programme focused its attention on investigating the epidemiology of the disease, training personnel, and setting up a network of mobile teams in order to direct and implement control activities in many areas of the country. Before the advent of DDT, these activities were limited to environmental management (large-scale drainage and filling in operations of marshes around
towns), use of larvicides and non-residual adulticides, active surveillance, and chemotherapy with quinine and quinacrine (Gabaldón and Berti, 1954; Berti et al., 1960). In 1945, the emphasis of the activities changed radically as a consequence of the introduction of residual spraying with DDT, and eradication seemed to be possible. That year, the Malaria Eradication Campaign started. DDT spraying allowed an increase in the coverage of malaria control programme activities and the mortality rate dramatically decreased to 8 per 100,000 inhabitants in 1950, and to 0.01 per 100,000 by 1959, when malaria was considered to be eradicated from 407,945 km² (47%) of the country, mainly in the North-central region (Berti et al., 1960). By the end of the 1950s, the distribution of the disease was confined to the Western, Eastern and Southern areas of the country (Figure 1.2, see page 26). Three types of situation were defined, according to the usefulness and efficacy of indoor DDT spraying: responsive, refractory and inaccessible malaria (Gabaldón, 1983).

**Responsive malaria** referred to the situation in those areas where residual indoor spraying effectively interrupted transmission. This corresponded to the central area of Venezuela where the main vectors, *An. albimanus* and *An. darlingi* were highly endophilic (resting indoors). Here malaria was successfully eradicated.

**Refractory malaria** referred to those areas where the disease persisted due to the vector’s behaviour (resting and biting patterns) or to physiological resistance to the insecticide. Here, malaria transmission persisted in spite of adequate spraying.
with DDT. These areas corresponded to the Western and Eastern areas of the country where the main vectors are *An. aquasalis* and *An. nuneztovari*.

**Inaccessible malaria** described those areas where the application of DDT was not possible due to a variety of factors such as: the area being inhabited by ethnic groups with nomadic habits, type of housing (e.g. no sprayable walls), difficult geographical access, and exophilic habits of the main vector. The *inaccessible* area corresponded to the South of the country (Bolivar and the Amazonas states) where *An. darlingi* prevails.

In areas with responsive malaria, the incidence decreased dramatically, as transmission was interrupted, while in areas of refractory malaria, the incidence decreased slowly, as transmission was reduced but not interrupted (Gabaldón, 1972, 1983).
Figure 1.2 Evolution of malaria eradication and control campaigns in Venezuela

a. Malaria was widespread in Venezuela before the eradication campaign started in 1945.
b. In 1962 two thirds of the country were freed of malaria.
c. Since 1982 previously malaria cleared areas have been re-infected.

Source: Gabaldón, 1983
Since 1962, Venezuela has experienced two periods of malaria resurgence. The first was in the early 1970s when the Annual Parasite Incidence\(^1\) (API) increased from 87 per 100,000 inhabitants in 1969, to 222 per 100,000 inhabitants in 1971. The other period of resurgence occurred at the end of the 1980s, when the malaria incidence rate rose to 244 per 100,000 inhabitants in 1988. This increase in incidence was related to the re-infection of formerly cleared areas in association with socio-economic changes, (an increase in mining activities in high risk areas, population mobility and migration) and to financial constraints affecting the malaria control programme (Figure 1.3, see page 30). By the beginning of the 1990s there was a decrease in the number of malaria cases, in line with the trend reported for the rest of America (WHO, 1996). This decrease coincided with a Global Malaria Strategy in which 21 countries in America were reorienting their programmes in order to identify the highest risk areas using epidemiological criteria and to concentrate the bulk of their resources on prevention and control in those areas. Following this strategy, countries made progress in providing early detection and treatment of cases (WHO, 1996). Moreover, from 1992 to 2000 the malaria control programme in Venezuela received an increase of its budget through national and international donors.

In 1995 the goal of the programme finally changed in accordance with the international strategies, and attention was focused on control rather than

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\(^1\) Annual Parasite Incidence (API): malaria cases/number of population at risk per 1000 (Warrell and Gilles, 2002).
eradication. The endemic regions of the country were stratified into “risk zones\(^2\)”,
depending on the API, to increase the effectiveness and targeting of control
activities (Figure 1.4, see page 30) (MSDS, 1995). Presently, the distribution of
malaria in the country is similar to that at the beginning of the 1960s. The
principal known vectors are *An. aquasalis* in the Eastern region, *An. nuñeztovari*
in the Western region and *An. darlingi* in the Southern region. Other potential or
suspected vectors are *An. albimanus, An. albitarsis, An. oswaldoi, An. pseudopunctipennis* and *An. emilianus*. The most frequently reported species
of *Plasmodium* is *P. vivax*, followed by *P. falciparum* and *P. malariae*. During the
year 2002, 29,336 cases of malaria were parasitologically diagnosed and 94% of
them were reported in the Sucre, Amazonas and Bolivar states, the former
corresponding with the North-eastern and the latter two with the southern region.
This number of cases represents 33% more than that expected according to the
prediction of the Ministry of Health for the seven years 1998 to 2004 (MSDS,
2002). Since the beginning of the 1990s, Venezuela has attempted a process of
State reform including decentralisation, which has been heterogeneously
implemented among federal entities and sectors. Health sector reform is still
ongoing. In accordance with a recommendation by the Pan American Health
Organization (PAHO) and the World Health Organization (WHO), antimalarial
activities are being progressively integrated into regional health services. One of
the problems in the decentralization process is the weakness of managerial and
operational capabilities at the regional and district level. For example, in several

\(^2\) Low risk IPA less than 1 malaria case per 1000 inhabitants. Moderate risk IPA 1 to 10 malaria
cases per 1000 inhabitants. High risk more than 10 malaria cases per 1000 inhabitants
(PAHO/WHO, 2002).
areas of the country there are no personnel trained to carry out entomological monitoring, or susceptibility testing either to insecticides or antimalarial drugs.

Currently, the main Malaria Control Programme activities are based on surveillance, early case detection, adequate treatment and focal vector control. The strategy is based on: a) improvement of local capacity to increase passive case detection; b) provision of early antimalarial treatment to individuals with a positive slide for malaria parasites; c) establishment of a national network for surveillance of antimalarial drugs efficacy, and d) implementation of vector control in places with high incidence, i.e. spraying with residual insecticides (organochlorines, organophosphates and pyrethroids) supplemented by fogging with peridomestic insecticides (pyrethroids and organophosphates).

Since 1999, Venezuela has subscribed to the goals of the global partnership initiative “Roll Back Malaria (RBM)” (Nabarro, 1999), and this has further accelerated the decentralisation of control activities, the establishment in the Amazon region of a surveillance system of drug susceptibility, and the continuous training of field workers for parasitological diagnosis and vector control. Among the control measures promoted by the RBM initiative is the introduction of Insecticide Treated Nets (ITNs) in transmission areas.
Figure 1.3 Annual Parasite Incidence (IPA) of malaria in Venezuela, 1954-2002

Figure 1.4 Malaria epidemiological map by risk level in Venezuela in 1999
1.3 The Amazonas state of Venezuela

The Amazonas state of Venezuela is located in the South of the country, with an area of 180,475 km², 20% of the total national territory. This state, together with the corresponding areas of Brazil, Colombia, Ecuador, Peru, Surinam, Guyana and French Guiana, constitute the Amazon region of South America. The population of Venezuelan Amazonas state is estimated as 110,324 inhabitants, 61% of whom live in the capital, Puerto Ayacucho, while the remaining 39% live in scattered communities in rural and more remote areas. Amazonas is the federal entity with the highest proportion of indigenous people in the country. Nineteen different ethnic groups make up half of the total indigenous population of Venezuela, with the Yanomami group being the largest (OCEI, 1992).

The health system of the state comprises seven districts: Atures in the state capital, San Fernando de Atabapo, Autana, Manapiare, Río Negro, Guainía and Alto Orinoco, the latter in the Upper Orinoco area. All of them are highly dependent, for administrative and operational support, on the capital district. Most of the health services, including the only hospital, are concentrated in the capital, while in the rural districts there is a network of Primary Health Centers (PHC). These are staffed occasionally by recently graduated medical doctors and, in most cases, by primary health care workers called Auxiliares de Medicina Simplificada.

During 2000 Amazonas was the state with the highest number of malaria cases reported in the country with an API of 40.3 per 1,000 inhabitants. The distribution
of cases is not homogenous throughout the state, Manapiare and Alto Orinoco being the most affected districts (MSDS, 2002).

1.4 The Alto Orinoco Region: the Yanomami

The Alto Orinoco Region (henceforth Alto Orinoco), is a large district located in the South-eastern part of Amazonas state. It is characterised by a large area of rain forest in the lowlands and pre-montane deciduous forest and grassland savannah in the highlands (Huber et al., 1984). The Yanomami Amerindian population is the main ethnic group living in this region (OCEI, 1992).

The Yanomami are considered to be the most numerous and least acculturated ethnic group in the country, and in the continent. They live in an extensive geographical area straddling the Brazilian-Venezuelan border in the Amazon region of South America (Figure 1.5). There are reports of intermittent contact with the criollos\(^3\) as early as the 18\(^{th}\) century, particularly between 1787 and 1800. Permanent contact did not begin until 1950, when the first Protestant mission was established in the area (Neel, 1970; Lizot, 1988). According to the latest indigenous census published in Venezuela, the Yanomami population was estimated at 15,012 dispersed over 82,662 km\(^2\) with a demographic density of 0.13 persons/km\(^2\), grouped in approximately 233 scattered communities (OCEI, 1992).

\(^3\) Criollo is the local and regional name for non-indigenous people.
Despite these estimates, neither Venezuela nor Brazil have carried out a truly reliable census of the Yanomami population due to their remoteness and the difficulty of movement through the tropical rain forest and high plain savannahs that characterise the area.

The Yanomami live traditionally in a single large circular house with a thatch single-pitch roof, open at the centre and with no solid walls, called shabono
(Figure 1.6, see page 35). More recently, however, individual family houses, with a closed double-pitch thatched roof made out of palm, and walls constructed with mud and/or palm leaves, have been built in some areas, especially around permanent mission settlements. The population of each shabono ranges from 10 to 300 inhabitants (Lizot, 1988) belonging to several closely related families. The daily activities of the Yanomami include vegetable (slash and burn) gardening, hunting, fishing, foraging for wild fruits and edible insects, collecting firewood, carrying water, and tool making (baskets, hammocks, arrows, accessories and colourful pigments which they use to paint their bodies). The component families of a shabono usually sleep in hammocks arranged around their fire.

The Yanomami are a very mobile group. Apart from shifting the location of their shabono every few years for social and/or ecological reasons (micro and macro movements), it is also common for individuals or families to leave the community for days, or weeks, in search of seasonal fruits in the forest\(^4\). Even more frequent movements include visits to friendly communities for ritual activities like funeral ceremonies or festive events that also involve negotiation of political, economic and marriage-exchange relationships. Finally, single families or people often travel to other communities to visit relatives living there. In all these contexts, networks of reciprocal exchange of objects, rituals, and news are maintained, for these are essential for the social well being of each community.

\(^4\) This hunting and mobilizations of communities is less frequent in shabonos settled near to the missionary establishments.
Figure 1.6 Yanomami *shabono:* above) typical multifamily house open at the centre and; below) individual covered family house
Chapter 1

The Yanomami have a complex set of beliefs regarding the aetiology, nosology and curing of illness. They perceive the person as having several non-material constituents beyond the body, organised in concentric circles reminiscent of the shabono's shape. In their cosmology, the forest and universe are populated with a range of spiritual entities. They also consider that enemy shamans and sorcerers, together with spiritual beings, are frequently the intentional agents responsible for an illness which involves either the theft, injury or other types of intrusion (non material objects) into one of the spiritual constituents of the body. Alongside these interpretations, the Yanomami often use the word shawara to describe illness in an epidemic situation (e.g. diarrhoea, respiratory diseases) which can hit communities or be brought in by individuals who come from communities where there is shawara. Therapeutic options include shamanic healing and the intake of plant preparations and, in places where there is a health post, the intervention of a medical doctor or Yanomami auxiliary. The course of action followed by an individual patient depends a lot on the particular case: the evolution of the illness, past experience, availability of resources, etc.. Shamans, however, even in places where there are medical doctors, are fundamental to the Yanomami culture in prevention, diagnosis and treatment of disease.

All this means that in the Yanomami culture, the understanding of disease causation is radically different from that in modern developed societies, and complicates relationships between social and supernatural factors determining the equilibrium between health, illness and death. The representation and
understanding of the causes of illness in the local culture are normally very important when designing control activities and operational research within a different culture (Chavasse et al., 1999).

Chagnon (1992) and Lizot (1998) classify the Yanomami into three groups according to their degree of acculturation and proximity to missionary and medical settlements: a) nearby villages, with the highest demographic density, high morbidity indices but with medical attention; b) intermediate villages and, c) distant or remote villages that have never had continuous medical assistance.

At present there are nine religious mission posts in the Alto Orinoco region, so the Yanomami communities living around these settlements are considered nearby villages. In these areas, some villagers have been in contact with the mission for up to 52 years and bilingual (Yanomami and Spanish) schools have been established and staffed with Yanomami teachers. Exposure to outsiders over the past decade has had a significant influence on their traditional way of life. This means, for example, that these communities are more sedentary and have more access than intermediate and distant shabonos to criollo culture and materials, including Western medicine.

Most of the PHCs have been constructed in localities along big rivers, like the Orinoco (and its tributaries), Padamo, Ocamo and Mavaca rivers, where Catholic missions settled during the 1960s. The communities of Ocamo and Mavaca, where the present study was carried out, are among the largest settlements, with the highest density and degree of contact with criollos. In each of them, there is a
Catholic mission, usually with 2 or 3 permanent members. They run educational programs and a cooperative trading system. The PHCs are usually attended by a rural medical doctor, medical students in their last year of training, and Yanomami personnel trained as primary health care workers or auxiliaries. The Yanomami population living nearby have direct access to these centres, and the health personnel visit the intermediate and distant villages at irregular intervals by boat, helicopter and/or on foot. However, the majority of the most remote communities have no access to health services. This "isolation" does not apply to the network of trading and economic exchange, as this extends far into the more distant communities from settlements where manufactured goods are available. In general, manufactured products obtained along the Orinoco river — in the villages nearby missions or by travelling from these communities to other settlements further down river or to Puerto Ayacucho — are exchanged further upriver for indigenous products. Manufactured products are also obtained from various sources along the Orinoco either through the economic cooperative at mission posts, or by individual purchase or exchange. In general, therefore, manufactured products flow up river and into the forest whereas indigenous ones flow down river.

The economic cooperatives are organised under the umbrella of the Shabonos Unidos del Alto Orinoco (SUYAO) organization. They are established in villages close to the Catholic mission posts, and make available industrial products such as: fishing tackle, machetes, knives, cooking pots, matches, etc., which are sold for money or exchanged for local craft products. The cooperative itself is usually
managed by men. Yanomami women are in charge of the *watota*. The *watota* is a place where women learn to sew clothes, such as pants and shirts. One year before this study began, the Yanomami women started to produce hammock nets in the *watota*, selling or exchanging them through the cooperative. This may represent a valuable opportunity for the implementation and spread of control measures such as ITNs through the Yanomami exchange system, which, as described above, is such an important part of their life and culture, and which partly motivated this study.

1.5 Malaria in the Alto Orinoco

Malaria in the Alto Orinoco region is caused mainly by *P. falciparum*, followed by *P. vivax* and, in a smaller proportion, by *P. malariae* (Figure 1.7, see page 41). Infections caused by *P. vivax* are more frequent during the dry season, while those caused by *P. falciparum* normally increase during the rainy season. The main malaria vector is *An. darlingi* and disease transmission occurs throughout the year, with seasonal peaks varying yearly.

Of all cases reported in the Amazonas state, Alto Orinoco provides approximately 40% (MSDS, 1999-2000). This figure does not take into account cases which are not registered in the local PHCs. Data obtained from the PHC from 1994 to 1998 show a decreasing trend in the API (Figure 1.8). This trend could be partially explained by a progressive improvement in the local health service and the malaria control programme. In particular, better access to prompt diagnosis and
drug treatment. In addition, environmental and climatic factors, such as cyclic flooding and drought, appear to have a major impact on malaria transmission in the area. For instance, the drought which occurred in 1998 seems to have contributed to the observed decrease in malaria transmission.
Figure 1.7 Percentage of malaria-positive blood slides by *Plasmodium* species from Ocamo and Mavaca localities, 1994-1998

Source: Magris *et al*., 1999

Figure 1.8 Annual Parasite Incidence (API) of malaria, Ocamo and Mavaca localities

Source: Magris *et al*., 1999
In recent years, several surveys have been carried out to improve understanding of the level of malaria endemicity and drug resistance in the area. These studies have mainly documented the prevalence of *Plasmodium* infection, splenomegaly and antibodies against malaria. The principal results are summarized in Table 1.1. Although some of these studies were carried out in the same localities, outcomes appear very different. These differences could be explained in several ways. First of all, they may reflect the differing seasons when the studies were carried out. Secondly, they may be due to the progressive improvements in accessibility and availability of the local health services during the time the studies were conducted. Thirdly, there may have been differences in the methodology used in data collection. Furthermore, all the studies mentioned above were cross-sectional surveys, and were not designed to observe longitudinal variations.
Table 1.1 Previous malaria surveys carried out in Ocamo and Mavaca localities

<table>
<thead>
<tr>
<th>Authors</th>
<th>Study villages</th>
<th>Period of cross-sectional survey</th>
<th>n&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Age range</th>
<th>Splenomegaly prevalence&lt;sup&gt;c&lt;/sup&gt;</th>
<th>Parasite prevalence&lt;sup&gt;d&lt;/sup&gt;</th>
<th>IgG prevalence&lt;sup&gt;e&lt;/sup&gt;</th>
<th>Mean Jib&lt;sup&gt;f&lt;/sup&gt; (g/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Torres et al., 1988</td>
<td>Ocamo, Mavaca</td>
<td>not specified</td>
<td>110</td>
<td>2-9 years</td>
<td>31%</td>
<td>3%</td>
<td>92%</td>
<td></td>
</tr>
<tr>
<td>Marcano, 1991</td>
<td>Ocamo, Mavaca</td>
<td>April - June, 1990</td>
<td>407</td>
<td>2-9 years</td>
<td>81%</td>
<td>33%</td>
<td>90%</td>
<td></td>
</tr>
<tr>
<td>Perez-Mato, 1998</td>
<td>Mavaca</td>
<td>October, 1992</td>
<td>103</td>
<td>2-9 years</td>
<td>94%</td>
<td>31%</td>
<td>91%</td>
<td>9.7 (sd: 5.1)</td>
</tr>
<tr>
<td>Villegas, 1997</td>
<td>Ocamo, Mavaca</td>
<td>June - August, 1997</td>
<td>256</td>
<td>2-9 years</td>
<td>64%</td>
<td>21%</td>
<td>10.9</td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup> All the villages in these studies were classified as nearby  
<sup>b</sup> n = Number of Yanomami included in the cross-sectional survey  
<sup>c</sup> Splenomegaly prevalence = number of individuals with palpable spleen per 100 examined  
<sup>d</sup> Parasite prevalence = number of individuals with parasites per 100 examined  
<sup>e</sup> Prevalence of anti *P. falciparum* IgG

The level of anaemia in the population was studied by Perez-Mato (1998). This author measured the haematocrit level, finding 94% of anaemia according to the definition given by WHO. High prevalence of anaemia is typically recorded in areas of malaria transmission (Menendez et al., 2000). In the Yanomami population, apart from the classical physiopathological changes caused by malaria infection, a syndrome called Hyperactive Malarious Splenomegaly (HMS) has been observed and documented by Torres et al. (1988). HMS is defined by the following features: 1) residence in a malarious area; 2) chronic splenomegaly; 3) serum IgM elevated more than two standard deviations above the local average; 4) high malaria antibody titres; 5) hepatic sinusoidal lymphocytosis, and 6) a clinical
and immunological response to long-term antimalarial prophylaxis (Marsden and Crane, 1976; Fakunle, 1981).

Data on the level of antimalarial drug resistance in the Alto Orinoco region are scarce. *In vitro* drug resistance tests carried out in 1994-1995 on *P. falciparum* samples from Yanomami living in Ocamo, confirmed the presence of resistance to 4-aminoquinolines (chloroquine and amodiaquine) but not to mefloquine and quinine (Magris, 1996). Later, Tami-Hirsch (1999), reported *in vivo* resistance to chloroquine at RIII levels in the same area.
Anopheles darlingi

Anopheles (Nyssorhynchus) darlingi (Root), is the most efficient human malaria vector in South America (Zimmerman, 1992) and the main vector in most of the Amazon basin. The first samples were collected and characterised in Rio de Janeiro, Brazil (Root, 1926). In South America An. darlingi is found from Colombia to north-eastern Argentina and in Central America in Belize, Guatemala, Honduras, El Salvador and Mexico, whereas it has not been officially reported in Nicaragua, Costa Rica and Panama. The species An. darlingi is usually identified morphologically (see review in: Rubio-Palis, 2000).

Anopheles darlingi lives mainly in warm-humid forest areas, along rivers and in lowland areas up to 1,500 m above sea level with a mean annual temperature between 25°C and 28°C. It can also be found in coastal and piedmont eco-regions, characterised by annual precipitation between 100 and 2,500 mm, as well as in tropical forest. In Guyana, Colombia, Honduras and Brazil An. darlingi has been found on the coast at <500 m above sea level, where the tropical forest reaches the sea (Rubio-Palis and Zimmerman, 1997).

This species normally breeds in partially shaded, unpolluted and relatively still pools of water with a neutral pH. In some areas of Belize, larvae have been found in floating debris (pieces of wood, dead leaves, flower and seed debris) or patches of submerged plants shaded or partly shaded in still or semi-still water. Less frequently the larvae have been collected in lake margins, small lagoons, and ground pools (Manguin et al., 1996). In Surinam, typical An. darlingi breeding
sites can be found along rivers and streams, in pools of water formed after 
flooding following the end of the rainy season. During the wet season An. darlingi 
larvae occur mainly between grass stems and debris floating in open and sunny 
places, in flooded forest areas and riverside swamps. Other breeding places can be 
found in newly opened-up areas of rainforest (often man made) such as pools 
associated with badly made drains (Rozendaal, 1990). In Ocamo (the locality 
where this study was carried out) larvae of An. darlingi have been found in 
lagoons with submerged macrophytes and in association with An. argyritarsis, 
An. marajoara, An. braziliensis, An. strodei, An. mediopunctatus and 
An. punctimacula, during surveys carried out in December 1997, during the dry 
season (Rubio-Palis, unpublished data) and in July 1997 during the rainy season 
(Rejmankova et al., 1999). Breeding site patterns were similar to those reported in 
Belize by Manguin et al. (1996).

The seasonal abundance of adult An. darlingi populations is known to vary 
throughout its distribution. Some of these differences might be related to 
geographical variations in seasonal temperatures, rainfall and river levels. In the 
Brazilian states of Amazonas, Roraima and Matto Grosso, the highest population 
densities were found during the dry season (Charlwood and Hayes, 1978), while 
in Costa Marques, state of Rondonia, An. darlingi populations peak during the late 
wet season and early dry season (Klein and Lima, 1990). In Surinam, population 
peaks occur during both the long and short dry seasons in some localities and in 
the long rainy season in others (Hudson, 1984; Rozendaal, 1987).
The biting pattern of *An. darlingi* also varies between localities (Rosa-Freitas *et al.*, 1992; Zimmerman, 1992). In some areas in Amazonas state Brazil, the peak biting pattern is bimodal, occurring in the early evening and morning (Lourenco-de-Oliveira *et al.*, 1989), while in other areas it varies from early evening to 23:30-02:00 h (Elliott, 1972; Charlwood and Hayes, 1978; Roberts *et al.*, 1987; Rozendaal, 1987). In Ocamo, *An. darlingi* biting activity occurs throughout the night, with an extended peak between midnight and 04:00 h (Rubio-Palis, 1995)(Figure 1.9).

**Figure 1.9** Biting activity of *An. darlingi* in Ocamo, Amazonas state, Venezuela (Rubio-Palis, 1995)

Studies of the resting behaviour of *An. darlingi* carried out in Venezuela, Brazil, Surinam and Guyana, concluded that this species is endophilic (Rubio-Palis,
2000), which explained the initial success of the DDT house spraying campaign in Venezuela.

In Quibdo, Department of El Chocó, in the north-west of Colombia, physiological resistance to DDT in *An. darlingi* was reported after many years of spraying by the malaria control programme (Suarez *et al.*, 1990). In a study carried out in the state of Bolívar, southern Venezuela, *An. darlingi* was found to be resistant to DDT and the pyrethroid cypermethrin, but susceptible to deltamethrin and lambda-cyhalothrin. No resistance to pyrethroids was found in *An. darlingi* from Amazonas state in Venezuela (Molina *et al.*, 1997).

*An. darlingi* has always been regarded as the most important vector of malaria within its geographical distribution, especially in the Amazon region, due to its anthropophilic habits, its longevity and its great susceptibility to infection by *P. falciparum, P. vivax* and *P. malariae* (Rubio-Palis, 2000). Arunda *et al.* (1986) analysed a total of 2,043 *An. darlingi* females, collected from the state of Para in Brazil, with the enzyme-linked immunosorbent assay (ELISA) for detection of *Plasmodium* Circum-sporozoite Protein (CSP). The percentage of infection was 4.2% *P. falciparum*, and 1.3% *P. vivax*. Only one mosquito sample reacted with *P. malariae*. Using the same technique Rubio-Palis *et al.* (1997) reported that the sporozoite rate in Ocamo was 0.42% for *P. falciparum*, 0.22% for *P. malariae* and 0.097% for *P. vivax*-247 in a sample of 7,196 mosquitoes.

Some reports have suggested that *An. darlingi* could be a species complex. Manguin *et al.*, (1999) studied samples of *An. darlingi* collected in seven
countries from America. Based on analysis with isozyme; random amplified polymorphic DNA, internal transcribed spacer 2, and morphologic markers, they concluded that all the *An. darlingi* populations examined demonstrated a genetic similarity that is consistent with the existence of a single species.

1.7 The malaria control programme in the Alto Orinoco Region

The history of malaria control in the Alto Orinoco can be divided into three periods according to the presence of different institutions in the area and the nature of the activities performed.

**First Period.** From the start of the Malaria Eradication Campaign in 1945 until the middle of the 1970s, Amazonas in general, and the Alto Orinoco in particular were considered to constitute an *inaccessible* focus of malaria. In the Alto Orinoco, the Malaria Control Programme started its activities through regional workers, on a top-down basis, in two surveillance posts built in 1959 in the localities of Mavaca and Mahekoto-theri. From these post the teams visited the Yanomami communities along the Orinoco, Ocamo and Mavaca rivers monthly, carrying out parasitological diagnosis and treatment of positive cases and, sporadically, spraying and fogging with insecticide. In remote villages, where no regular visits could be ensured, mass prophylactic treatment was often carried out (G. Bórtoli and María Blaker personal communication).
Second Period from the middle of the 1970s to 1992. In 1974 the surveillance posts were dismantled and personnel were moved to Puerto Ayacucho (two days by river from the Alto Orinoco). Until the beginning of the 1990s, regional Ayacucho teams visited the area. Visits were very sporadic, with low coverage and at great expense. Missionary nurses, starting approximately from the middle of 1970s, took over the distribution of drugs obtained from the regional programme and nearly every local case of fever was treated with antimalarial drugs irrespective of parasitological diagnosis. During this period medical assistance started to become more regular and continuous in the area.

Third period. Since 1992 local teams and medical doctors have been trained to follow the activities of the Malaria Control Programme, focusing on early diagnosis and case-treatment, with the author of this thesis being mostly responsible for the coordination of training activities.

There are six PHCs in the area, each with a medical doctor, a nurse and a microscopist. The medical care is mainly for out-patients. Severe cases of any medical condition are referred, when possible, to the hospital in Puerto Ayacucho – two hours flying in small aeroplanes. The villages that are not close to a health post are visited weekly, monthly or annually, depending on the resources available. The coverage is still very low – estimated at less than 20% of the Yanomami population – and morbidity and mortality remain high. During this period prospective studies were also carried out on malaria transmission and antimalarial drug sensitivity to understand the epidemiology of the disease in
order to propose alternative anti-vector control measures and more efficient and acceptable drug regimens, considering the socio-cultural aspects of the Yanomami population.

1.8 Malaria control by mosquito nets

Insecticide Treated Nets (ITNs) have emerged during recent years as an important tool in malaria prevention, used not only at country and regional levels in malaria control programmes, but also at the individual level by people wishing to use better ways of protecting themselves and their families from mosquito nuisance or mosquito-borne infections (Lines and Zaim, 2000).

Many studies have now shown the efficacy of ITNs in reducing malaria morbidity and mortality in a variety of epidemiological conditions. A systematic review carried out by Lengeler (2003) of all randomised controlled studies at village level, showed that the use of ITNs can reduce mild episodes of malaria by around 50% in areas of Africa with stable malaria transmission and 40% in areas with low malaria transmission such as in Asia and South America where either *P. vivax* or *P. falciparum* are prevalent. In addition, in Kenya (Nevill *et al.*, 1996) ITNs reduced severe malaria morbidity by 44% among children aged 1-59 months. The effect of ITNs on both overall and malaria-specific mortality in children was examined in areas of stable malaria in different African countries: The Gambia

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5 In stable malaria areas transmission is high and endemicity is relatively insensitive to environmental changes. Variation in the transmission is minimal over many years, although seasonal fluctuations do occur and transmission can continue even with very few vectors. The immunity of the population is high (Warrell and Gilles, 2002).
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(Alonso et al., 1991; D'Alessandro et al., 1995a), Kenya, (Nevill et al., 1996) Ghana (Binka et al., 1996) and Burkina Faso (Habluetzel et al., 1997; Diallo et al., 1999; Habluetzel et al., 1999). It was estimated that 6 deaths per year could be prevented per 1,000 children provided with nets. Making an extrapolation to the under five population at risk of malaria in Sub-Saharan Africa (14% of 400 million population, or 56 million), it was estimated that approximately 336,000 child deaths could be avoided if every child could be protected by an ITN (Lengeler, 2003).

However, operational issues such as large-scale use, and development of systems for insecticide re-treatment, have posed serious problems for the implementation of this measure of malaria vector control. Randomised control trials and meta-analysis have been carried out on the efficacy of treated versus untreated nets or the use of no nets at all. Results of these studies show that the protective efficacy of untreated nets could be approximately half of that of ITNs (Choi et al., 1995; Jana-Kara et al., 1995; Maxwell et al., 1999; Abdulla et al., 2001; Clarke et al., 2001; Guyatt and Snow, 2002; Lengeler, 2003).

Although the short-term protective efficacy of ITNs in the context of well-controlled, randomised trials has been confirmed in several studies, the consequences of their long-term use on epidemiological parameters are not yet completely clear. Particularly in areas with high levels of malaria transmission, it has been argued that reducing the transmission intensity from high to intermediate level could delay the natural acquisition of malaria immunity in children, with a
consequent mortality rebound in later childhood (Trape and Rogier, 1996; Snow et al., 1997b). This issue is considered in the General Discussion (page 203).

1.9 Effect of ITNs on the vector population

Untreated bednets have long been used as an individual protective measure against mosquito bites as they constitute a physical barrier between humans and mosquitoes. However, a high level of protection depends on factors like good maintenance of the net and its correct use. The protective effect of a bednet can be enhanced by treatment with an insecticide. Mosquitoes are attracted by the carbon dioxide and odour of the person sleeping under the net, and once they approach the treated bednet they are repelled and/or killed by the insecticide. ITNs are more specific than residual house spraying because the insecticide is placed in the path of the host-seeking mosquito, whereas with house spraying mosquitoes absorb the insecticide only if they rest on the walls, generally after feeding. If the vector is highly exophilic, house-spraying may not be effective. For example, one of the reasons for the poorer than expected impact of house spraying in the district of Garki in Nigeria in the 1970s was thought to be the presence of a heterogeneous population of An. gambiae sensu stricto, with only one part of the population endophilic and another part of the population exophilic. The indoor residual spraying left the exophilic population unexposed to the insecticide and this fraction of the population was considered to be sufficient to maintain the transmission of malaria (Molineaux et al., 1979).
In addition, there is evidence that an ITN with large holes protects as well as an untreated intact bednet, reducing biting by up to 95% (Lines et al., 1987; Curtis et al., 1992) and often bednets and curtains in the field are torn.

The impregnation of bednets with a synthetic pyrethroid reduces malaria transmission in two ways: by providing protection to individuals sleeping under treated bednets (or in houses with treated curtains) through a repellent effect and inhibition of biting; and by killing mosquitoes. If treated bednets/curtains are widely used in the community a reduction in the density of the local vector population may occur, called the mass killing effect. In addition reduction in longevity occurs, i.e. the mosquitoes are killed before the parasite has completed its cycle and can be transmitted (Lines, 1996).

The mass killing effect has been studied in Africa. In particular, it has been documented in Burkina Faso (Cuzin-Ouattara et al., 1999), Tanzania (Magesa et al., 1991; Maxwell et al., 1999) and Kenya (Howard et al., 2000; Hawley et al., 2003). In The Gambia, however, it was not possible to show such an effect (Quinones et al., 1998) and the observed protection against malaria seen in children using treated bednets was thought to be primarily due to personal protection. No studies have been so far carried out on the mass killing effect in America.
Chapter 1

If the vector population density is reduced by the extensive use of bednets in the community, those people who do not have bednets are also protected against malaria.

One aspect of choosing the right insecticide compound for net treatment is its irritancy. If the insecticide is too irritant, because of its specific chemical properties or a too high concentration is applied, the irritated mosquito may fly away before absorbing a lethal dose of insecticide through tarsal contact. In this case the net may provide a good personal protection with no effect on the density of the vector population. In addition, the use of such nets might increase the risk of biting of unprotected people sleeping in the same house or community (Rozendaal and Curtis, 1989).

Hodjati and Curtis (1997) investigated the effects of bednets impregnated with 200 and 500 mg/m² permethrin on pyrethroid resistant and susceptible strains of *An. stephensi* under experimental conditions. They showed that in these conditions, the higher dose provoked more irritation, but lower knockdown and mortality rates, whereas the lower dose was less irritating and hence more effectively insecticidal. Thus a dose of 200 mg/m² is preferable to 500 mg/m² for the permethrin impregnation of nets for malaria vector control.
1.10 The pyrethroids: insecticides used for the treatment of mosquito nets

The impact of ITNs on reducing the intensity of malaria transmission relies on the efficacy of the insecticide used. Pyrethroids are currently the only class of insecticide approved for use on nets due to their low toxicity to mammals and high toxicity to insects (Zerba, 1988; Zaim et al., 2000). They are synthetic analogues of the natural pyrethrins contained in flowers of the genus Chrysanthemum. They constitute, together with chlorinated hydrocarbons (DDT, dieldrin, lindane), organo-phosphorus compounds (parathion, malathion, diazinon) and carbamates (methylcarbamate esters such as bendiocarb, carbosulfan and carbaryl) one of the four major classes of insecticides. Pyrethroids are neurotoxins and have been grouped into two subclasses (Type I and II) based on chemical structure and the symptomatology after acute intoxication of insects and mammals. Type I pyrethroids include non-α-cyano-pyrethroids such as permethrin that induce a tremor syndrome (T-syndrome) characterized by hyper-excitation, ataxia and convulsion followed by prostration and flaccid paralysis. Type II pyrethroids are characterized by the presence of an α-cyano group. They produce a choreoathetosis syndrome in rodents with salivation and finally paralysis. Although this classification system is widely employed, it has several shortcomings for the identification signs found following oral administration of various pyrethroids (Soderlund et al., 2002). In mammals and insects the principal molecular mode of action of synthetic pyrethroids is considered to be an alteration of sodium channel kinetics (Vijverberg and van den Bercken, 1982). They act on the nerve membrane, modifying the sodium
channels, probably by preventing protein conformational changes at the lipid-protein interface. The effects on the nervous system include repetitive firing, blockage of impulse conduction or of neuromuscular transmission, and spontaneous depolarization of the resting potential (Zerba, 1988). However, additional mechanisms have been described such as inhibition for Ca\textsuperscript{++}-channels, ATPases and the receptors for acetylcholine, serotonin and benzodiazepine (reviewed by Zlotkin, 1999). Whether any of these interactions are responsible for the toxicological effects of pyrethroids in higher animals remains unclear.

The pyrethroids currently recommended for net treatment are: alphacypermethrin, cyfluthrin, deltamethrin, lambdacyhalothrin and permethrin and one non-ester pyrethroid (etofenprox). A careful assessment of the risk associated with the use of one of the pyrethroids, deltamethrin, on treated bednets was published recently by Barlow et al. (2001) who concluded that the risks in using this insecticide are low, and are greatly outweighed by the benefits of using treated bednets in reducing malaria morbidity and mortality.

Lambdacyhalothrin was the insecticide chosen for the treatment of nets in the present study. It belongs to the sub-group of pyrethroids characterized by an α-cyano group. It has only moderate acute mammalian toxicity and it is listed in the WHO pesticide classification (WHO, 1988) as being only moderately hazardous (class II), like most other pyrethroids. An evaluation undertaken of the safety for operators and users of nets impregnated with lambdacyhalothrin, has shown that absorption of the insecticide is minimal with no significant change of vital signs
Minimal side effects, such as a burning sensation in the eyes, lacrimation and a runny nose, have been reported from close contact with freshly treated nets in Tanzania (Njunwa et al., 1991).

1.11 Studies with ITNs in America

Few studies have documented the effectiveness of treated nets on malaria control in Central and South America. A summary of published studies of malaria control using insecticide treated nets in the continent is presented in Table 1.2, see pg. 61).

In the Amazon region of Brazil, in the locality of Costa Marquez, Rondonia, Santos et al. (1998) conducted a study to evaluate the effectiveness of impregnated bednets in preventing malaria. In this study two groups of 20 houses were selected. One group received deltamethrin impregnated bednets ($20 \text{ mg/m}^2$) and the other received untreated bednets. Clinical, parasitological and entomological evaluations were performed every two months for one year. The results of this study showed a higher risk of malaria infections in the untreated bednets group during the high transmission season but no significant difference during the low transmission season. At the end of the study, a decrease in spleen size and an increase of haematocrit to normal levels were observed in both groups and the difference between the groups was not significant. The authors suggested that these effects were primarily due to the reduction of the malaria incidence rate by 86% recorded in the entire area rather than due to the intervention. Another factor that could have contributed to the lack of difference between the two study
arms was that the peak of activity of the main vector, *An. darlingi*, is at dusk and dawn in this area, when the majority of the people are unprotected. In addition, the sample size in this study was probably too small (20 houses per study arm) to detect differences between intervention and control group in an area with moderate transmission.

In northern Guatemala, where the principal vectors are *An. albimanus* and *An. vestitipennis*, and approximately 90% of the malaria cases are due to *P. vivax* infections, Richards et al. (1993) studied the impact of treated bednets on malaria incidence and prevalence. Three villages were selected. One received permethrin (500mg/m²) treated bednets, one received untreated bednets and one was used as a control (no nets). In addition, 100 households in another two villages were randomly allocated to a treated bednets or no nets group. The authors found that all bednets, treated and untreated, reduced the malaria incidence by 57% and 47% respectively and suggested that this reduction was mainly due to a personal protection effect due to the nets providing a physical barrier against the vector.

Only one community randomized study has previously been carried out in America and it is included in the Lengeler review (Lengeler, 2003). In this trial Kroeger et al. (1995) studied the effectiveness of pyrethroid treated nets in different hypoendemic malaria areas in Ecuador, Colombia and Peru. The study design consisted of a community randomized controlled trial, with no blinding, where villages were paired according to size, geographical location, net coverage and malaria incidence at baseline. The intervention group received treated nets
and the control group received untreated nets. The effectiveness of the bednets was measured in terms of reduction of malaria incidence estimated from resident’s self-diagnosed cases. The analysis of all areas together indicated that the incidence rate was significantly lower in the villages with pyrethroid treated nets as compared to villages with untreated nets. The average reduction of malaria incidence was 41% over a four-month period and 28% over a two-week period. Results of the studies for each area are summarized in Table 1.2.

A trial in Surinam, cited in Zimmerman and Voorham (1997), where the main malaria vector is *An. darlingi*, reported that permethrin (500mg/m²) treated hammock nets reduce malaria prevalence from 15-20% to less than 1% after 36 months of the intervention. According to Zimmerman and Voorham (1997) the variation in the epidemiology of malaria in these areas can lead to results which are difficult to interpret. For instance, *P. vivax* infections are characterized by a high rate of relapse (if the parasite is not treated with primaquine) making it difficult to measure malaria incidence. Moreover the vector’s twilight biting habit, in some areas, makes it difficult to evaluate the benefit of impregnated bednets.
Table 1.2 Review of the studies on Insecticide Treated Nets in America.

<table>
<thead>
<tr>
<th>Country &amp; References</th>
<th>Insecticide</th>
<th>Study design</th>
<th>Predominant Plasmodium Infection</th>
<th>Predominant vector species</th>
<th>Outcomes</th>
<th>Main results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brazil: Porto Murtinho, Costa Marques (Santos et al., 1998)</td>
<td>Deltamethrin 20 mg /m²</td>
<td>Non-randomized allocation of 40 households in two villages -20 received treated bednets and 20 untreated.</td>
<td>63% <em>P. vivax</em></td>
<td><em>An. darlingi</em></td>
<td>Malaria incidence, splenomegaly prevalence and haematorcrit level</td>
<td>No significant differences between study arms</td>
</tr>
<tr>
<td>Colombia coast: Pobado, San Juan River, Choco Department (Kroeger et al., 1995)</td>
<td>Lambdacyhalothrin 10-30 mg /m²</td>
<td>Randomized control trial, 22 villages were paired according to size, geographic location, net coverage and malaria incidence at baseline. Within each pair one village was randomized to receive the intervention (promotion, selling and and impregnation of bednets). Control remained with untreated nets.</td>
<td>68.5% <em>P. falciparum</em></td>
<td><em>An. nevali</em></td>
<td>2 weeks and 4 months malaria incidence rate measured on self-diagnosed cases of malaria reported to researchers</td>
<td>Significant differences between study arms. Reduction of about 71-81% of malaria incidence.</td>
</tr>
<tr>
<td>Ecuador coast: Muisne, Esmeralda Province (Kroeger et al., 1995)</td>
<td>Permethrin 200mg /m²</td>
<td>Study design: <em>ibidem</em>. Sample size: 14 villages</td>
<td>51% <em>P. vivax</em></td>
<td><em>An. albimans</em></td>
<td><em>Ibid.</em></td>
<td>No significant differences between study arms. Reduction of about 20-22.7% of malaria incidence</td>
</tr>
<tr>
<td>Peru coast: Catacos, Piura Department (Kroeger et al., 1995)</td>
<td>Lambdacyhalothrin 9 mg/m² (Year 1) Permethrin 550 mg/m² (Year 2)</td>
<td>Study design: <em>ibid.</em> Sample size: 12 villages</td>
<td>100% <em>P. vivax</em></td>
<td><em>An. albimans</em></td>
<td><em>Ibid.</em></td>
<td>No significant differences between study arms. Reduction up to 10% of malaria incidence</td>
</tr>
</tbody>
</table>
### Table 1.2 continued from previous page

<table>
<thead>
<tr>
<th>Country/Organization</th>
<th>Insecticide</th>
<th>Study design</th>
<th>Sample size</th>
<th>Anopheles</th>
<th>Malaria incidence</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peru Amazon: Tambopata District, Madre de Dios Department (Kroeger et al., 1995)</td>
<td>Permethrin 200 mg/m²</td>
<td>Study design: <em>ibid.</em> Sample size: 36 villages</td>
<td></td>
<td>100% <em>P. vivax</em></td>
<td><em>An. evansae</em></td>
<td>Ibid. No significant differences between study arms. Reduction of about 33-40% of malaria incidence</td>
</tr>
<tr>
<td>Nicaragua coast el Viejo, Chinandega department (Kroeger et al., 1999)</td>
<td>Lambdacyhalothrin 12.5 mg/m²</td>
<td>Study design: <em>ibid.</em> Sample size: 12 villages in 1994 and 26 in 1996</td>
<td></td>
<td>99% <em>P. vivax</em></td>
<td><em>An. albimanus</em></td>
<td>4 months malaria incidence rate measured on self-diagnosed cases of malaria reported to researchers. The protective efficacy varied according to coverage: ~68% with 31-70% coverage and significant differences between study arms 322% with 16-30% coverage and significant differences between study arms 0% with less than 16% coverage</td>
</tr>
<tr>
<td>Guatemala: Los Amantes (Richards et al., 1993)</td>
<td>Permethrin 500 mg/m²</td>
<td>Non-randomized allocation of treatment in 3 villages: treated, untreated nets and no nets. A further 50 households in 2 additional villages were chosen and 25 randomly assigned treated bednets.</td>
<td></td>
<td>90% <em>P. vivax</em></td>
<td><em>An. albimanus</em></td>
<td>Malaria incidence -ITNs vs NNs, reduced significantly malaria incidence (about 57%) -UNs vs NNs, reduced significantly malaria incidence (about 47%) -ITNs vs UNs, reduced malaria incidence (about 19%) but the difference was not significant</td>
</tr>
<tr>
<td>Surinam (Voorham, 1997)</td>
<td>Lambdacyhalothrin 25mg/m²</td>
<td>Non-randomized study. No contemporary control. Intervention in 270 houses with impregnated wide-mesh gauze that covered the openings in the wooden walls of huts</td>
<td></td>
<td>95% <em>P. falciparum</em></td>
<td><em>An. darlingi</em></td>
<td>Malaria prevalence before and after the intervention Malaria prevalence was significantly reduced from 35-40% to 5-10% after 36 months of intervention.</td>
</tr>
</tbody>
</table>
1.12 Organization of the thesis

This thesis presents the results of the first evaluation of insecticide treated hammock nets (ITHNs) for malaria control among the Yanomami population in the Amazon region of Venezuela.

The general Introduction (Chapter 1) presented the situation of malaria in the study area, Alto Orinoco, and more generally in Venezuela and South America. Particular attention is given to the description of the Yanomami ethnic group, the study population, of which generally little is known. A description is given of An. darlingi, the main malaria vector in the area and in the Amazon region. At the end of the Chapter a review of the major studies carried out on the impact of Insecticide Treated Nets (ITNs) on malaria is presented.

In Chapter 2 the aims and objectives of the present study are described as well as the general methodology used for the treatment of hammock nets and the implementation of the intervention. This Chapter includes the selection of the sample size in the particular situation of the Yanomami population where small villages are scattered in a large area.

In Chapter 3 methodological and operational studies relative to the insecticide treatment of hammock nets are described, including different impregnation procedures, frequency of net-washing, residual insecticide effects and maintenance of nets in relation to the habits of the population studied. All technical aspects of this kind of intervention are discussed in the light of future projects of the Malaria Control Programme, to provide ITNs in the same area.
Chapter 4 describes the methodology used to assess the impact of the intervention on the \textit{An. darlingi} vector population, and the results are presented, analysed and discussed.

Chapter 5 includes methods, results, analysis and discussion of the longitudinal follow-up and cross-sectional surveys carried out in order to measure the effect of the intervention on the malaria incidence rate, which was the main outcome of the present research. This chapter also presents the results of the intervention on malaria associated factors, such as prevalence of parasitaemia, splenomegaly and haemoglobin level.

Finally, a general discussion of the results, conclusions and suggestions for future work are presented in Chapter 6.
CHAPTER 2. Rationale, general objectives, study design and general aspects of the intervention

2.1 Rationale of the study

Indoor Residual Insecticide Spraying (IRS) and fogging were for many years the only vector control measures carried out in Yanomami villages of the Alto Orinoco region of Southern Venezuela. The spraying was limited to those areas which could easily be reached, and the frequency of the treatment was sporadic, due to financial constraints and scarce availability of trained personnel. In 1992, some Yanomami communities started using hammock nets, spontaneously to avoid the nuisance of mosquitoes at night and of black flies (Simuliidae) during the day. In 1996, it was proposed that the women’s co-operative start to make, sell and/or exchange nets (whose fabric was subsidised by the Rotary Club). The use of hammock nets was considered to be more suitable than house spraying in the local socio-cultural context. This was, firstly, because the typical Yanomami house has a very small surface to be sprayed, and secondly, because the Yanomami often sleep in the forest at night, far from their village, and hammock nets can easily be transported and used. The main malaria vector in the region, An. darlingi, tends to show an endophagic-exophilic behaviour and, in the study area, the highest risk of infection is at night, as the local An. darlingi shows increased biting activity from midnight to early morning, when most people are sleeping (M. Magris, unpublished observations). All this suggested that the introduction of impregnated nets might have advantages over previous vector control methods for malaria vector control in the Alto Orinoco region. This study
represents the first effort to evaluate, epidemiologically and entomologically, the impact of this type of intervention for malaria control in the Amazonas state of Venezuela. It is also the first community randomised placebo controlled trial to be undertaken in an area where *An. darlingi* is the vector; this species is the most important malaria vector throughout the Amazon basin.
2.2 Aim of the study

This project aimed, firstly, to evaluate the impact of community-wide use of Insecticide Treated Hammock Nets (ITHNs) in comparison with Placebo Treated Hammock Nets (PTHNs), on parasitological and clinical indicators of malaria (malaria morbidity) among the Yanomami population, and secondly the effects of ITHNs on entomological indices of the malaria vectors in the Alto Orinoco region of Southern Venezuela.

Specific objectives for the study were:

- To introduce a net distribution programme in the Yanomami communities of two localities, Ocamo and Mavaca, of the Alto Orinoco region.

- To determine the impact of the intervention on malaria incidence, prevalence, and clinical indicators in the study population.

- To characterise malaria transmission in the study area in terms of mosquito density and sporozoite rate and to monitor the impact of the intervention on these entomological indices.

- To investigate technical aspects of net-treatment methods and factors affecting durability of nets and insecticide
2.3 General methodology

2.3.1 Study design

The optimum design for most intervention trials is that in which allocation into
different groups is done at random at the individual level. This approach may not
be desirable for trials on ITNs as the use of an impregnated net by one individual
may have an effect on the risk of malaria for those sleeping nearby. An alternative
is to allocate the intervention at the household level. In Yanomami villages,
however, it is common for a member of one house to sleep in another house in the
same village on some nights. For these reasons, in this study, it was decided to use
a village (shabono) as the unit of allocation.

Another component of an ideal intervention trial is the double blinding. But, in
practice, in studies where the intervention is an ITN, it is difficult to maintain the
blindness, as the effect of the insecticide on mosquitoes is easy to recognise.
Furthermore, object exchange is frequent between Yanomami, even between
individuals from different shabonos. Thus there was the possibility that nets with
and without insecticide could be exchanged. It was therefore decided that the
principal investigator should know the allocation of the nets in order to be able to
identify any exchange between insecticide-treated and placebo-treated nets.

The study was then designed as a single blind randomised study with matched-
pairs of villages. The study villages were matched into pairs by API data from
1997\(^6\) (Table 2.1, see page 76) in order to minimise the potential confounding variables. This means that in the absence of the intervention we would have expected the same risk of malaria between villages of the same pair. Then, one village of each pair was assigned, at random, to one intervention group (ITHN) and one to the other (PTHN). The randomisation within the matched-pairs was done by tossing a coin: heads for ITHN and tails for PTHN.

A comparison between treated nets and no nets at all would have been methodologically desirable because net use was very rare when the study began, and because this comparison would have maximised the probability of finding a clear impact of ITHNs. However, since this was considered ethically unacceptable, in the present study there were two arms: insecticide treated hammock nets compared with placebo treated hammock nets. The disadvantage of this design is that if untreated nets are well used and maintained they may confer a good level of personal protection. In this case, there might be a large reduction in malaria in both groups, as compared with past incidence and prevalence rates, and little difference between them.

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\(^6\) Malaria incidence in 1997 instead of 1998 was chosen to match the villages. This is because 1998 was, in many ways, an exceptional year, with a long drought, extensive and repeated forest fires, and completely dry breeding sites. As a result an unusual and dramatic reduction in malaria incidence was seen.
2.3.2 Study area

As already described in the Introduction (Chapter 1, page 32), a geographical classification of Yanomami communities has been proposed by Chagnon (1992) and Lizot (1998) in relation to the distance between the *shabono* and the settlements with PHC or missions. In this sense communities are classified into *nearby*, *intermediate*, and *distant or remote* categories.

*Nearby* villages, are those Yanomami settlements in the immediate vicinity of missions or PHCs. Of those located in areas of malaria transmission, the Ocamo and Mavaca localities have the highest density. *Intermediate* and *distant* villages are those accessible by travelling from 3 to 10 hours by boat or 1 to 2 days on foot through the jungle from the nearby villages. *Remote* villages are extremely remote settlements needing a helicopter to reach them or more than two days walking through the jungle.

The *intermediate* and *remote* categories are more representative of the conditions of traditional Yanomami communities but regular surveillance is not possible. Thus in this study it was necessary to restrict the study frame to *nearby* villages. For this reason, the sample size (measured as the number of pairs of villages) is just large enough.

The study area comprised Yanomami villages located in the localities of Ocamo (2°47'8.5"N; 65°12'57"W) and Mavaca (2°30'38"N, 65°09'28"W) in the Upper Orinoco basin of Amazonas state in Venezuela, ranging in altitude from 110 to 120 m above sea level. The climate is characteristic of tropical rain forest,
according to the Haldrige classification (Ewel *et al.*, 1976), with an annual rainfall between 3,750 and 5,000 mm³, an average temperature of 26-27°C, and 80% relative humidity. Although there is virtually no season without rain, the months with less precipitation are usually from October to March and the rainy season occurs from April to October.

The Ocamo locality comprises ten villages (*shabonos*): Santa María de los Guaicas, Carlitos, Clavotheri, Iyewēi-theri, Hishinowēi-theri, Mario, Kashorawē, Shashanawē, Yohoope and Lechoza, located along the Orinoco and Ocamo rivers. Mavaca is composed of eight *shabonos*: Mosho, Kopariwē, Piegrita, Motorema, Purima, Hatakoa, Shakita and Warapana, located along the Orinoco and Mavaca rivers (Figure 2.1). The distance between the Mavaca and Ocamo localities is approximately of 25 km, and between their component villages is no more than 2 km.
Figure 2.1 Map of the study area in Alto Orinoco, Venezuela. The nine villages used as controls (Placebo Treated Hammock Net) are marked with green circles and the nine that received lambdacyhalothrin treated hammocks nets are marked with red circles. The numbers correspond to the paired villages as is shown in Table 2.1.
2.3.3 Sample size calculation

The sample size was calculated by the method proposed by Hayes and Bennett (1999) for a matched-pair trial. The protocol required an 80% power for detecting a 30% reduction of annual malaria incidence from 513.5 per 1000-person years at risk (incidence of 1997, see Table 2.1 page 76) in the control group to 359 per 1000-person years at risk in the intervention group. These calculations showed that with the 18 available communities, the sample size would be inadequate with just one season of data, but would be adequate with a study duration of two seasons (18 months). According to the parameters described below, the number of clusters required is given by the following expression.

\[ c = 2 + \frac{(Z_{\alpha/2} + Z_{\beta})^2 [\lambda_0 + \lambda_1/y] + k^2 (\lambda_0^2 + \lambda_1^2)}{(\lambda_0 - \lambda_1)^2} \]

where \( c \) is the required number of clusters in each arm of the study. \((Z_{\alpha/2} + Z_{\beta})^2\) is 7.84 for 80% power. \( \lambda_1 = 0.359 \) and \( \lambda_0 = 0.513 \) are the assumed annual malaria incidence rates in the presence and absence of the intervention respectively. \( k = 0.02 \) is the coefficient of variation within pairs and \( y \) is the number of person years of follow up which in this case was estimated as 888 per 1.5 years.

With these parameter values, the minimum number of clusters required was 8 in each arm, for a total of 16 villages (8 matched pairs).
2.3.4 Census

A pre-intervention census of the 18 villages was carried out in November 1998, in order to register the number of individuals living in each village. The following data were recorded for each member of the household: name, age, date of birth (where possible), sex, and residence status (whether resident or visitor in the village). To assess the age of the Yanomami, birth records were taken from the PHC, the missionary posts, and the records made by Cocco (1987) and Lizot (unpublished). When records did not exist, the age was estimated using the following methods recommended by Lizot (pers comm.): a) determining whether individuals know if they are younger or older than other people in the community; b) in the case of women, knowing the number of living children; and c) enquiring about the memory of past events experienced by the Yanomami which have taken place in a particular year known to the researchers or the missionaries.

The census was updated in each cross-sectional survey in order to register new births, deaths, and migrations.

2.3.5 Characteristics of the study population

The census of 1998 recorded a total study population of 924 inhabitants, 559 (60.5%) from Mavaca and 365 (39.5%) from Ocamo. The range of population size by village was from 12 to 136. The numbers of inhabitants by village and
study arm are shown in Table 2.1. There was no significant difference between study arms in the age distributions of either male or females.
Table 2.1 Number of inhabitants by pair of villages assigned to Insecticide Treated Hammock Nets (ITHN) and Placebo Treated Hammock Nets (PTHN) group and Annual Parasite Incidence (API) of the study villages during 1997. This API was used in the design and sample size calculation for this trial

<table>
<thead>
<tr>
<th>Region</th>
<th>Pair</th>
<th>Village</th>
<th>ITHN</th>
<th>PTHN</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>API</td>
<td>Total</td>
<td>Female</td>
</tr>
<tr>
<td>Mavaca</td>
<td>1</td>
<td>Hatakao</td>
<td>1018</td>
<td>51</td>
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<tr>
<td></td>
<td>2</td>
<td>Purima</td>
<td>857</td>
<td>54</td>
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<td>3</td>
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<td>4</td>
<td>Kopariwe</td>
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<td>Lechoza</td>
<td>596</td>
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<td>Ocamo</td>
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<td>Shashanawe</td>
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<tr>
<td></td>
<td>8</td>
<td>Clavotheri</td>
<td>222</td>
<td>12</td>
</tr>
<tr>
<td></td>
<td>9</td>
<td>Mario</td>
<td>*</td>
<td>22</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Overall</td>
<td>429</td>
<td>198</td>
</tr>
</tbody>
</table>

*Annual Parasite Incidence=(number of malaria cases/number of inhabitants per village) per 1000

*In pair 9 villages do not have API for 1997 because both villages were constituted with families from Sta. Ma. de los Guaicas in the end of 1998 and it was decided to make a pair with Mario and Hishinowei-theri.
2.3.6 Organization of field work

The study was conducted in collaboration with the Yanomami communities of Ocamo and Mavaca, the Alto Orinoco Health District (AOHD), the School of Higher Studies in Public Health "Dr. Arnoldo Gabaldón", and the Centro Amazónico de Investigación y Control de Enfermedades Tropicales (CAICET). The parasitological and most of the entomological laboratory work was done in field stations in the Alto Orinoco. Bioassays were performed in the Department of Research of the School of Higher Studies in Public Health "Dr. Arnoldo Gabaldón" in Maracay, Aragua state. Immunoassays were done in CAICET with the collaboration of Dr. Adeline Chan from Centers for Disease Control and Prevention, Entomology Branch, Atlanta, Georgia, USA.

a) Field team

The data were collected by a research team composed of the principal investigator (M. Magris), two medical doctors, three entomology technicians, two Yanomami assistants for field entomological collections, one Yanomami nurse, two Yanomami microscopists and two boat drivers. The principal investigator trained all the personnel. In addition, a Venezuelan entomologist (Dr. Yasmin Rubio-Palís) and her malarialogy inspectors, collaborated with the project and they were instrumental in the training of the entomological technicians and Yanomami assistants.
b) Logistics

During the project, existing field laboratories at the PHC post were up-graded and provided with equipment (microscopes, electricity generators, fridges, solar panels, etc.). The medical doctors resided in the PHC posts, one in Ocamo and one in Mavaca localities, during the study period. The entomology technicians travelled each month from Maracay and/or Puerto Ayacucho to the study area by light aircraft and stayed there for 15 days per month. As there are no roads in the study area, all transport was by boat and on foot.

2.3.7 Ethical considerations

In 1996, several meetings were held with the Yanomami from Ocamo and Mavaca localities in order to find out the opinion of the population about the use of hammock nets to prevent mosquitoes bites, the possible design of those nets, possible problems that could arise with their use — e.g. use close to the fire, etc. There was also discussion about how to raise funds to prove the efficacy of hammock nets for malaria control if it was agreed that the community wanted them. In agreement with the villagers in 1996, a project was submitted to the Ministry of Health (MoH) of Venezuela for financial support. The project was included in a wider research agenda for endemic disease control programmes that was funded by the Venezuelan government and the World Bank, agenda known as Endemic Diseases Control Project (EDCP-MoH/WB).
By the beginning of 1997 the project had obtained technical approval and there were no impediments to its initiation following the criteria of the EDCP-MoH/WB. It had also been submitted to and approved at the Regional level of the Ministry of Health in Amazonas. The planned starting date was the middle of 1997. By that time, the regional and central authorities of the MoH had changed. The new authorities demanded a re-appraisal of technical issues and the constitution of an ethical committee specifically for this particular project, following instructions by the new Minister of Health. The constitution of the committee took several months. The technical details of the project were re-evaluated in 1998 by a scientific expert committee with members of the Instituto Venezolano de Investigación Científica (IVIC); the Instituto de Medicina Tropical de la Universidad Central de Venezuela (IMT-UCV) and CAICET, who reported back to the Regional Health Council. That year the project obtained ethical clearance from an ad hoc ethical committee of the Venezuelan Ministry of Health. Technical and ethical approval was finally released, for the second time, in September 1998 (see Annex 1).

New community meetings with the residents of the study villages of Ocamo and Mavaca localities were held before starting the interventions in order to explain again the aims of the project, the study design, and the procedures for monitoring. Before the introduction of the hammock-nets, in December 1998, informed consent was obtained, household by household, by the PI from all residents of the villages involved (see Annex 2).
In addition, each time blood samples for the parasitological diagnosis of malaria were required, verbal consent was sought from the participants in the study. In the case of children, consent was sought from their parents or guardians.

Parasitological diagnosis and haemoglobin tests were done in the field on the day the samples were collected. The results were returned to the community within 24 hours. Both positive and negative slide results were reported. Individuals found positive were treated according to *Plasmodium* species and clinical severity. If, during the clinical examination, other conditions were detected (pneumonia, severe anaemia, skin infections, scabies, diarrhoea, etc.), appropriate treatment or referral was provided.

Lambdacyhalothrin was chosen for this study. This insecticide has been endorsed by the World Health Organisation Pesticides Evaluation Scheme for use on nets against malaria mosquito vectors (WHO Specification VBC/IS/91.23) (Zaim et al., 2000) see also Chapter 1, page 56).

### 2.3.8 Data management

All data collection forms were evaluated in the field during the pre-intervention period. Each participating subject was identified by a unique code as well as by his/her corresponding cross-sectional data collection form as follows: one digit for the number of the cross-sectional survey in which s(he) participated, and three last digits for the individual identification number. Cross-sectional forms were
checked daily for accuracy and completeness and entered on to a computer after each cross-sectional survey. Data from active and passive case detection were identified with name, village and the locality of each individual and then assigned an identification number according to the census. These forms were entered onto the computer monthly. Databases were designed and double-entered in Epi-info (version 6.0; Centers for Disease Control and Prevention, Atlanta-Georgia, USA), which can make logical checks. A validation programme was run with both entries and any inconsistencies were corrected using the original questionnaire. The statistical analysis was performed in Stata (version 7.0; Stata Corporation College Station, Texas).

2.3.9 Strategy of analysis and statistical methods

Before starting the analysis, the distribution of each variable was tested and transformations were done, when necessary, to normalize the data for analysis assuming a normal distribution. When this was not possible, the original distribution was fixed in the model for analysis. Descriptive statistics of the parasitological, clinical and entomological data were initially performed.

To see the effect of the intervention on malaria incidence and morbidity, means and proportions by villages were first compared by paired t-test, and then using a multiple regression model the outcome was adjusted for covariance and the predicted residual was estimated. The observed outcome value was then divided by the predicted residual. To test the null hypothesis – no effect of the
intervention- a paired or un-paired t-test was carried out. These methods were proposed by Bennett et al. (2002).

In addition, protective efficacy was estimated for each outcome. Detailed descriptions of the specific analysis procedures relevant to each objective are given in the corresponding chapter.
2.4 Intervention

2.4.1 Characteristics of the nets

Before starting the study, members of the villages were invited to hold a meeting to discuss the design of the hammock nets. The discussion addressed issues relating to the size, colour, model and use or not of a zipper. The communities decided that they preferred a green rectangular net without a zipper. Two long sleeves at each end were designed in order to allow passage of the rope to hang the hammock. Four small pieces of netting were added to the ends, so that two sticks could be fitted to spread the net. The hammock nets were manufactured in Venezuela with the following dimensions: 200cms x 150cms x 75cms (≈10 m²) at a unit cost of US$8. The netting (green) was made of 100% polyamide (nylon) of which 79% was 40 denier and 21% 15 denier. The mesh was less than 0.2cm in order to ensure exclusion of black flies, which are a major biting nuisance outdoors and in the day time (Figure 2.2).

Before impregnation, each net was given a code number. A four-colour code was used to identify insecticide treated nets and placebo treated nets to minimise the possibility of users knowing which kind of net they received. Each colour card had an identification number with protective plastic covering, which was stapled on to the nets.
Figure 2.2 Design of the hammock nets
2.4.2 Impregnation

Mosquito nets were first impregnated by project staff in January 1998, at CAICET. After that, re-impregnation of the nets was planned every 6 months in the village by villagers with close supervision by the project staff.

The nets used in the intervention group received lambdacyhalothrin (Icon®) 2.5 CS (capsule suspension) supplied free of charge by Zeneca Public Health. The target dose was 10mg/m². The protocol for dipping each net was as follows: the insecticide concentrate was placed in a large plastic bowl and water added to make a solution containing 0.05% of lambdacyhalothrin. The nets were soaked in the solution for a few minutes, after which nets were removed from the bowl and any excess liquid squeezed out. The treated nets were hung on a line to dry in the sun. Anyone who dipped a net or nets was provided with a pair of rubber gloves and boots.

The impregnation of nets for use in the control group was done with a “blank” formulation (i.e. comprising the same solvents but without the active ingredient) provided by the insecticide manufacturers, and was carried out in the same way as for lambdacyhalothrin.

Before each re-impregnation, villagers were asked to wash their nets thoroughly with their usual soap and bring them to a central place in each village on a fixed day. A Yanomami team was trained to carry out the impregnation at community level and the research team supervised the operation (Figure 2.3, see page 88). Ninety millilitres of lambdacyhalothrin were measured into an empty plastic
container previously calibrated for the purpose and then poured into a large plastic bowl with 20 litres of water. This amount was sufficient to treat 20 nets. The remaining mix of lambdacyhalothrin was thrown away in the soil. This was acceptable because the insecticide is degraded rapidly and does not produce residues that are spread into the environment (IPCS, 1990).

The initial impregnation of the nets took place in January 1998, before distribution. For unavoidable reasons (explained in page 78), distribution of the treated nets did not take place until December 1998. In the meantime the nets were stored in plastic bags in the PHC post in Ocamo. Because of the delay, the quality of the insecticide deposit was tested in Oct/Nov 98 with gas liquid chromatography, and with bioassays, and was found to be satisfactory (see results, page 102). The second impregnation was carried out in July 1999. During the start of the third impregnation in February 2000, a problem was noticed with the insecticide that was being used. In fact the batch used, compared with the batch of previous impregnations, did not have the same strong smell, was less irritating to human nasal membranes, and failed to kill insects which landed on the freshly treated nets. It was then confirmed, using bioassays, that the new batch did indeed have much less insecticidal activity compared to those previously used. Nets freshly treated with this batch produced zero mortality in a Aedes aegypti (Rockefeller strain) bioassay.
A new batch was therefore requested from, and supplied by, Zeneca. This was tested using bioassays and found to be satisfactory. It was therefore used to re-treat all the study nets in March 2000.

In August 2000 a sample of the insecticide used in the third impregnation was sent to Zeneca in order to establish the nature of the problem but no feedback has been received to date.
Figure 2.3 Impregnation of hammock nets at village level
CHAPTER 3. Technical aspects of net-treatment methods and factors affecting durability of nets and insecticide

3.1 Introduction

During the evaluation of an intervention with ITNs, besides assessing the effects on entomological and epidemiological parameters, it is important to evaluate technical aspects that can influence either the conduct of the intervention or its consequences. Some of these aspects are related to the impregnation procedure, net-washing and net-maintenance habits of the local population, which could affect the concentration of the insecticide in the net and how long the net lasts. These aspects should be further considered for the design and implementation of a Malaria Control Program based on ITNs. Some of these technical operational issues arose during the course of the present study and are discussed later in this chapter.

3.1.1 Does the insecticide target dose achieved depend on how nets are dried?

The pioneering studies on insecticide treated nets carried out in The Gambia in Kenya in the late 1980s (Snow et al., 1987, 1988; Sexton et al., 1990) have been influential in many ways. Most of the techniques developed during these works have since been adopted by malaria control projects elsewhere. The techniques used to impregnate nets with pyrethroid insecticides are one example. These methods were vindicated by the clear epidemiological impact seen in these trials.
However, some elements of the procedures were adopted mainly as precautionary measures, and the question of whether they are actually necessary has never been tested experimentally.

Many projects recommend that when nets have been dipped in the insecticide-water mixture, they should be dried flat. This perhaps reflects the idea that if the nets were hung vertically to dry, the insecticide would drip to the bottom, leading to an uneven distribution. It is even more common to recommend drying in the shade. This suggestion is presumably based on the fact that natural pyrethrum is highly photo-labile (Palchick, 1996).

A small experiment was therefore designed to compare the effect on the insecticide concentration in netting of drying in the sun rather than in the shade, and of drying vertically rather than flat. The mean insecticide concentrations in samples of lambdacyhalothrin-treated nets were compared between nets that were dried in the sun and nets that were dried in the shade, and either hanging vertically or folded flat.

3.1.2 Residual activity of the insecticide

Generally, bednets become very dirty within a few months. The dirt in itself apparently does not limit the effectiveness of the insecticide (Lines, 1996), but users may decide to wash them. For example, in The Gambia 53% of nets were washed at least once a month (Alonso et al., 1993). A similar frequency of
Chapter 3

washing was described by Evans (1994) in Dar es Salaam (Tanzania), where residents washed nets as regularly as once a month, usually because kerosene from the lamps used for lighting creates soot, which dirties the nets.

The conventional recommendations to achieve and maintain dosages of pyrethroid insecticides on mosquito nets suggest that nets should be retreated at 6 to 12 monthly intervals. However, in principle and ideally, the dosage and frequency of re-impregnation should be adjusted to the frequency of washing, due to the fact that each wash decreases the amount of insecticide in the net. Miller et al. (1991) found that after 3 washes, nets treated with cypermethrin, lambdacyhalothrin, permethrin or pirimiphos-methyl had lost between 85 and 96% of the active ingredient. It has been pointed out that if nets are washed only once or twice a year, their re-treatment requirements will be different from those which are washed fortnightly (Miller et al., 1999).

In the present study it was important to determine the concentration of insecticide in the net achieved by the impregnation operation, and to monitor this concentration and its insecticidal activity during the period of study in operational circumstances. The insecticide concentration can be assessed by Gas Chromatography and Mass Spectrometry (GC-MS) analysis and the insecticidal effect through bioassay.

The standard WHO bioassay method currently recommends a 3 minute exposure time to insecticide treated nets with mortality assessed after 24 hours (WHO, 1981). Exposure is achieved by confining adult mosquitoes to the net under a
WHO bioassay cone. With pyrethroids, which have strong repellent properties, this may not be ideal; there is a risk of the mosquitoes resting on the cone and not on the net. An alternative method consists wrapping up part of an intact bednet round a frame made of two intersecting circles of wire about 15 cm in diameter. The netting is held round the frame in such a way that a “sleeve” is left, through which mosquitoes can be introduced and removed with an aspirator. This method forces the mosquitoes to rest on the netting during the exposure period. Another variant method consists of scoring the median time to knockdown, rather than percentage mortality after 3 minutes. An odd number (usually 11) of mosquitoes are introduced, and observed until the \((n+1)/2\) individual (e.g. the 6th out of 11) is knocked down. This requires a clear definition of knockdown, but in practice, it was found to give a sensitive indication of the effect of washing and re-treating nets, whereas a standard 3 minute exposure of a susceptible strain/population to an alpha-cyano pyrethroid tends to give 100% mortality in all tests (WHO, 1998).

In practice during the present study, it was found to be difficult to observe knockdown through the dark green netting used. Therefore, to evaluate the residual effect of the lambdacyhalothrin during regular use, bioassays were performed using the standardized WHO method of 3-minute exposure to a piece of the net under a bioassay cone. Mortality scoring was done after 24 hours. In addition, a larger sample of nets was studied during the follow up in order to assess the net-washing habits of the Yanomami of the study area.
3.1.3 Damage of the nets after regular use

The interval of net replacement affects the cost of the intervention as a regular control measure. This interval is in turn influenced by the value placed on the nets and the care given to them by the population who use them as well as local environmental conditions.

As noted in Chapter 1 page 32, the use of nets is relatively recent in the Yanomami area. In Yanomami traditional houses (*shabonos*), domestic life tends to centre around a small fire; hammocks are hung close to the flames; nets are exposed to sparks and soot. In order to have some measure of net maintenance habits, and the life-time of nets in the area, a sample of nets was evaluated at the end of the study to assess the number and size of holes as well as signs of repairs to the nets.
3.2 Materials and methods

3.2.1 Determination of insecticide concentration in samples of nets

A Gas Chromatography-Mass Spectrometry (GC-MS) analysis was carried out in order to determine the insecticide concentration in treated nets dried either in the sun or in the shade, and either hanging vertically or folded flat.

During the mass treatment of nets for the intervention study, forty nets were treated as described in Chapter 2, page 85 using the same bowl of diluted insecticide, with the same time being allowed for dipping. The target dosage was 10 mg/m². Twenty of the freshly treated nets were put to dry in the sun. Ten of them were hung vertically with the roof of the net at the top and the hem at the bottom. They were hung from a wire suspended across a small football pitch, facing the sun. The other ten nets were folded three times, so they comprised 16 layers of netting fabric, and were laid flat on a plastic sheet on the concrete surface of the pitch. Twenty other nets were hung in the shade, under a conical thatch canopy about 4m across, built on a metal frame with no walls and a concrete floor. As before, ten of them were hung from wires and ten were folded flat on a plastic sheet.

The weather was hot, 38°C, with a slight breeze. There were no clouds and very little haze in the sky, so the sun was uncomfortably strong. The nets were put in position between 10 and 11 am, and were left there for 6 to 7 hours. The nets hanging in the sun were completely dry in about 40 minutes; those hanging in the shade dried in 60 to 90 minutes. The nets laid flat in the sun took several hours to
dry completely, while some of those laid flat in the shade were still damp at the bottom at the end of the day. Between 5 pm and 6 pm samples of about 15 cm square were cut from the nets. Two samples were taken from each net, one near to the top and one from the very bottom. In the case of the sun-dried nets, the samples were taken from the side that had been more exposed to the sun. Samples were labelled and individually wrapped in aluminium foil until the GC-MS analysis was performed.

The GC-MS analysis was performed on sub-samples of netting (1cm x 1cm) in Salford University by Edward Magbity as part of his PhD studies. The methods are described in his thesis (Magbity, 1999) and in Annex 3.

3.2.2 Bioassays

To evaluate the residual efficacy of the lambdacyhalothrin treated nets, bioassays were performed to determine the insecticide activity. In March 2000, forty new nets were impregnated and distributed at random in villages with treated nets in the Ocamo locality in exchange for the older treated nets that the owners had been using since the time of the intervention in December 1998. A series of pieces from the roof, side-top and side-bottom were cut one month after impregnation, and subsequently at month three and month six. Each net sample was wrapped in aluminium foil, codified and kept at 4°C until bioassay examination. The holes left in the nets by the sampling procedure were repaired with pieces of nets given by the research team to women from the communities who were hired for this
purpose. After the six-month assessment, new treated nets were distributed to replace the repaired ones.

The bioassays were done in the Research Laboratory of the School of Malariology (Maracay). Since there is no laboratory colony of An. darlingi in Venezuela, the nets were tested with Ae. aegypti (Rockefeller strain) susceptible to pyrethroids, using the WHO bioassay cones (WHO, 1981). Batches of ten mosquitoes were exposed for 3 min to treated net pieces; untreated net pieces served as the control. Mortality was recorded after 24 h. Three replicates of roof, side top and side bottom pieces of each sampled net were analysed through bioassays.

After impregnation the nets were marked with water-soluble ink in order to record how frequently they were being washed. The nets were inspected fortnightly. Washed nets were recognised by the disappearance of the water-soluble mark, and subsequently noted (see Annex 4).

In order to assess the net-washing habits, a bigger sample of hammock nets (n=340) was selected among villages from the Ocamo and Mavaca study localities, including both insecticide treated net villages and placebo treated net villages. The sampling of the nets was done using the code number of the net given at the beginning of the study and using a table of random numbers following the procedure described in Smith and Morrow (1996). The hammock nets sampled were followed fortnightly for six months. Using a questionnaire (see Annex 4), the nets were inspected fortnightly. Washed nets were recognised by the disappearance of the water-soluble mark and subsequently noted.
3.2.3 Damage to hammock nets

In order to describe the physical conditions of the hammock nets after two years of intervention, a survey was conducted at the end of the study. One hundred and sixty three hammock nets were chosen, at random, from villages with placebo or insecticide treated nets. A chart was designed to record the number, location and size of holes, as well as signs of repairs to the nets. The size of holes was classified using a chart (see Annex 5) showing areas from 1 cm$^2$ to 17 cm$^2$ and the numbers of holes and position were recorded in a form (see Annex 6).

3.2.4 Analysis

Concentrations of lambdacyhalothrin were determined by GC-MS analysis in netting that had been dried by different procedures. In order to estimate the concentration mean (and 95% confidence intervals) achieved with each procedure, a multiple regression analysis was done using the Generalized Linear Model (GLM) approach with gamma distribution. Initially, the model included all covariates: drying position (flat or vertical), drying place (sun or shade) and localization of the net piece (top or bottom). Then, least significant covariates were excluded by stepwise elimination using the Likelihood Ratio Test (LRT). Also interaction parameters were tested. The final model was checked by looking at the appropriate residual plot.
Bioassays were performed to determine the mosquito mortality produced by the insecticide treatment and to relate this mortality to exposure variables such as length of time of use and number of washes of the hammock-nets. Bioassays results were analysed using a binomial model with GLM. This permits the analysis of repeated measures on the same individual net, allowing for within-net clustering, using Huber/White/sandwich estimate of variance (Stata, 2001).

The mean percentage of mosquito mortality for each level of the exposure variables was calculated from the Odds estimated in the model, applying the following formula: 

$$ p = \frac{Odds}{1 + Odds} \times 100. $$
3.3 Results

3.3.1 Insecticide concentration in treated nets dried in different ways

The insecticide concentrations obtained by GC-MS analysis in pieces of net dried in four positions (sun-vertical, shade-vertical, sun-flat and shade-flat) were compared and are presented in Table 3.1. In general, there was a very wide range of insecticide concentrations within all groups, whatever the method of drying. No significant variation was found between mean concentrations from pieces taken from top and bottom of nets dried vertically (in the shade $P=0.29$ and in the sun $P=0.26$) while significant differences were found between pieces taken from nets dried flat (in the shade $P=0.007$ and in the sun $P=0.002$)\(^7\). However, this was not consistent: in the shade-flat dried nets, the mean of the “bottom” pieces was greater than that of the “top” pieces, while among the sun-flat dried nets, the reverse was the case. The reason for these inconsistencies is unclear, and for subsequent analysis the mean of the top and bottom was used for each net.

Distributions (relative frequency) of the concentration of lambdacyhalothrin in pieces taken from top and bottom by drying procedure are shown in Figure 3.1. The mean concentration of lambdacyhalothrin obtained in nets dried sun-flat was only 2.9 mg/m\(^2\) (95% CI 1.5-5.4). This group reached the target dose of 10 mg/m\(^2\) in only one replicate net sample. The mean concentration of insecticide detected in the other three groups was closer to expectation, and the means did not differ

\(^7\) Note that these $P$-values were calculated from the difference between top and bottom concentrations with each net, and are valid despite the wide overlap between the confidence intervals shown in Table 3.1.
significantly between the three groups (Figure 3.2). However the least variation between nets (as reflected in the width of the 95% CI) was in the sun-.vertical group.

Table 3.1 Insecticide concentration determined by Gas Chromatography and Mass Spectrometric (GC-MS) analysis in pieces of lambdacyhalothrin treated nets according to drying procedure

<table>
<thead>
<tr>
<th>Dry position</th>
<th>n</th>
<th>Mean (mg/m²)</th>
<th>95% CI</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Shade-flat</td>
<td>20</td>
<td>11.2</td>
<td>6.4-19.6</td>
<td>0.9-45</td>
</tr>
<tr>
<td>Top</td>
<td>10</td>
<td>8.8</td>
<td>4.7-16.3</td>
<td>0.9-30</td>
</tr>
<tr>
<td>Bottom</td>
<td>10</td>
<td>13.6</td>
<td>7.7-24.5</td>
<td>1.2-45</td>
</tr>
<tr>
<td>Shade-vertical</td>
<td>22</td>
<td>10.7</td>
<td>7.1-16.0</td>
<td>1-26.4</td>
</tr>
<tr>
<td>Top</td>
<td>11</td>
<td>11.6</td>
<td>7.9-17.2</td>
<td>1-22.1</td>
</tr>
<tr>
<td>Bottom</td>
<td>11</td>
<td>9.7</td>
<td>5.8-16.2</td>
<td>1.3-26</td>
</tr>
<tr>
<td>Sun-vertical</td>
<td>26</td>
<td>12.9</td>
<td>10.4-15.8</td>
<td>2.6-30</td>
</tr>
<tr>
<td>Top</td>
<td>13</td>
<td>12.3</td>
<td>9.2-16.5</td>
<td>3.4-30</td>
</tr>
<tr>
<td>Bottom</td>
<td>13</td>
<td>13.3</td>
<td>10.5-16.9</td>
<td>2.6-24</td>
</tr>
<tr>
<td>Sun-flat</td>
<td>18</td>
<td>2.9</td>
<td>1.5-5.4</td>
<td>0.4-13</td>
</tr>
<tr>
<td>Top</td>
<td>9</td>
<td>3.6</td>
<td>1.8-7.3</td>
<td>0.5-13</td>
</tr>
<tr>
<td>Bottom</td>
<td>9</td>
<td>2.2</td>
<td>1.2-4.1</td>
<td>0.4-7</td>
</tr>
</tbody>
</table>

*a Number of pieces of net

b Mean concentration and 95% CI of insecticide obtained with the generalized linear model

c Max and min insecticide concentration in mg/m²
Figure 3.1 Distribution of lambdacyhalothrin concentration in pieces taken from impregnated nets according to location and dry procedure.
3.3.2 Residual concentration and insecticidal activity of the insecticide after one year of storage

After impregnation in January 1998, the nets were stored in plastic bags, at room temperature (~25 °C) for eight months before they were distributed. To determine the concentration and insecticidal activity of the lambdacyhalothrin, chemical analysis by Gas Liquid Chromatography (GLC) and bioassays were carried out on pieces of net after the eight month period of storage and before the beginning of the intervention.
Five out of 900 nets stored were chosen from different batches and storage positions and 20cm² pieces of material were cut from the roof, top-side and bottom-side. Each sample was wrapped in aluminium foil and sent to Zeneca Venezuela S.A. for chemical analysis. The quantity of lambdacyhalothrin in the fibre netting piece was determined using GLC (Reading Scientific Services Ltd.). The results are present in Table 3.2.

Table 3.2 Concentration of lambdacyhalothrin determined by gas liquid chromatography (GLC) on nets after 8 months storage (data supplied by Zeneca)

<table>
<thead>
<tr>
<th>Net</th>
<th>Lambdacyhalothrin a.i./m²</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Roof</td>
</tr>
<tr>
<td>1</td>
<td>20</td>
</tr>
<tr>
<td>2</td>
<td>19</td>
</tr>
<tr>
<td>3</td>
<td>19</td>
</tr>
<tr>
<td>4</td>
<td>6</td>
</tr>
<tr>
<td>5</td>
<td>12</td>
</tr>
</tbody>
</table>

Note that these nets had been dried by hanging in the sun for about two hours, and the range of concentration was consistent with that seen in the “sun-vertical” nets used in the experiment described above.

Pieces of 20 cm² cut from roof, top-side and bottom-side of 20 nets, also chosen from different batches and storage positions, were sent for bioassays to the
Department of Medical Entomology of the Institute of Tropical Medicine at Central University of Venezuela. Bioassays were performed with *Ae. aegypti* (Rockefeller strain) susceptible to pyrethroids following the methodology given by WHO, 1981. The 24 h mortality was 100% for all samples.

### 3.3.3 Insecticidal activity of treated nets after regular use and washing

A total number of 651 bioassays were carried out after a period of regular use of nets impregnated with lamdacyhalothrin: 345 assays were done after one month of use, 189 after 3 months of use, and 117 after 6 months of use. In total, 6,870 mosquitoes were tested. Table 3.3. shows the mean percentage mortality of mosquitoes exposed to pieces of insecticide impregnated net. There is a decreasing trend of the mean mosquito mortality with time of regular use.

<table>
<thead>
<tr>
<th>Washes</th>
<th>Months 1</th>
<th>Months 2</th>
<th>Months 6</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>n=38</td>
<td>n=21</td>
<td>n=13</td>
</tr>
<tr>
<td></td>
<td>97.8%</td>
<td>81.3%</td>
<td>89.2%</td>
</tr>
<tr>
<td>1</td>
<td>n=0</td>
<td>n=0</td>
<td>n=3</td>
</tr>
<tr>
<td></td>
<td>*</td>
<td>*</td>
<td>89.3%</td>
</tr>
<tr>
<td>2</td>
<td>n=0</td>
<td>n=0</td>
<td>n=1</td>
</tr>
<tr>
<td></td>
<td>*</td>
<td>*</td>
<td>47.7%</td>
</tr>
</tbody>
</table>

Table 3.3 Results of bioassays according to month after impregnation and number of washes expressed in average percentage mortality

n= number of tested nets
Despite the small sample size of washed nets (only three were washed once and one was washed twice) the data of Table 3.3 were subject to multiple regression analysis to separate the effects of time and of washing. The results are presented in Table 3.4.

Table 3.4 shows the mean percentage of mosquito mortality related to time after impregnation and number of washes of nets sampled. Site of the piece (roof, side-top and side-bottom) was excluded for the multivariable analysis because there was no significant difference in the univariable analysis ($P>0.3$). The multiple regression analysis shows that mosquito mortality declined significantly ($P<0.001$) after two and six months of regular use when it is compared with the first month -from 98% to 81% (95% CI 73-87) at month two and to 89% (95% CI 77-95) at month six. The univariable analysis gave similar results. The results obtained show that one wash did not have a significant effect on mosquito mortality ($P=0.9$), but two washes reduced it significantly ($P<0.01$).
Table 3.4 Crude odds and mean percentage mortality of *Ae. aegypti* exposed to lambdacyhalothrin treated nets after time of regular use, number of washes and location of the pieces analyzed

<table>
<thead>
<tr>
<th></th>
<th>Crude analysis</th>
<th>Adjusted analysis*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pieces (Nets)</td>
<td>Mean % (95% CI)</td>
</tr>
<tr>
<td>Month</td>
<td></td>
<td>P</td>
</tr>
<tr>
<td>1</td>
<td>115 (39)</td>
<td>98 (96-99)</td>
</tr>
<tr>
<td>2</td>
<td>63 (21)</td>
<td>81 (73-87)</td>
</tr>
<tr>
<td>6</td>
<td>51 (17)</td>
<td>87 (75-93)</td>
</tr>
<tr>
<td>Washes</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>217 (73)</td>
<td>91 (88-94)</td>
</tr>
<tr>
<td>1</td>
<td>9 (3)</td>
<td>89 (58-98)</td>
</tr>
<tr>
<td>2</td>
<td>3 (1)</td>
<td>29 (--)</td>
</tr>
<tr>
<td>Localization</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Roof</td>
<td>76</td>
<td>90 (86-93)</td>
</tr>
<tr>
<td>Top</td>
<td>76</td>
<td>91 (87-93)</td>
</tr>
<tr>
<td>Bottom</td>
<td>77</td>
<td>92 (88-94)</td>
</tr>
</tbody>
</table>

*Analysis adjusted for washes and month
(-) 95% CI was not possible to estimate because only one net was washed twice
n.s. no significant differences

The frequency of washing registered in surveys of randomly sampled nets is shown in Table 3.5. During the follow up period of six months, 88.5% of the nets were washed once or not washed at all. The frequency of washes was higher in villages from Mavaca than the Ocama locality.
Table 3.5 Frequency of nets washing during six month of regular use

<table>
<thead>
<tr>
<th>Number of washes</th>
<th>Ocamo (n=130)</th>
<th>Mavaca (n=210)</th>
<th>Total (n=340)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Frequency</td>
<td>%</td>
<td>Frequency</td>
</tr>
<tr>
<td>0</td>
<td>104</td>
<td>80.0</td>
<td>12</td>
</tr>
<tr>
<td>1</td>
<td>21</td>
<td>16.2</td>
<td>164</td>
</tr>
<tr>
<td>2</td>
<td>5</td>
<td>3.8</td>
<td>13</td>
</tr>
<tr>
<td>3</td>
<td>0</td>
<td>0</td>
<td>7</td>
</tr>
<tr>
<td>4</td>
<td>0</td>
<td>0</td>
<td>11</td>
</tr>
<tr>
<td>5</td>
<td>0</td>
<td>0</td>
<td>3</td>
</tr>
</tbody>
</table>

3.3.4 Net damage after regular use

In order to describe the extent and location of damage and repairs, in this study nets were divided in two areas: the upper area or “protective area” beside and above the occupant, and the lower area below the occupant between the hammock and the floor. The “protective area” is about 3/4 of the total area of the net Figure 3.3.
Figure 3.3 The yellow arrow shows the protective area of the net while the orange arrow shows the lower area.
Of 162 nets selected at random, 5%(8) were greatly damaged (~50% of the total protective area was lost). 32,516 holes were found in 154 nets with an average of 211 holes/net. Table 3.6 shows the distribution of un-repaired holes according to their locations in the net and their dimensions. Out of the total number of holes found, nearly 90% were less than 1 cm$^2$, and 55% were located in the protective area.

Table 3.6 Dimensions and location of 32,516 holes (in percentage) found in 154 nets after two years of regular use

<table>
<thead>
<tr>
<th>Holes</th>
<th>Protective area</th>
<th></th>
<th>Lower area</th>
<th></th>
<th>Total</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Un-repaired n</td>
<td>%</td>
<td>Un-repaired n</td>
<td>%</td>
<td>n</td>
<td>%</td>
</tr>
<tr>
<td>&lt;1 cm$^2$</td>
<td>17,820</td>
<td>54.8</td>
<td>11,189</td>
<td>34.4</td>
<td>29,009</td>
<td>89.2</td>
</tr>
<tr>
<td>1 - 9 cm$^2$</td>
<td>2,190</td>
<td>6.7</td>
<td>1,089</td>
<td>3.3</td>
<td>3,279</td>
<td>10.1</td>
</tr>
<tr>
<td>10 - 17 cm$^2$</td>
<td>120</td>
<td>0.4</td>
<td>108</td>
<td>0.3</td>
<td>228</td>
<td>0.7</td>
</tr>
<tr>
<td>Overall</td>
<td>20,130</td>
<td>61.9</td>
<td>12,386</td>
<td>38.1</td>
<td>32,516</td>
<td>100.0</td>
</tr>
</tbody>
</table>

Table 3.7 shows the total number of holes (un-repaired and repaired) as well the percentage of repaired holes by location in the net. Of 154 examined nets, 94% had one or more holes repaired, 7 nets did not have any repairs and were in good condition, without holes. Bigger holes were more frequently repaired than those of less than 1 cm$^2$ and more repairs had been done in the “protective area” than in the “lower area”.

109
Table 3.7 Number of holes (repaired and un-repaired) and percentage of repairs by location after two years of regular use

<table>
<thead>
<tr>
<th>Holes</th>
<th>Protective area</th>
<th>Lower area</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Un-repaired (n)</td>
<td>Repaired (n) %</td>
</tr>
<tr>
<td>&lt;1 cm²</td>
<td>17,820</td>
<td>251</td>
</tr>
<tr>
<td>1 - 9 cm²</td>
<td>2,190</td>
<td>1,760</td>
</tr>
<tr>
<td>10 - 17 cm²</td>
<td>120</td>
<td>295</td>
</tr>
</tbody>
</table>
3.4 Discussion

In this study different procedures for drying nets after dipping for a target dose of 10mg/m² were evaluated. No differences were found between the mean insecticide concentrations of nets that were dried vertically in the sun or in the shade, or those dried flat in the shade. However, the mean dose was considerably lower in nets dried flat in the sun. The reason for this is unclear. Achieving a consistent dosage is also desirable. Variation between nets, shown by the width of the confidence intervals in Table 3.1 page 100, was least in the group of nets dried vertically in the sun.

Alonso et al. (1993) in a village-level study in The Gambia, found very wide variation in insecticide concentrations between pieces taken from the same net, and between nets. The differences were greater between individual nets than within nets. These findings were mainly attributed to the loss of insecticide caused by drying nets on mattresses. Miller et al. (1991) in laboratory conditions, impregnated nets with different insecticides using the technique described by Lines et al. (1987). The latter technique consists of rubbing and squeezing the net in a plastic bowl containing insecticide solution, then laying the net on a polythene sheet and repeatedly turning it over as it dries. The resultant mean insecticide concentration varied considerably from the target dosage for each insecticide. Under-dosage was found for lambdacyhalothrin, cypermethrin, deltamethrin and over-dosage for pirimiphos-methyl. However, permethrin came closest to the target dose. The mean concentration found in nets impregnated with
lambdacyhalothrin and dried flat in the shade in Miller's study (2.6 mg/m²) was very similar to that found in the present study when nets were dried flat in the sun (2.9 mg/m²).

In the present study significant variation was found between insecticide concentrations in pieces cut from the top and bottom of nets only when they were dried flat, either in the shade and or in the sun. Previous studies (Lindsay et al., 1991; Miller et al., 1991) presented similar results when nets were dried flat in the shade. Rozendaal and Curtis (1989) showed that ITNs hung and dried vertically had a slight downward gradient in insecticide concentration.

The results of the experiments reviewed and those found in the present study suggest that more homogeneous concentrations are achieved by hanging the nets to dry them. However, the variability within and between studies associated with different drying positions indicate that this kind of experiment should be systematically repeated, to ensure validity and reliability, before recommending a specific drying procedure.

It is difficult to explain the low concentration of insecticide obtained in nets dried flat in the sun compared with nets in the other groups. All the nets were impregnated in the same mixing bowl with the same dilution of insecticide and at the same time. The discrepancy is therefore attributed to the drying procedure and not to the insecticide dipping.

Another important aspect to be considered during impregnation at village-level, is that villagers might expect to use the nets on the day of impregnation. This was
particularly important in the case of the Yanomami. They ask to start the
impregnation early in the morning in order to have enough time to dry the nets
before the evening. In the present study nets laid flat in the shade were still damp
at the end of the day and even those laid flat in the sun took several hours to dry.

There actually seems to be no evidence against the recommendation of drying
impregnated nets vertically and in the sun. It is a fast procedure that will satisfy
expectations for immediate net use by the community and - according to the
present study results - the target dose is well achieved with little variation of
insecticide concentration within the net.

Bioassays showed that mosquito mortality on lambdacyhalothrin treated nets
decreased significantly from 98 to 87% towards the end of 6 months. The decline
was related to the duration of regular use as well as to the number of washes. The
results showed that regular use reduces the mean mortality from 98% to 89% six
months after the impregnation. One wash during the last three months does not
affect the mean percentage mortality while one net, which was washed twice gave
a mortality of 83%. Despite the fact that the percentage of mosquito mortality
decreased significantly after six months, the level was still acceptable. These
results are similar to those reported for lambdacyhalothrin treated nets in
experimental trials for An. gambiae s.l. (Miller et al., 1991; Curtis et al., 1996) as
well as in trials at village level in Tanzania (Njunwa et al., 1991; Maxwell et al.,
1999) and for An. culicifacies in India (Sampath et al., 1998).
Prior to the beginning of the intervention, before the nets were distributed, no particular recommendation was given to the population regarding net washing frequency. In other studies, the populations have been asked to avoid washing the nets. In the present study the Yanomami could have been asked not to wash the nets in order to maintain the insecticidal effect. But this particular recommendation would have represented a counter-message to the persistent discourse on hygiene coming from the health post personnel “wash your clothes, wash your hands, clean your house”. It was decided to observe, on a “natural” basis, the attitudes and practices of the population toward the newly acquired object; the nets. It was observed that after six months of regular use almost 90% of the nets had been washed only once or not washed at all. This washing frequency was much lower than that reported in other ITN trials (Alonso et al., 1993; Miller et al., 1999). It seems that in this particular area there is no need for a supplementary information campaign to minimize washing of nets.

At the end of the intervention, after two years of regular use, the nets examined had various levels of damage (Table 3.6). Probably the evaluation of the nets conditions should had been done earlier and a number of nets particularly damaged replaced in due course. However, Maxwell et al. (2002) in Tanzania showed evidence that ITNs with quite large holes (>20 holes less than 2cm or, >5 holes sized 2-5 cm or, >2 holes more than 5 cm in diameter) performed as well as intact nets in reducing prevalence of parasitaemia, splenomegaly and anaemia in children under 2 years of age. Almost 94% of the samples had some evidence of repair (see Table 3.7) that can be considered as a sign of the value given to the
nets by the Yanomami population. This complements observations gathered by the research team, and other health professionals working in Amazonas, that nets are objects of exchange with Yanomami of other areas, even remote areas (see Chapter 6, page 203).
CHAPTER 4. Effect of the intervention on the population of *Anopheles darlingi* Root (Diptera: Culicidae)

4.1 Introduction

The primary rationale of malaria vector control with insecticide is to prevent a high proportion of mosquitoes surviving for 12 - 14 days, the time necessary to complete the sporogonic cycle of the parasite (Warrell and Gilles, 2002).

Pyrethroid impregnated nets act both as a physical barrier, by protecting the sleeper from mosquito bites, and as a chemical barrier, by repelling mosquitoes or killing them if they stay long enough in contact with the net (see Chapter 1, page 53). Therefore insecticide treated nets provide not only personal protection, but also, if they are used by a large proportion of the community, they can cause a mass killing effect on the local vector population. In those areas where the malaria vector is highly anthropophilic, community use of treated nets is expected to kill a large proportion of the local vector population before mosquitoes can reach the age at which the malaria parasite reaches maturity, thus reducing the malaria risk for the whole community (Curtis, 1992). The mass killing effect can be observed as a reduction in mosquito density, parity and sporozoite rate. Such effects have been reported in several endemic areas, such as, Tanzania (Magesa *et al.*, 1991; Curtis *et al.*, 1998; Maxwell *et al.*, 1999, 2002), Kenya (Gimnig *et al.*, 2003a, 2003b; Hawley *et al.*, 2003), in Burkina Faso (Carnevale *et al.*, 1988; Robert and Carnevale, 1991; Cuzin-Ouattara *et al.*, 1999).
To know whether insecticide treated nets can cause a mass killing effect in the intervention area is very important for planning the implementation of malaria control programmes. In fact if the mass killing effect is shown to occur then, it is particularly important to reach maximum coverage otherwise the nets will not be optimally effective.

In South America the effect of ITNs on entomological parameters has not been properly studied. In the present study malaria incidence was the primary outcome of interest, and entomological evaluation was carried out to supplement the results of the epidemiological evaluation. In order to study the impact of ITHNs on *An. darlingi* population density in the study area, CDC-light traps were used. The sampling was carried out monthly in villages with and without ITHNs. To detect any impact of the intervention on the longevity and infection rate, parity and sporozoite rates were measured in a sub-sample of the collected specimens.
4.2 Materials and methods

4.2.1 Anopheline survey

Mosquito catches were performed using CDC-light traps set beside occupied intact nets and run from rechargeable batteries (Lines et al., 1991). The use of CDC-light traps in epidemiological studies, instead of Human Biting Catches (HBC), is desirable because it avoids the need to expose people to mosquito biting. Moreover this method is easier to standardize compared to HBC.

Pre-intervention catches were carried out in Ocamo and Mavaca localities in 1998 from June to December, and during the intervention from January to December in 1999. In 2000 only a few villages in Ocamo locality were surveyed. Generally one light trap was installed during four consecutive nights per month per village (see Table 4.2, page 126). With the house owner’s consent the light trap was located indoors, hung from the roof at 1.5m from the floor and run from 19.00 to 06.00 hr. During the pre-intervention period in 1998, light traps were placed beside a person sleeping without a net whereas, during the intervention period, traps were placed besides a person sleeping under an insecticide or placebo treated net. Villagers were asked to switch off the traps in the morning, after having tied up the neck of the collecting bag. The collected specimens were transported to the field laboratory in Santa Maria de los Guaicas in Ocamo locality or to the PHC in Mavaca locality. Mosquitoes were killed by freezing, morphologically identified to species using the key of Cova-Garcia and Sutil (1977), and counted. Data were recorded on a special form (see Annex 7). Specimens were stored in pools of no more than 25 mosquitoes,
in 1.5ml Eppendorf tubes previously perforated. Tubes were coded by species, village, house and date and stored at room temperature in boxes containing silica gel. Samples were transported to CAICET laboratories in Puerto Ayacucho for further analysis. The silica gel was checked every week until the analysis was performed and changed before it become completely hydrated.

Rainfall and river height were gathered from records kept by personnel of the Ministerio de Ambiente y Recursos Naturales (MARN) at the meteorological station of Santa Maria de Los Guaicas in Ocamo locality.

### 4.2.2 Parity rate

Parity dissections were performed on a few of the mosquito specimens collected by CDC-light traps from August to November 2000 in the villages of the Ocamo locality with insecticide and placebo treated nets.

Live mosquitoes were removed from the traps early in the morning with an aspirator, stored in paper cups, identified to species, counted and classified by gonadotrophic stage. The unfed *An. darlingi* specimens were dissected and analyzed for parity determination following the technique described by Detinova (1962), i.e. presence or absence of tracheolar coils. To avoid possible bias related to the net treatment the slides were coded by village and the scoring was blind.
4.2.3 Detection of sporozoites

A sub-sample for testing was selected from the total number of mosquitoes captured in Ocamo locality. Because of the large number collected, it was not possible to test them all for sporozoites. This sub-sample represented 5% of the An. darlingi caught during 1998 (pre-intervention period) and 9.7% of those caught during 1999-2000 (post-intervention period). This sub-sample was chosen at random (Smith and Morrow, 1996) from the mosquitoes collected monthly for each village. An. darlingi specimens were analyzed with ELISA and VecTest® to determine the sporozoite rate for P. falciparum, P. vivax (variants 210 and 247) as described by Ryan et al. (2002).

4.2.4 Analysis

4.2.4.1 Mosquito density

The distributions of mosquito counts by village, and for all villages, were first checked for normality. The results showed that the mosquito density distribution was skewed to the right so, in an attempt normalize the data, they were log-transformed to In (x_i+1), where x_i is the number of mosquitoes in trap i. This procedure was considered suitable since the number of traps with zero counts was relatively small (91/1266=7%). The village distributions after log-transformation are shown in Figure 4.1.
Figure 4.1 Distributions (cumulative frequency) of the log-transformed count of mosquitoes collected by CDC-light traps

The overall distribution and the normal probability plot (Figure 4.2) indicated that the logarithmic transformation helped to normalize the distribution, but still left it not entirely normal.

Figure 4.2 Overall distribution and normal plot probability of log transformed mosquito count
Therefore, for descriptive purposes, the Williams' mean rather than the arithmetic mean was used, whereas, for purposes of statistical inference, to analyze the effect of the intervention, the analysis was done using the negative binomial distribution. The latter is considered more appropriate when data are over-dispersed.

The Williams' mean (Mw) (in Clements, 1999) was estimated as,

\[ M_w = \text{anti ln} \left( \frac{\sum_{i} \ln(x_i + 1)}{n} \right) - 1 \]

where \( x_i \) is the total number of mosquitoes collected in trap \( i \), and \( n \) the total number of traps.

The degree of over-dispersion was measured as the variance mean ratio (VMR),

\[ VMR = \frac{S^2}{m} \]

which for a Poisson distribution equals 1, and for an over-dispersed (aggregated) distribution is greater than 1, as is illustrated in Table 4.1.
Table 4.1 The mean, range, standard deviation and VMR of mosquito counts for the 18 villages during the study period

<table>
<thead>
<tr>
<th>Village</th>
<th>n</th>
<th>Mean</th>
<th>$M_w$</th>
<th>Range</th>
<th>sd</th>
<th>$s^2$</th>
<th>VMR</th>
<th>CV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sta. Ma. de los Guaicas</td>
<td>116</td>
<td>72.4</td>
<td>26.2</td>
<td>0-720</td>
<td>121.6</td>
<td>14,786.6</td>
<td>204.3</td>
<td>1.7</td>
</tr>
<tr>
<td>Iyewet-therl</td>
<td>104</td>
<td>193.8</td>
<td>76.1</td>
<td>0-1,540</td>
<td>261.7</td>
<td>68,467.9</td>
<td>353.3</td>
<td>1.4</td>
</tr>
<tr>
<td>Clavotheri</td>
<td>81</td>
<td>289.5</td>
<td>55.0</td>
<td>0-5,430</td>
<td>723.5</td>
<td>523,423.3</td>
<td>1,808.1</td>
<td>2.5</td>
</tr>
<tr>
<td>Carlitos</td>
<td>98</td>
<td>407.5</td>
<td>56.0</td>
<td>0-5,150</td>
<td>836.8</td>
<td>700,182.5</td>
<td>1,718.3</td>
<td>2.1</td>
</tr>
<tr>
<td>Kashorawe</td>
<td>40</td>
<td>317.2</td>
<td>77.7</td>
<td>0-2,980</td>
<td>593.1</td>
<td>351,788.3</td>
<td>1,109.1</td>
<td>1.9</td>
</tr>
<tr>
<td>Lechoza</td>
<td>115</td>
<td>95.5</td>
<td>17.9</td>
<td>0-908</td>
<td>172.6</td>
<td>29,801.5</td>
<td>312.1</td>
<td>1.8</td>
</tr>
<tr>
<td>Shashanawe</td>
<td>80</td>
<td>154.3</td>
<td>57.4</td>
<td>0-938</td>
<td>209.4</td>
<td>43,856.7</td>
<td>284.2</td>
<td>1.4</td>
</tr>
<tr>
<td>Yohoope</td>
<td>78</td>
<td>202.2</td>
<td>98.6</td>
<td>0-848</td>
<td>203.5</td>
<td>41,420.1</td>
<td>204.9</td>
<td>1.0</td>
</tr>
<tr>
<td>Warapana</td>
<td>52</td>
<td>95.9</td>
<td>25.0</td>
<td>0-1,060</td>
<td>195.0</td>
<td>38,013.2</td>
<td>396.3</td>
<td>2.0</td>
</tr>
<tr>
<td>Shakita</td>
<td>36</td>
<td>12.3</td>
<td>2.2</td>
<td>0-116</td>
<td>29.0</td>
<td>839.0</td>
<td>68.3</td>
<td>2.4</td>
</tr>
<tr>
<td>Hatakao</td>
<td>46</td>
<td>16.4</td>
<td>3.2</td>
<td>0-230</td>
<td>43.1</td>
<td>1,856.7</td>
<td>113.3</td>
<td>2.6</td>
</tr>
<tr>
<td>Purima</td>
<td>52</td>
<td>102.7</td>
<td>58.3</td>
<td>0-1,155</td>
<td>166.7</td>
<td>27,790.8</td>
<td>270.6</td>
<td>1.6</td>
</tr>
<tr>
<td>Motorema</td>
<td>51</td>
<td>199.4</td>
<td>87.9</td>
<td>0-1,236</td>
<td>263.9</td>
<td>69,646.3</td>
<td>349.3</td>
<td>1.3</td>
</tr>
<tr>
<td>Piegrita</td>
<td>44</td>
<td>31.9</td>
<td>13.6</td>
<td>0-138</td>
<td>37.1</td>
<td>1,375.2</td>
<td>43.2</td>
<td>1.2</td>
</tr>
<tr>
<td>Kopariwe</td>
<td>47</td>
<td>119.3</td>
<td>30.5</td>
<td>0-950</td>
<td>221.5</td>
<td>49,081.3</td>
<td>411.6</td>
<td>1.9</td>
</tr>
<tr>
<td>Mosho</td>
<td>51</td>
<td>47.2</td>
<td>18.2</td>
<td>0-341</td>
<td>69.0</td>
<td>4,763.5</td>
<td>100.8</td>
<td>1.5</td>
</tr>
<tr>
<td>Mario</td>
<td>92</td>
<td>792.7</td>
<td>253.9</td>
<td>0-5,145</td>
<td>1,055.9</td>
<td>1,114,821.3</td>
<td>1,406.4</td>
<td>1.3</td>
</tr>
<tr>
<td>Hishinowel-therl</td>
<td>83</td>
<td>1,015.8</td>
<td>442.1</td>
<td>0-5,970</td>
<td>1,163.7</td>
<td>1,354,255.9</td>
<td>1,333.2</td>
<td>1.1</td>
</tr>
</tbody>
</table>

- Number of light trap nights (one trap per village per night)
- Mean, $m = \sum_{i=1}^{n} \frac{x_i}{n}$, $x_i$ is the total number of mosquitoes collected in trap $i$, and $n$ the total number of traps
- William's mean, $M_w = \text{anti} \ln \left( \frac{\sum_{i=1}^{n} \ln(x_i + 1)}{n} \right) - 1$
- Range min and max number of mosquitoes counts
- Standard deviation = sd
- Variance = $s^2$
- Variance Mean Ratio = $\frac{\text{VMR}}{s^2}$
- Coefficient of variation = $CV = \frac{\text{VMR}}{sd}$

123
4.2.4.2 Analysis of the effect of the intervention on the *Anopheles darlingi* population

The approach described by Bennett *et al.* (2002) was used to measure the effect of the intervention on mosquito density.

First, for the unadjusted analysis, a unpaired $t$-test on the ln of the William's mean of each cluster was used for hypothesis testing. Then the intervention effect was adjusted for the pre-intervention mosquito density. This was done using a binomial regression model. The post-intervention data (i.e. the individual trap-catches) were regressed on the mean catch in each village during the pre-intervention period. In this way, the pre-intervention village means were used to generate an expected catch for each village during the post-intervention period. This expected value was calculated excluding the possible effect of the intervention. The ratio of observed and expected mean trap catches was then calculated, by village, and the log of these ratios were then subjected to an unpaired $t$-test comparing ITHNs and PTHNs groups.
4.3 Results

4.3.1 CDC-light trapping

In December 1997 mosquito catches were carried out in the Ocamo locality to establish a work routine for the entomological team. Catches from June to December 1998 constitute the baseline data (pre-intervention period) and those performed from January 1999 to December 2000 constitute the intervention period.

At the beginning of the study, the light traps were well accepted by the local population. However, late in 1999 some Yanomami complained about the noise and light produced by the traps and disconnected the batteries or covered the traps with cloths. In a few exceptional cases light traps were used for other purposes, like hunting at night.

A total of 1,310 captures were carried out in the present study. Forty four of them (corresponding to 3% of the total) were excluded from the analysis for a number of reasons, i.e. in 31 cases traps had damaged batteries, in 10 cases the batteries were inverted by villagers, in 1 case the trap was covered with a cloth and in 2 cases traps were invaded by flying ants.

Table 4.2 shows the number of CDC-light traps by month and village during the study period.
### Table 4.2 Number of CDC light trap catches per months and villages

<table>
<thead>
<tr>
<th>Village</th>
<th>Pre-intervention</th>
<th>Post-intervention</th>
<th>2000</th>
<th>TOTAL</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1997</td>
<td>1998</td>
<td>1999</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Dec</td>
<td>Jan</td>
<td>Feb</td>
<td>Mar</td>
</tr>
<tr>
<td>Ocarno</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sta. Ma. de las Guacanas</td>
<td>4</td>
<td>5</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>Iyesu-theri</td>
<td>2</td>
<td>2</td>
<td>4</td>
<td>3</td>
</tr>
<tr>
<td>Clavotheri</td>
<td>2</td>
<td>2</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>Carlitos</td>
<td>4</td>
<td>2</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>Kashorawe</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>Lechoza</td>
<td>2</td>
<td>2</td>
<td>4</td>
<td>3</td>
</tr>
<tr>
<td>Shashanawe</td>
<td>1</td>
<td>2</td>
<td>4</td>
<td>3</td>
</tr>
<tr>
<td>Yohoobo</td>
<td>2</td>
<td>4</td>
<td>4</td>
<td>3</td>
</tr>
<tr>
<td>Mario</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hishinowei-theri</td>
<td>3</td>
<td>3</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>16</td>
<td>33</td>
<td>43</td>
<td>34</td>
</tr>
</tbody>
</table>

Chapter 4
4.3.2 Relative abundance of mosquitoes species in CDC-light traps

A total of 332,355 female *Anopheles* were collected in the light traps in December 1997 and between June 1998 and December 2000 of which 90% and 10% were collected in Ocamo and Mavaca localities respectively. Table 4.3 shows the relative abundance of *Anopheles* species during the study period.

Table 4.3 Relative abundance of Anopheline catches by CDC-light traps

<table>
<thead>
<tr>
<th>Species</th>
<th>Locality</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Ocamo n (%)</td>
<td>Mavaca n (%)</td>
</tr>
<tr>
<td>An. darlingi</td>
<td>299,281 99.9</td>
<td>32,724 99.7</td>
</tr>
<tr>
<td>An. triannulatus</td>
<td>3 &lt;0.01</td>
<td>0 0</td>
</tr>
<tr>
<td>An. oswaldoi</td>
<td>101 0.03</td>
<td>86 0.30</td>
</tr>
<tr>
<td>An. strodei</td>
<td>0 0</td>
<td>8 0.02</td>
</tr>
<tr>
<td>An. benarrochi</td>
<td>19 0.01</td>
<td>14 0.04</td>
</tr>
<tr>
<td>An. mattogrossensis</td>
<td>5 &lt;0.01</td>
<td>1 &lt;0.01</td>
</tr>
<tr>
<td>An. spp</td>
<td>113 0.04</td>
<td>0 0</td>
</tr>
<tr>
<td>Overall</td>
<td>299,522(90)* 100</td>
<td>32,833(10)* 100</td>
</tr>
</tbody>
</table>

*Percentage corresponding to the mosquitoes caught per locality in relation to the total

The predominant species was *Anopheles* (*Nyssorhynchus*) *darlingi* (Root 1926) (99.9%). The remaining 0.1% comprised *An. (Nys) triannulatus* (Neiva & Pinto 1922), *An. (Nys) oswaldoi* (Peryassú 1922), *An. (Nys) strodei* (Root 1926), *An. (Nys) benarrochi* (Gabaldón, Cova García & López 1941) and *An. (Anopheles)*
mattogrossensis (Lutz & Neiva 1911). This pattern was consistent between villages, months and years. For the statistical analysis only *An. darlingi* was considered because it is the principal vector of malaria in the area (Rubio-Palis, 2000).

4.3.3 Temporal and spatial patterns of mosquito density

In order to ascertain the temporal (monthly) pattern of mosquito density and its relationship to environmental variables like rainfall and river depth, the Williams’ mean of densities per month was plotted for the whole duration of the project (1997 to 2000). Figure 4.3 and Figure 4.4 show the relationship between mosquito density and rainfall or river depth respectively. Mosquito densities were plotted for each locality and for the whole study area. Rainfall (in mm) and river depth (in m) data correspond to the month when the captures were performed.

August-September and November-December 1998, May-June 1999 and July-September 2000 were the periods of time with the highest mosquito density. The overall mosquito density was lower in the villages in Mavaca compared to Ocamo. Time series analysis was not performed, however, In-mosquito density (calculated as \[\ln(x+1)\]), was positively and significantly correlated with river depth (\(r=0.6, 27 \text{ d.f., } P=0.001\)) and rainfall (\(r=0.4, 27 \text{ d.f., } P=0.05\)). Using a lag of up to 2 months between rainfall/river depth and mosquito density did not improve the fit.
Figure 4.3 Williams’ mean of mosquito density and rainfall by month

a) Columns correspond to Williams’ mean density of mosquitoes, combining all villages in Ocamo and Mavaca localities. Dotted lines denote the rainfall recorded in Ocamo meteorological station for the value observed in the particular month of capture.

b) The Williams’ mean values are calculated for Ocamo (grey columns) and Mavaca (black columns) localities separately. Note: sampling was in Ocamo throughout the whole all study, in Mavaca up to December of 1999.
Figure 4.4 Williams’ mean of mosquito density and river depth by month

a) Columns correspond to Williams’ mean density of mosquitoes, combining all villages in Ocamo and Mavaca localities. Lines denote the Orinoco river depth in the Ocamo locality area observed in the particular month of capture.

b) The Williams’ mean values are calculated for Ocamo (grey columns) and Mavaca (black columns) localities separately. Note: sampling was in Ocamo throughout the whole study in Mavaca up to December 1999.
4.3.4 Pattern of mosquito density related to intervention

Figure 4.5 shows the temporal distribution of the Williams' mean of mosquito densities with insecticide and placebo treated hammock nets from 1998 to 2000. The overall seasonal pattern was broadly similar in ITHNs villages and PTHNs villages. Sampling was confined to Ocamo during 2000, but the sampling effort was similar in placebo and insecticide treated village throughout the study. Therefore, possible differences in mosquito density are not due to differential sampling in villages with insecticide or placebo treated nets.

![Figure 4.5 Williams' mean (Mw) of mosquito density by treatment status. Thick line refers to insecticide treated group and thin line to placebo group. Note: sampling was made in Ocamo throughout the whole study; in Mavaca up to December 1999.](image-url)
Table 4.4 shows the Williams’ mean of mosquito densities in 1998 (pre-intervention period). During the pre-intervention period, the communities that later received insecticide treated nets had higher mosquito densities than those that received placebo treated nets.

Table 4.4 Williams’ mean of mosquito density catches with CDC-light trap during the pre-intervention period (1998)

<table>
<thead>
<tr>
<th>Pair</th>
<th>Village</th>
<th>ITHN</th>
<th>PTHN</th>
<th>Overall</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Hatakoa</td>
<td>5</td>
<td>25.7</td>
<td>10 - 118</td>
</tr>
<tr>
<td>2</td>
<td>Purima</td>
<td>9</td>
<td>73.7</td>
<td>2 - 1,155</td>
</tr>
<tr>
<td>3</td>
<td>Shakita</td>
<td>5</td>
<td>25.7</td>
<td>9 - 120</td>
</tr>
<tr>
<td>4</td>
<td>Kopariwe</td>
<td>5</td>
<td>17.7</td>
<td>0 - 136</td>
</tr>
<tr>
<td>5</td>
<td>Lechoza</td>
<td>21</td>
<td>185.3</td>
<td>4 - 908</td>
</tr>
<tr>
<td>6</td>
<td>Iyewei-theri</td>
<td>18</td>
<td>170.1</td>
<td>5 - 940</td>
</tr>
<tr>
<td>7</td>
<td>Shashanawe</td>
<td>22</td>
<td>74.3</td>
<td>3 - 544</td>
</tr>
<tr>
<td>8</td>
<td>Clavotheri</td>
<td>20</td>
<td>78.5</td>
<td>0 - 681</td>
</tr>
<tr>
<td>9</td>
<td>Mario</td>
<td>6</td>
<td>2404.0</td>
<td>1753 - 3782</td>
</tr>
<tr>
<td></td>
<td>Overall</td>
<td>111</td>
<td>90.8</td>
<td>0 - 3782</td>
</tr>
<tr>
<td></td>
<td>Overall</td>
<td>119</td>
<td>38.6</td>
<td>0 - 1972</td>
</tr>
</tbody>
</table>

*Pair of villages matched by malaria incidence

bNumber of trap-nights

Williams’ mean (Mw) = number of female An. darlingi per light-trap-catch in the villages that were going to be designated as the Placebo Treated Hammock Net (PTHN) group or the Insecticide Treated Hammock Net (ITHN) group.

The matching of villages into pairs at the beginning of the project was done using only data for malaria incidence (the principal outcome chosen for this study) and not by mosquito density. Therefore an unmatched analysis was considered more appropriate to measure the effect of the intervention on mosquito density (Diehr et al., 1995; Donner and Klar, 2000).
4.3.5 Effect of lambdacyhalothrin impregnated hammock nets on Anopheles darlingi population

In order to study the effect of the intervention on the mosquito density, only data collected after the beginning of the intervention, during 12 months in 1999, were included in the analysis. Catches performed in 2000 were excluded since only a few villages in the Ocamo locality were sampled (see Table 4.2, page 126). The intervention effect was estimated firstly without taking into account the pre-intervention data (unadjusted analyses), and secondly taking into account the pre-intervention data (adjusted analyses).

4.3.5.1 Unadjusted analysis of Anopheles darlingi densities

The light-trap catches, measured as the number of female An. darlingi per trap, in placebo and insecticide treated villages were compared for the year 1999. An unpaired t-test on the In of the Williams' mean showed no significant differences between the two groups (t= 0.88, d.f.=16 P = 0.39) (Table 4.5, page 134).
Table 4.5. Results of the unadjusted analysis of the intervention effect on *An. darlingi* population

<table>
<thead>
<tr>
<th>Village</th>
<th>ITHN*</th>
<th>PTHNb</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n&lt;sup&gt;c&lt;/sup&gt;</td>
<td>M&lt;sub&gt;w&lt;/sub&gt;&lt;sup&gt;d&lt;/sup&gt;</td>
<td>ln(M&lt;sub&gt;w&lt;/sub&gt;)</td>
<td>n&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Hatakaoa</td>
<td>41</td>
<td>2.4</td>
<td>0.9</td>
<td>45</td>
</tr>
<tr>
<td>Purima</td>
<td>43</td>
<td>55.5</td>
<td>4.0</td>
<td>43</td>
</tr>
<tr>
<td>Shakita</td>
<td>31</td>
<td>2.2</td>
<td>0.8</td>
<td>41</td>
</tr>
<tr>
<td>Kopariwe</td>
<td>42</td>
<td>32.5</td>
<td>3.5</td>
<td>41</td>
</tr>
<tr>
<td>Lechoza</td>
<td>47</td>
<td>28.9</td>
<td>3.4</td>
<td>20</td>
</tr>
<tr>
<td>Iyewi-theri</td>
<td>40</td>
<td>123.1</td>
<td>4.8</td>
<td>40</td>
</tr>
<tr>
<td>Shashanawe</td>
<td>42</td>
<td>70.3</td>
<td>4.3</td>
<td>43</td>
</tr>
<tr>
<td>Clavotheri</td>
<td>28</td>
<td>191.5</td>
<td>5.3</td>
<td>35</td>
</tr>
<tr>
<td>Mario</td>
<td>43</td>
<td>833.4</td>
<td>6.7</td>
<td>40</td>
</tr>
<tr>
<td>Overall</td>
<td>357</td>
<td>44.7</td>
<td>3.7</td>
<td>348</td>
</tr>
</tbody>
</table>

Unadjusted analysis of *Anopheles* density: Ratio = 0.50; 95% CI: 0.33 - 0.75; t = 0.88; d.f. 16; P = 0.39

* Insecticide Treated Hammock Net (ITHN) group
* Placebo Treated Hammock Net (PTHN) group
* Number of trap-nights
* Williams' mean
4.3.5.2 Adjusted analysis of *Anopheles darlingi* densities

It was noted that the year before the intervention, the overall vector density in the insecticide treated villages was higher than in the placebo treated villages (see Table 4.4 in page 132). In fact, from 1998 to 1999, the density increased in most placebo villages, while it decreased or remained roughly constant in most treated villages. These data are shown in Figure 4.6.
Figure 4.6 William’s mean mosquito density by pair and treatment status for 1998-1999

In a) pair of villages that received Placebo Treated Nets and b) those that received Lambdacyhalothrin treated nets
Therefore, the intervention effect was adjusted for pre-intervention mosquito density using a negative binomial regression model. Baseline data, the logarithm of the arithmetic mean trap-catch within villages during 1998, were transformed as the ln of the arithmetic mean of the catches within each village and used in the regression model.

The village-specific mean density ratios of observed vs. expected mosquito count were ln transformed and then used for hypothesis testing (Table 4.6). The unpaired t-test on the adjusted mean density ratio showed a significant ($t=2.45$, d.f.=16, $P=0.03$) difference between study group (Mosquito density ratio: 0.38, 95% CI: 0.30-0.48). In other words the adjusted mean density in the ITHNs group was 62% (CI: 52-70%) lower than that in the PTHNs group (Table 4.6).

Anopheles density data were analysed also with a paired t-test. The paired t-test on the unadjusted mean density ratio showed no significant ($t=-1.27$, d.f.=8, $P=0.2$) differences between study groups, while the paired t-test on the adjusted mean density ratio showed significant ($t=-2.45$, d.f.=8, $P=0.04$) difference (data not shown). Similar results were thus obtained from both paired and unpaired t-test analysis.
### Table 4.6 Results of the adjusted analysis of the intervention on *An. darlingi* population

<table>
<thead>
<tr>
<th>Village</th>
<th>ITHN</th>
<th></th>
<th>PTHN</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n(^a)</td>
<td>Observed number</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>of <em>An. darlingi</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>(O)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Expected number</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>of <em>An. darlingi</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>(E)(^b)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>O/E e</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>ln(O/E)(^d)</td>
<td></td>
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</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hatakoa</td>
<td>41</td>
<td>541.0</td>
<td>4,074.8</td>
<td>0.13</td>
</tr>
<tr>
<td>Purima</td>
<td>43</td>
<td>3,463.0</td>
<td>12,755.7</td>
<td>0.27</td>
</tr>
<tr>
<td>Shakita</td>
<td>31</td>
<td>381.0</td>
<td>3,080.9</td>
<td>0.12</td>
</tr>
<tr>
<td>Kopariwe</td>
<td>42</td>
<td>5,343.0</td>
<td>4,822.8</td>
<td>1.11</td>
</tr>
<tr>
<td>Lechoza</td>
<td>47</td>
<td>4,408.0</td>
<td>17,615.1</td>
<td>0.25</td>
</tr>
<tr>
<td>Iyewei-theri</td>
<td>40</td>
<td>9,552.0</td>
<td>16,281.4</td>
<td>0.59</td>
</tr>
<tr>
<td>Shashanawe</td>
<td>42</td>
<td>7,987.0</td>
<td>9,397.7</td>
<td>0.85</td>
</tr>
<tr>
<td>Clavotheri</td>
<td>28</td>
<td>17,470.0</td>
<td>8,192.5</td>
<td>2.13</td>
</tr>
<tr>
<td>Mario</td>
<td>43</td>
<td>51,670.0</td>
<td>70,217.6</td>
<td>0.74</td>
</tr>
<tr>
<td>Overall</td>
<td>357</td>
<td>100,815.0</td>
<td>146,438.5</td>
<td>0.69</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Village</th>
<th>n(^a)</th>
<th>Observed number</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>of <em>An. darlingi</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>(O)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Expected number</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>of <em>An. darlingi</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>(E)(^b)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>O/E e</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>ln(O/E)(^d)</td>
<td></td>
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</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Motorema</td>
<td>45</td>
<td>9,850.0</td>
<td>5,202.9</td>
<td>1.89</td>
</tr>
<tr>
<td>Mosho</td>
<td>43</td>
<td>2,019.0</td>
<td>4,705.5</td>
<td>0.43</td>
</tr>
<tr>
<td>Warapana</td>
<td>41</td>
<td>4,523.0</td>
<td>4,046.9</td>
<td>1.12</td>
</tr>
<tr>
<td>Piegrita</td>
<td>41</td>
<td>1,389.0</td>
<td>849.4</td>
<td>1.64</td>
</tr>
<tr>
<td>Kashorawe</td>
<td>20</td>
<td>10,557.0</td>
<td>3,846.7</td>
<td>2.74</td>
</tr>
<tr>
<td>Yohoope</td>
<td>40</td>
<td>8,644.0</td>
<td>12,975.5</td>
<td>0.67</td>
</tr>
<tr>
<td>Sta. Ma. Guicas</td>
<td>43</td>
<td>3,729.0</td>
<td>6,916.3</td>
<td>0.54</td>
</tr>
<tr>
<td>Carlitos</td>
<td>35</td>
<td>30,873.0</td>
<td>16,234.9</td>
<td>1.90</td>
</tr>
<tr>
<td>Hishinowei-theri</td>
<td>40</td>
<td>65,470.0</td>
<td>29,821.9</td>
<td>2.20</td>
</tr>
<tr>
<td>Overall</td>
<td>348</td>
<td>137,054.0</td>
<td>84,599.9</td>
<td>1.46</td>
</tr>
</tbody>
</table>

---

\(^{a}\) n, number of trap-nights; (O) Observed number of *An. darlingi*; (E) The expected *An. darlingi* density estimated by a negative binomial regression adjusted for the ln of the arithmetic mean of mosquito count in 1998 (baseline *An. darlingi* density) excluding the intervention effect. Then the observed number of *An. darlingi* was divided by the expected by village and them were ln transformed; a un-paired t-test on the adjusted ln of the mean *Anopheles* ratio was applied to test the null hypothesis RR=1.

---

*Anopheles* density ratio=0.38; 95% CI: 0.30 - 0.48; \(t=2.45\); d.f. 16; \(P=0.03\)

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4.3.6 Parity rate

A total of 989 specimens collected were dissected. The number dissected varied from month to month reflecting changes in population density, and were sometimes very small especially in the insecticide treated group. Results (presented in Table 4.7) showed that the parous rate was slightly but not significantly higher in the placebo group ($\chi^2_{MH}=1.56$, stratifying by month, d.f.=1, $P=0.2$). There is therefore no significant evidence for an impact of the intervention on the survival rate of the mosquito population in the insecticide treated villages.

| Table 4.7 Parous rate of *Anopheles darlingi* from CDC-light trap collections in villages with placebo treated hammock nets (PTHN) or insecticide treated hammock nets (ITHN) |
|---|---|---|---|---|
| Month | PTHN Parous (%) | No. examined | ITHN Parous (%) | No. examined |
| Aug-00 | 57.1 | 308 | 51.1 | 233 |
| Sep-00 | 47.5 | 137 | 50.3 | 165 |
| Oct-00 | 83.3 | 6 | 55.6 | 27 |
| Nov-00 | 75.0 | 8 | 41.9 | 105 |
| Overall | 54.9 | 459 | 49.2 | 530 |

A total of 956 mosquitoes were classified according to the gonotrophic stage (results are shown in Table 4.8). The proportion of mosquitoes caught in the gravid stage was very similar in the two groups. However, when these gravid
females were excluded, and the fed-unfed ratio was compared between treatment groups, it was found that the proportion unfed was significantly higher in the insecticide treated group ($\chi^2_{\text{MH}}=24.79$, stratifying by month, d.f.=1, $P<0.0001$).

Table 4.8 CDC-light trap collection of female *Anopheles darlingi* classified by gonadotrophic stage (U: unfed, F: fed, G: Gravid) as percent of total females in the villages with placebo treated hammock net (PTHN) or insecticide treated hammock nets (ITHN)

<table>
<thead>
<tr>
<th>Month</th>
<th>PTHN</th>
<th>ITHN</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>U</td>
<td>F</td>
</tr>
<tr>
<td>Sep-00</td>
<td></td>
<td></td>
</tr>
<tr>
<td>%</td>
<td>41.5</td>
<td>37.8</td>
</tr>
<tr>
<td>n</td>
<td>200</td>
<td>182</td>
</tr>
<tr>
<td>Oct-00</td>
<td></td>
<td></td>
</tr>
<tr>
<td>%</td>
<td>56.9</td>
<td>3.4</td>
</tr>
<tr>
<td>n</td>
<td>33</td>
<td>2</td>
</tr>
<tr>
<td>Nov-00</td>
<td></td>
<td></td>
</tr>
<tr>
<td>%</td>
<td>85.6</td>
<td>11.4</td>
</tr>
<tr>
<td>n</td>
<td>113</td>
<td>15</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td></td>
</tr>
<tr>
<td>%</td>
<td>51.5</td>
<td>29.6</td>
</tr>
<tr>
<td>n</td>
<td>346</td>
<td>199</td>
</tr>
</tbody>
</table>

4.3.7 Sporozoite rate

Out of 299,281 mosquitoes collected in Ocamo locality, a sample of 30,205 (10%) was tested by ELISA for the presence of *P. falciparum*, *P. vivax*-210 and *P. vivax*-247 CSP. Of this sample 26,780 were tested by the author of this thesis in the CAICET laboratories and 3,425 at the laboratories of the London School of Medicine.
Hygiene and Tropical Medicine (LSHTM) by Rix (1999). Of these, 4,075 were collected in the pre-intervention period (December 1997 to December 1998) and 26,130 in the post-intervention period (January 1999 to September 2000). Mosquitoes were tested in pools of 10. Out of all mosquitoes tested only two pools were positive for CSP. One pool positive for *Pf* CSP, contained mosquitoes collected in Hishinowei-theri in December of 1997 during the pre-intervention period. The other positive pool for *Pv*-247 CSP was collected in Yohoope (placebo village) during the post-intervention period.

It was not possible to estimated exactly the precise number of trap-nights represented by 30,205 mosquitoes tested by ELISA. The EIR in the study area before and after the intervention was therefore calculated as the estimated sporozoite rate multiplied by the estimated biting density. Lines *et al.* (1991), working with *An. gambiae*, found that, on average, two HBC collected as many mosquitoes as 3 CDC-light traps. Assuming the same relative sampling efficacy with *An. darlingi* the EIR is estimated as the sporozoite rate multiplied by the mean number of females *Anopheles* mosquitoes per light trap per night multiplied by 1.5. The estimated EIRs for the pre and post-intervention period are presented in Table 4.9.
Table 4.9 Sporozoite and annual Entomological Inoculation Rate (EIR) during the pre and post-intervention

<table>
<thead>
<tr>
<th>Study period</th>
<th>Sporozoite rate% (^a)</th>
<th>Number of An. darlingi caught</th>
<th>Number of CDC-light trap</th>
<th>Mean catches (^b)</th>
<th>Annual EIR (^c)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre-intervention</td>
<td>0.025% (1/4,075) 95% CI: 0.0006-0.136</td>
<td>56,651</td>
<td>194</td>
<td>292.0</td>
<td>39</td>
</tr>
<tr>
<td>Post-intervention</td>
<td>0.008% (1/12,050) 95% CI: 0.008-0.046</td>
<td>138,806</td>
<td>315</td>
<td>440.7</td>
<td>20</td>
</tr>
<tr>
<td>Control</td>
<td>(0% (0/14,080) 95% CI: 0-0.026</td>
<td>105,501</td>
<td>378</td>
<td>279.1</td>
<td>0</td>
</tr>
<tr>
<td>ITHNs</td>
<td>(0% (0/14,080) 95% CI: 0-0.026</td>
<td>105,501</td>
<td>378</td>
<td>279.1</td>
<td>0</td>
</tr>
</tbody>
</table>

\(^a\) The Confidence Intervals are calculated according to Limits of expectations of Binomial and Poisson Distributions (based on W. L. Stevens)

\(^b\) Numbers of An. darlingi caught divided by the numbers of CDC light traps used

\(^c\) Mean catches x 1.5 x sporozoite rate x 365 (Lines et al., 1991)
4.4 Discussion

4.4.1 Malaria transmission in the study area

*Anopheles darlingi* is the primary vector of human malaria in the Amazon region (Foratini, 1962; Rubio-Palis and Zimmerman, 1997) and it is the major contributor to the present resurgence of this disease in South and Central America (Zimmerman, 1992). The seasonal pattern of fluctuations in the *An. darlingi* population varies in different ecological areas. For instance, in Brazil, Charlwood (1980) found in Amazonas a peak in mosquito density (measured by HBC) at the end of the long dry season, whereas in Mato Grosso a peak in density was observed at the end of the long rainy season. In the rain forest of Surinam, in a study made on anthropophilic anophelines along the Upper-Surinam and Upper-Marowijine Rivers, it was found that the seasonal fluctuations in abundance of *An. darlingi* varied between sampling stations, reflecting local differences in the availability of breeding sites (Rozendaal, 1991). *An. darlingi* often breeds in shallow tributaries to large rivers, which may be dry or flooded depending on the overall river level. It was suggested that density variation in different ecological areas and years depends on the relationship between the level of the rain and height of the river. Heavy rains might flush out breeding sites whereas in the dry season, the limited amount of water may cause a decrease in the population with some exceptions, i.e. in the forest where breeding can continue.

In the present study, *An. darlingi* accounted for 99% of all anophelines collected in light-traps located beside a person overnight in Ocamo and Mavaca localities,
Upper Orinoco. The vector was present all year round with a peak of density during the rainy season, when the river was high and suitable breeding sites were generated. The same pattern of distribution was observed before and after the intervention. A similar observation was reported by Hudson (1984) in the Surinam rain forest where the peak abundance of *An. darlingi* occurred in the middle of the rainy season, when the river was at its highest level. In contrast, in a previous study carried out in the same area as the present study (Ocamo locality), no correlation was found between rainfall, river level and mean number of *An. darlingi* (Rubio-Palis et al., 1997). However, in that study, HBC were carried out weekly, but in one house only, for one year, and therefore it would be inappropriate to generalize those conclusions to the whole area.

This previous study also provides the only other estimate of the sporozoite rate in Ocamo. This was carried out on mosquitoes collected by monthly HBC in 1994 in Ocamo locality. It found an overall sporozoite rate of 0.764%, and the annual entomological inoculation rate (EIR) was estimated to be 127 infective bites per person per year (Rubio-Palis *et al.*, 1997). Because of the low sporozoite infection rate in the area, large samples of mosquitoes need to be analyzed in order to estimate the EIR and to detect any possible difference between intervention and control.
4.4.2 Impact of intervention on vector population

The present study was not designed to detect a mass killing effect. As mentioned in Chapter 1, page 45 An. darlingi is highly anthropophilic (Lourenco-de-Oliveira et al., 1989; Tadei and Dutary-Thatcher, 2000). Nevertheless, typical Yanomami villages are small and surrounded by the jungle, and it might be expected that village An. darlingi populations would be mixed with, or be part of, a larger sylvatic population, most of which is virtually never exposed to the insecticide. However, studies carried out in the Brazilian Amazon found that An. darlingi was almost the only species present inside houses and its abundance decreased moving away from the house into the forest. In other words, An. darlingi was the most abundant species in and around houses, and was more common there than in the forest (Lourenco-de-Oliveira et al., 1989). In the rain forest of Surinam similar results were obtained by Rozendaal (1991). Gliglioli (1956) pointed out that the preference for biting in human settlements might be due to some degree of adaptation to man. Hence it is possible that village An. darlingi populations tend to remain more or less in or near the village, and that there is only limited mixing with sylvatic populations, and limited exchange between village populations. In this case, ITNs might be expected to produce a mass effect which should be detectable as a decrease in An. darlingi density, parity and sporozoite rate.

In the present study An. darlingi densities in the insecticide treated villages were slightly, but non-significantly, lower than those in placebo treated villages during the first year of intervention. However, given the fact that the vector densities in
the baseline period were higher in the insecticide group than in the placebo, another analysis was carried out adjusting *Anopheles* density in each village for that of the pre-intervention year. Significant differences in densities were found. This suggested that mean density in insecticide treated villages was reduced by 62% (Density ratio: 0.38, 95% CI 52-70%) compared to placebo villages ($P=0.03$). This supports the hypothesis that there was a mass killing effect due to ITHNs.

However, it should be remembered that in this study, the CDC-traps in insecticide treated villages were hung next to treated nets, while those in the placebo villages were hung next to placebo-treated nets. Thus the observed reduction in the mean mosquito density could be due to a mass effect on the overall vector abundance, but it could be also due to repellency of mosquitoes, i.e. the proximity of the treated net to the trap may prevent mosquitoes from entering the trap. If there had been a major effect on density due to mortality, then an effect on parous and sporozoites rates should be observed. In the present study the parity dissection was done on samples collected in 2000, when the nets were becoming damaged and net usage was declining. Nevertheless, no significant differences was found in parous rates between mosquitoes caught in villages with insecticide and placebo treated nets. However the proportion of fed mosquitoes caught in the light traps was significantly lower ($P<0.01$) in the villages with insecticide treated nets compared to villages with placebo treated nets. This is evidence for a personal protection caused by the excito-repellent effect of lambdacyhalothrin. A similar result was found with *An. darlingi* in Surinam in a study carried out in
experimental huts, comparing a hut with a single sleeper protected by a net impregnated with permethrin (0.5g/m²) with a hut with a sleeper using an untreated net. The result showed that the blood feeding rate in the mosquitoes collected in the exit traps was significantly lower ($P<0.001$) for the huts with treated nets (Rozendall et al. 1989).

Regarding the sporozoite rate, only one specimen positive for *P. vivax*-247 CSP was found, out of 26,130 tested collected after the intervention. Therefore, the estimated sporozoite rate in the placebo group was 0.008% vs. 0% in the insecticide treated group. With so few positive mosquitoes, it is not possible to reach any conclusion regarding the effect of the intervention on the sporozoite rate.

Therefore, the general reduction in vector density in the villages with insecticide treated nets together with the reduction in the blood feeding can be attributed tentatively to a mass killing effect resulting from community usage of impregnated nets.

4.4.3 Methodological issues related to entomological assessment of the impact of ITHNs.

Regarding the methodology used to assess the mosquito density, light traps were hung in villages with ITHNs (see page 118 in this Chapter). Lines *et al.* (1991) recommended that CDC-light traps should be placed next to a person sleeping
under an untreated net in a separate sentinel house or room within a village where the majority of people were using treated nets. However, given the particular setting of hammocks within the household as well as the traditional shape of the Yanomami huts (shabono described at page 32 in Chapter 1), to place a light trap beside an untreated net would have been probably ineffective because of the closeness of the other treated nets. To avoid interference with sleeping habits, it was decided to hang the light traps beside a person sleeping under an insecticide impregnated net within the shabono. No published data are available showing whether there is a difference in the functioning of CDC-light traps placed beside a treated or an untreated net. The only data available come from a small study (Paine, 1998 unpublished work) carried out within the frame of a larger trial in Tanzania with An. gambiae and An. funestus. No significant differences were found in the mean number of mosquitoes caught with CDC-light traps placed beside either an insecticide treated or untreated net, although the number of replicates was not sufficient to draw a firm conclusion.

In a study carried out in Sierra Leone, Magbity et al. (2002) compared the efficacy of light trap vs. HBCs in villages with treated nets and villages without nets. In treated villages, light-trap catches were carried out in houses that in most cases contained treated nets, although the nets in the same room as the trap were always untreated. In villages with treated nets, there was some evidence of a reduction of the relative sampling efficiency of light traps at high densities. However, the author concluded that the magnitude of this effect was not large enough to be of practical significance. Therefore the author suggested that CDC
light traps can be used as a substitute for HBC to estimate the biting rates of *An. gambiae s.l.* mosquitoes in villages where a large proportion of the inhabitants use ITNs.

It was not possible to show an impact of the intervention on the sporozoite rate, because only one post-intervention specimen was found positive for CSP antigens. Before the intervention one out of 4,075 mosquitoes (in pools of ten) analyzed was positive. Therefore, the estimated sporozoite rate was 0.02%. From this result it was estimated that to reveal a reduction of 50% in the CSP rate as a result of the intervention, a sample of about 140,000 mosquitoes should have been analyzed by ELISA (95% level of significance and 80% power) in each of the study arms (for the sample size calculation see Kirkwood and Sterne (2003)) which represents an impractically large amount of work.

4.4.4 Mass killing effect in other studies

The effect of insecticide treated nets at village level on entomological parameters has not been properly studied in South America. Only a small study carried out by Santos *et al.* (1999) reported in Costa Marques, Rondonia, Brazil, that the use of deltamethrin (20mg/m²) impregnated nets significantly decreased the vector density during high anopheline density periods in comparison to untreated nets. But this was a small study and it can not be confidently concluded that these density differences were due to the intervention.
In Africa, a clear mass killing effect has been found on *An. gambiae* populations in studies carried out in Burkina Faso, Tanzania, Zaire and Kenya using different pyrethroids.

In Burkina Faso, a major impact on the entomological parameters, vector densities and sporozoite rate was shown by Cuzin-Ouattara *et al.* (1999) using permethrin treated curtains (1g/m$^2$). In this study the density of mosquitoes was greatly reduced by the intervention and the sporozoite rate was 11.5% in control areas and 4.1% in the intervention area.

In Tanzania the mass killing effect was studied in a highly malaria endemic area in the north-east of the country. Firstly, in a study reported by Magesa *et al.* (1991) effect of treated nets on mosquito density, parity rate, ovarian age grade and sporozoite rate were all shown. Secondly, in a study carried out in late 1995-early 1996 by Curtis *et al.* (1998), four villages were provided with polyester bednets treated with lambdacyhalothrin (10mg/m$^2$) and the comparison groups were villages with untreated nets (control) and villages without nets but with IRS. Later on, in 1996 in the same area, the four villages which had been the untreated controls in the nets vs. spraying trial, were provided with nets treated either with lambdacyhalothrin or, for comparison, nets treated with alphacypermethrin (20mg/m$^2$) (Maxwell *et al.*, 1999). Both studies reported a significant reduction in the densities of anopheline vectors caught in light traps in untreated sentinel bedrooms in the treated villages and in the sporozoite rates in these mosquitoes. The reduction in the entomological inoculation rate was 89.7% in the first study.
and 95.5% in the second. In both of the above mentioned studies it was found, in addition to the mass killing effect, a reduction in the number of blood-fed mosquitoes found in the rooms with treated nets or in window exit traps on these rooms, giving evidence of personal protection of individual net users from being bitten (54.5% and 81.7% of reduction in blood fed mosquitoes). Most recently Maxwell et al. (2003) showed an impacts of treated nets on mosquito densities in high and low altitude villages but the observed reduction in mean sporozoite rates did not reach statistical significance.

In Zaire, the effect of deltamethrin (25mg/m²) treated nets was compared to untreated nets and no nets in three villages. After the intervention it was found that ITNs reduced the density of An. gambiae s.l. by 94% and the entomological inoculation rate by 98% (Karch et al., 1993).

In Kenya, in a large-scale, group-randomized controlled trial, high coverage of ITNs was associated with a community-wide suppression of mosquito populations detectable in neighboring villages lacking ITNs (Gimnig et al., 2003a, 2003b). Results of the spatial analysis showed also a protective effect of ITNs on compounds lacking ITNs located within 300 meters of compounds with ITNs for child mortality, moderate anemia, high-density parasitaemia, and hemoglobin levels thus strongly supporting the existence of a community mass killings effect (Hawley et al., 2003).

However, in The Gambia, with the same vector (An. gambiae s.l.) as in Tanzania, Zaire, Kenya and Burkina Faso, no evidence of a mass killing effect comparing
villages with permethrin (250mg/m²) treated nets and villages with untreated nets was observed. No significant differences were found in the An. gambiae density, parous rate or sporozoite rate (Quinones et al., 1998). It was hypothesized that mosquitoes from treated and untreated villages were sharing common breeding sites in The Gambia river so that the two populations were mixed, confounding the effects of the ITNs on entomological parameters. The impact of ITNs was attributed to personal protection rather than to a mass killing effect at village level (Lindsay et al., 1993; Quinones et al., 1998).

In other areas such as Thailand the absence of a mass effect following the use of ITNs was explained by the exophagic, zoophilic and early biting habits of An. minimus species A in that country (Somboon et al., 1995). While in Assam, India where the same vector is endophilic and anthropophilic with a biting peak after midnight, a mass killing effect of treated nets was reported by Jana-Kara et al. (1995).

4.4.5 Conclusion

Malaria transmission in the study area is characterized by a low sporozoite rate but the high abundance of the vector An. darlingi maintains high levels of malaria transmission and therefore high incidence of the disease in the population.

ITHNs had a positive impact on the reduction of malaria incidence in the intervention area (see Chapter 5) and this can be partially attributed to a mass
killing effect and partially to personal protection. Further work should be carried out to confirm the impact of ITHNs on *An. darlingi* population in this particular setting.
CHAPTER 5. Impact of the intervention on malaria incidence, mean haemoglobin level, prevalence of splenomegaly and parasitaemia

5.1 Introduction

The efficacy of intervention programs based on the use of ITNs for reducing malaria can be measured in different ways. The main parameters that have been used in field trials are: mortality (all-cause or malaria specific), incidence of mild malaria illness (defined in various ways), and the prevalence of diverse indicators including prevalence of parasitaemia, splenomegaly and anaemia; level of haemoglobin, and nutritional status. In malaria endemic areas of America, the mortality due to this disease is much lower than in sub-Saharan countries and the main parameters used to measure the impact of control and intervention programs are the annual parasite incidence and slide positivity percentage (WHO/RBM, 2000; PAHO/WHO, 2002).

Malaria incidence is the main epidemiological parameter studied in the present thesis and it is defined as the number of new clinically and parasitologically confirmed cases in the overall population at risk, over a period of time. It is difficult to give a universally appropriate standard definition of a malaria case, as the sensitivity and specificity of any one definition varies with the level of malaria in the study area and the natural immunity in the population. For instance, in some studies, mainly in areas where P. falciparum is the most prevalent species, a malaria case has been defined when the parasite was detected and the patient had fever \( \geq 37.5^\circ \text{C} \) (Snow et al., 1987; Snow et al., 1988). Other researchers defined a
new case using, in addition to positive slide and clinical symptoms, the parasite density, with threshold densities that in some cases varied according to age group (Marbiah et al., 1998).

When studying the effect of, for example, vector control on malaria incidence in areas where the malaria transmission is intense and most of the population is permanently infected with the parasite, as in sub-Saharan countries, one approach is first to clear the pre-existing infection by treating the whole study population (Sexton et al., 1990; Curtis et al., 1998; Maxwell et al., 1999; Fanello, 2002). In this case the acquisition of a new parasite infection is determined by weekly or fortnightly parasite diagnosis of those individuals who remain negative. In areas of low malaria transmission, usually, positive cases are few and are treated, once identified, in the pre-intervention survey (Kamol-Ratanakul and Prasittisuk, 1992; Luxemburger et al., 1994). In the studies carried out in South and Central America by Kroeger et al. (1995, 1999), the malaria incidence rate was measured based on self-diagnosed cases of malaria reported during the last two or four weeks to researchers. In these latter studies, although no systematic parasitological confirmation was done, “a pilot phase established that about 88-96% of the self-diagnoses were based on the same criteria used by health professionals” (Kroeger et al., 1995).

A further problem arises in areas with high prevalence of P. vivax where 10-20% of cases are likely to suffer a relapse in the first year (Sinha et al., 1989; Adak et al., 1998; Dua and Sharma, 2001; Duarte et al., 2001). These cases are not
affected by the use of ITNs. Neither can the problem be solved by the use of primaquine since this is generally ineffective in preventing relapses, either because of low compliance, or because the prescribed dosage is inadequate. Therefore *P. vivax* relapses, which are misdiagnosed as new infections, can cause an overestimation of malaria incidence. For this reason a careful definition of a new malaria case is needed in such areas. In America most malaria is due to *P. vivax* (30-100%). This issue has been addressed in one study carried out in Guatemala by Richards *et al.* (1993) where a new case of *P. vivax* was described as follows i) when a new episode occurred after the entire course of radical treatment was given and ii) the new infection occurred more than six months after the previous episode.

Prevalence of parasitaemia is defined as the percentage of persons parasite positive (with or without symptoms) divided by the study population size at one time point (cross-sectional survey). The results obtained with this parameter in intervention studies or in control programs depend on the season when the cross-sectional survey is carried out. Low parasite prevalence is normally found during the low transmission season and high parasitaemia in the high transmission season. Therefore for comparison purposes it is better that surveys are carried out during the same season.

Another measure used to evaluate the efficacy of ITNs is the presence of a palpable spleen and the degree of splenomegaly. The impact of the intervention on the prevalence of splenomegaly is commonly measured during cross-sectional
successive surveys through direct palpation and reported as presence/absence of palpable spleen or according to the Hackett scale (WHO, 1963).

Regarding anaemia and haemoglobin level as outcome measures, it is normally recorded in areas of intense malaria transmission (Menendez et al., 2000). Although anaemia can be caused by other reason than malaria, it has been found to be sensitive to intervention in previous ITN trials (Curtis et al., 1998; Yadav et al., 1998; Marchant et al., 2002; Maxwell et al., 2002, 2003; Hawley et al., 2003; ter Kuile et al., 2003). Mean Packed Cell Volume (PCV) or haemoglobin levels in g/dl are used to measure anaemia in the field. Comparison between results collected with the two methods is done using a standard conversion factor of 3:1 i.e. 1g/dl=3%PCV (Lengeler, 2003).

The effect of ITNs upon the nutritional status of infants is generally assessed through the following anthropometric parameters i) weight-for-age, ii) weight-for-height iii) upper arm circumference and iv) weight. These parameters have been considered in three studies carried out in The Gambia (D'Alessandro et al., 1995a), Kenya (Snow et al., 1997a) and Tanzania (Shiff et al., 1996). In all these three trials a positive impact of ITNs on weight-for-age and weight-for-height was found. In the first two trials they compared ITNs with untreated nets while in the third one the control group did not have nets.

The outcomes chosen in the present study to measure the epidemiological effect of ITHNs compared to PTHNs as control were: malaria incidence rate (defined as the number of individuals with malaria symptoms plus any parasitaemia, divided
by the person-time at risk), prevalence of parasitaemia and splenomegaly, and haemoglobin level.
5.2 Materials and methods

In December 1998, 18 villages were selected for this study. As described in Chapter 2, villages were paired according to the malaria incidence rates observed during 1997, and were then randomly allocated to different treatment groups. Half of the villages (Hatakoe, Purima, Shakita and Kopariwê from Mavaca locality and Lechoza, Iyewëi-theri, Shashanawë, Clavotheri and Mario from Ocamo locality) received hammocks nets treated with lambdacyhalothrin 10mg/m². The other half of the villages (Motorema, Mosho, Warapana, Piegrita from Mavaca locality and, Kashorawë, Yohoopë, Santa María de los Guaicas, Carlitos and Hishinowëi-theri from Ocamo locality) received placebo treated hammock nets.

5.2.1 Malaria surveillance-longitudinal follow-up

In order to record malaria cases and calculate malaria incidence in the villages, continuous active and passive case detection was carried out after the intervention between January 1999 and December 2000.

Active case detection was carried out in all villages fortnightly. Each house of the study area was visited and the members of the family who were present were asked about malaria symptoms at the time of the visit and in the two days prior to the visit. Their axillary temperatures were measured with a digital thermometer, and those with ≥ 37.5°C, and/or reporting either fever or shivering in the previous
48 hours, were asked to provide an ear-prick blood-sample for thick and thin blood-smears for malaria diagnosis. Data were collected in a form (see Annex 8). No attempt was made to follow up members of the family who were absent.

Passive case detection was carried out every day including weekends at the PHC of each locality. Ear-prick blood-samples were taken from individuals using the same criteria as for active case detection. Data were recorded in a form (see Annex 9).

During the case detection, the ear-prick instead of finger-prick was used, because in Venezuela it is considered to be less painful and more acceptable, and is therefore the common method used by the malaria control programme.

5.2.2 Clinical, haematological and parasitological cross-sectional surveys

To study the effect of the intervention on parasitaemia prevalence, three cross-sectional surveys (S1, S2, and S3) were done measuring haemoglobin concentration and presence or absence of palpable spleen. The pre-intervention survey (S1) was done in December 1998, before the distribution of ITHNs and PTHNs nets. S2 was done six months after the intervention in July 1999. S3 was carried out in December 2000 at the end of the intervention.

Before each cross-sectional survey, a meeting was held with the population of each village. During this meeting the methodology to be used during the survey
was explained, and the day of the visit was fixed. The population was requested to remain in the village on the day of the survey.

At each cross-sectional survey, the village population was examined clinically and blood-samples were taken. Results were recorded on individual questionnaires (see Annex 10). Details of the procedure for obtaining informed consent are give in Chapter 2, see page 78.

5.2.1.1 Clinical data collections

Consenting individuals attending the cross-sectional survey voluntarily were investigated for clinical symptoms, spleen enlargement, haemoglobin level and malaria parasites. Each person, or parent in the case of young children, was asked about their health at the moment of the interview and in the preceding two days; presence of fever, diarrhoea, shivering, headache, vomiting, and spleen pain were recorded. A physical examination including measuring axillary temperature, and spleen and liver palpation was carried out. A digital thermometer (Gerber Ref. 78072) was used to measure axillary temperature. The spleen palpation was done with the person in the supine position and the examiner sitting below and in front of the examined individual, as described by Wernsdorfer and McGregor (1988). Pregnant women were excluded from spleen examination. Any other obvious disease was recorded under the “Notes” section of the questionnaire. In order to decrease bias, the same medical doctor made the clinical examination in all cross-sectional surveys.
5.2.2.2 Parasitological and haemoglobin sample collection

During the cross-sectional surveys, blood samples were collected by finger-prick because this is the standard method for determining haemoglobin concentration. Haemoglobin levels were measured using a portable β-Haemoglobin photometer (HemoCue®, HemoCue AB, Angelholm, Sweden). Thick and thin blood-smears were taken for microscopic examination.

5.2.3 Malaria diagnosis

Thick and thin blood-smears, collected during case detection and cross-sectional surveys, were stained. When dry, thick smears were dipped in distilled water to remove haemoglobin. Thin smears were dipped in methanol to fix the blood components. Slides were left to air dry and then stained with Giemsa 5% (Merck Ref. 109204). The stain was prepared by diluting 150μl of stock solution of Giemsa in 3ml buffer at pH 7.2 (sodium phosphate Na₂HPO₄ 40.55g + potassium phosphate KH₂PO₄ 0.45g + distilled water 1 l). Slides were placed horizontally face down for 20 min, and then stood upright to dry. Slides were labeled with the patient’s code and type of detection, e.g. active case detection (BA), passive case detection (BP) and cross-sectional survey (S1, S2 or S3).

Slides were examined using a light microscope (Nikon Alphaphot YS2-H3) at 1000x magnification. For passive and active case detection, slides were considered negative if no parasites were found in 200 fields. In the cross-sectional
surveys, up to 500 fields were examined. Blood smears were classified as negative or positive for *P. falciparum*, *P. vivax*, *P. malariae*, and mixed infections were recorded. To provide objective and blind quality control, all blood slides were re-examined by an experienced microscopist in an independent reference laboratory (the Laboratory of Malaria at Maracay, Aragua State) which was not involved in the field study. In cases of discrepancy, the PI reviewed the slides and decided the diagnosis.

5.2.4 Malaria treatment

When the slides were taken in the village, during active case detection and the cross-sectional surveys, the team returned to the village to report the results to the population and to offer treatment, on the same or the following day. Parasite-positive cases, with or without symptoms, were treated following regional guidelines. *P. falciparum* cases were treated with quinine at 10mg/kg every 8h for 7 days. A complete daily course of quinine treatment was handed to each patient every day, and only the taking of the first dose of the day was directly observed in the majority of cases. Chloroquine (25 mg/kg over 3 days) was used for treatment of *P. vivax* and *P. malariae*. Individuals with *P. vivax* infections received 3 doses of primaquine at 0.7 mg/kg per day, in addition to their chloroquine, in order to treat liver stages.
5.2.5 Analysis

In order to measure the effect of the intervention on the malaria case incidence rates, only new malaria cases were included in the analysis, following the case definition described below. To avoid any confusion between new infections and infections contracted prior to the intervention, positive cases were considered from 1 month after intervention.

5.2.5.1 Case definition

A definition of new case was established in order to estimate the malaria incidence rate. Malaria cases or parasite positive cases found during the cross-sectional surveys were not taken into account in estimating the malaria incidence rate.

A new case of *P. vivax* was defined as: A person with fever or shivering observed at the time, or reported in the previous two days, plus a *P. vivax* positive slide, detected through the fortnightly active case detection in the community or through passive case detection at the clinic, and without a previous record of *P. vivax* infection in the preceding six months.

A new case of *P. falciparum* was defined as: A person with fever or shivering observed at the time, or reported in the previous two days, plus a *P. falciparum* positive slide, detected through fortnightly active case detection in the community...
or through passive case detection at the clinic, and without a previous record of *P. falciparum* infection in the preceding month.

Therefore, a new episode of slide positive malaria was considered to be a relapse or recrudescence if it occurred within six months (*P. vivax*), or one month (*P. falciparum*), after a previous episode of acute malaria diagnosed during the study.

### 5.2.5.2 Calculation of the time at risk

The *population at risk* was defined as the total number of inhabitants of any age, living permanently in each village of the study area and who received nets at the beginning of the intervention. Individuals who died or emigrated permanently out of the study area during the research period were excluded from the analysis, from the time they left. For those persons that did not emigrate permanently but travelled temporarily out of the study area, the time at risk was estimated as follows. Three censuses were carried out, one just before the intervention (December 1998), one in the middle of the first year post-intervention (July 1999) and one at the end of the study (December 2000). To estimate the time at risk of each person, the study period was divided in two periods corresponding to the intervals between these censuses. Then, for each participant, the time at risk was estimated for each of these two intervals. These two periods were then added to obtain the total time at risk. The different possible combinations used to estimate the time at risk for each person are presented in Table 5.1. Then, following the
case definition given in section 5.2.5.1, when a person had a new episode of
*P. falciparum* or *P. vivax*, he/she was excluded from the time at risk analysis for 1
or 6 months respectively. In other words this period was subtracted from the total
time at risk.
Table 5.1. Parameters used to estimate the time at risk for each person who participated in the intervention study

<table>
<thead>
<tr>
<th>Census 1</th>
<th>Census 2</th>
<th>First event</th>
<th>Time at risk 1</th>
<th>Census 2</th>
<th>Census 3</th>
<th>First event</th>
<th>Time at risk 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Y</td>
<td>Y</td>
<td>Y</td>
<td>$C_2 - C_1$</td>
<td>Y</td>
<td>Y</td>
<td>Y</td>
<td>$C_3 - C_2$</td>
</tr>
<tr>
<td>Y</td>
<td>Y</td>
<td>N</td>
<td>$C_2 - C_1$</td>
<td>Y</td>
<td>Y</td>
<td>N</td>
<td>$C_3 - C_2$</td>
</tr>
<tr>
<td>Y</td>
<td>N</td>
<td>Y</td>
<td>$(E - C_1) + (C_3' - E)/2$</td>
<td>Y</td>
<td>N</td>
<td>Y</td>
<td>$(F - C_2) + (C_3' - F)/2$</td>
</tr>
<tr>
<td>Y</td>
<td>N</td>
<td>N</td>
<td>$(C_3' - C_1)/2$</td>
<td>Y</td>
<td>N</td>
<td>N</td>
<td>$(C_3' - C_2)/2$</td>
</tr>
</tbody>
</table>

$C_1$, date of the first census; $C_2$, date of the second census; $C_3$, date of the third census; $C_3'$ are the date of the second and third census for individuals that are not seen during the corresponded censuses; $E$, $F$, date of the first time a person was seen during the case detection between the first and second census or between the second and third census, respectively; $Y$, if the individual has been seen; $N$, if the individual has not been seen.

For example during the first period the different options are:

1. If a person was seen at the first and second census, the time at risk is the time between the first and second census, independently if the person was seen or not during the case detection. This corresponds to the first two rows in the table.

2. If a person was seen during the first census but not at the second one (and the person was seen during the case detection), the time at risk was calculated as the time between the first census and the first time that was seen during the case detection plus half of the time between when the patient was seen during the case detection and the second census. This corresponds to the third row of the table.

3. If a person was seen during the first census but not at the second one (and the person was not seen during the case detection), the time at risk was estimated as half of the time between the two censuses. This corresponds to the fourth row in the table.
5.2.5.3 Analysis of the intervention effect on malaria incidence rate, haemoglobin level and prevalence of parasitaemia and splenomegaly

To analyse the effect of intervention on each outcome, the method proposed by Bennett et al. (2002) was used.

Malaria incidence rate was estimated as the total number of new malaria cases divided by the number of years at risk and was expressed as incidence per 1000 person years at risk. Prevalence was estimated as number of persons with parasites (or a palpable spleen) divided by the total number of persons examined during each cross sectional survey. The mean haemoglobin rate was estimated as the sum of haemoglobin concentration divided by the total number of persons examined. For each outcome, an adjusted rate for each study arm was calculated, to allow for potential confounding factors such as age, sex and excluding the intervention factor, using a multiple regression model. The intervention effect was measured as the adjusted rate ratio (for the malaria incidence), risk ratio (for prevalence of parasitaemia and splenomegaly) and mean ratio (for haemoglobin level). Because the study design was randomised by village, the calculation of significance levels and confidence intervals must allow for the correlation within villages, so a paired t-test or Wilcoxon rank test for the significance of the RR was carried out, using residuals from the regression model aggregated at the village level. A 95% test-based confidence interval for the adjusted RR was also obtained from the analysis of the aggregated residuals.
The effect measures estimated for each endpoint are shown in Table 5.2, as well as the regression model used for the adjusted analysis, and the statistic used to test the null hypotheses.
Table 5.2 Effect measures estimated for each endpoint, and the regression models used for the adjusted analyses

<table>
<thead>
<tr>
<th>Outcome</th>
<th>Parameters estimated to measure the intervention effect</th>
<th>Confounder</th>
<th>Regression model</th>
<th>Statistical test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Malaria Incidence</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>All <em>Plasmodium</em> species</td>
<td>Incidence rate ratio (IRR)</td>
<td>Age, sex, pair</td>
<td>Negative binomial</td>
<td>Paired t-test on the ln of the unadjusted and adjusted rate</td>
</tr>
<tr>
<td><em>P. falciparum</em></td>
<td>Incidence rate ratio (IRR)</td>
<td>Age, sex, pair</td>
<td>Negative binomial</td>
<td>Paired t-test on the ln of the unadjusted and adjusted rate</td>
</tr>
<tr>
<td><em>P. vivax</em></td>
<td>Incidence rate ratio (IRR)</td>
<td>Age, sex, pair</td>
<td>Negative binomial</td>
<td>Wilcoxon rank test on the unadjusted and adjusted rate ratio</td>
</tr>
<tr>
<td>Haemoglobin concentration (Hb)</td>
<td>Geometric mean Hb ratio</td>
<td>Age, sex, pair, presence or absence of palpable spleen</td>
<td>Linear</td>
<td>Paired t-test on the ln of the unadjusted and adjusted rate</td>
</tr>
<tr>
<td>Parasite prevalence</td>
<td>Risk ratio (RR)</td>
<td>Age, sex, pair</td>
<td>Binomial</td>
<td>Wilcoxon rank test on the unadjusted and adjusted rate ratio</td>
</tr>
<tr>
<td>Splenomegaly prevalence</td>
<td>Risk ratio (RR)</td>
<td>Age, sex, pair</td>
<td>Binomial</td>
<td>Wilcoxon rank test on the unadjusted and adjusted rate ratio</td>
</tr>
</tbody>
</table>
5.2.5.4 Efficacy of the intervention

The incidence rate ratio (IRR) is calculated from the ratio between the malaria incidence rates in the ITHN arm and that in the PTHN arm. The risk ratio (RR) is calculated from the ratio between the parasite or splenomegaly prevalence in the ITHN arm and that in the PTHN arm. The Protective Efficacy (PE) was calculated as $PE=(1-IRR)^*100$ in the first case and as $PE=(1-RR)^*100$ in the latter case (Kirkwood and Sterne, 2003).
5.3 Results

At the start of the study, in December 1998, 429 participants received ITHNs treated with lambdacyhalothrin and 495 received PTHNs. During the next two years, from January 1999 to December 2000, 924 individuals were followed up.

5.3.1 Malaria episodes

A total number of 3,054 blood smears were collected during the intervention period, 1,691 (55%) from people living in the ITHNs villages and 1,363 (45%) from people in the PTHNs villages. The percentage of overall and species-specific slide positivity are given in Table 5.3.
Table 5.3 Overall slide positivity percentages by species and intervention group during the intervention period.

<table>
<thead>
<tr>
<th></th>
<th>ITHN</th>
<th>PTHN</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total number of persons</td>
<td>429</td>
<td>495</td>
<td>924</td>
</tr>
<tr>
<td>Total number of smears examined</td>
<td>1691</td>
<td>1363</td>
<td>3054</td>
</tr>
<tr>
<td>Percentage slide positivity</td>
<td>10.7 (181)</td>
<td>16.9 (231)</td>
<td>13.5 (412)</td>
</tr>
<tr>
<td>95% CIa</td>
<td>9.2 -12.2</td>
<td>15.0 - 18.9</td>
<td>12.3 - 14.7</td>
</tr>
<tr>
<td>Percentage positivity for P. vivax</td>
<td>5.1 (86)</td>
<td>7.0 (96)</td>
<td>6.0 (182)</td>
</tr>
<tr>
<td>95% CIa</td>
<td>4.0 - 6.1</td>
<td>5.7 - 8.4</td>
<td>5.1 - 6.8</td>
</tr>
<tr>
<td>Percentage positivity for P. falciparum</td>
<td>5.2 (88)</td>
<td>8.4 (115)</td>
<td>6.6 (203)</td>
</tr>
<tr>
<td>95% CIa</td>
<td>4.1 - 6.3</td>
<td>7.0 - 9.9</td>
<td>5.8 - 7.5</td>
</tr>
<tr>
<td>Percentage positivity for P. malariae</td>
<td>0.2 (3)</td>
<td>1.0 (13)</td>
<td>0.5 (16)</td>
</tr>
<tr>
<td>95% CIa</td>
<td>0.0 - 0.4</td>
<td>0.4 - 1.5</td>
<td>0.3 - 0.8</td>
</tr>
<tr>
<td>Percentage positivity for Pv + Pf</td>
<td>0.2 (4)</td>
<td>0.4 (7)</td>
<td>0.4 (11)</td>
</tr>
<tr>
<td>95% CIa</td>
<td>0.0 - 0.5</td>
<td>0.1 - 0.9</td>
<td>0.1 - 0.6</td>
</tr>
</tbody>
</table>

a 95% confidence intervals = $p \pm (1.96 \times SE)$. The absolute numbers of positive slides are given in parenthesis.

Of the 412 malaria positive smears, 304 (74%) were found by passive case detection and 108 (26%) by active case detection.
5.3.2 Relapses and/or recrudescence episodes

During the study 412 malaria cases were confirmed by parasite diagnosis. 182 (44%) of these episodes were identified as *P. vivax*, 203 (49%) as *P. falciparum*, 16 (4%) as *P. malariae* and 11 (3%) mixed (*P. vivax* and *P. falciparum*) infections. Following the case definition given previously (page 164 in this Chapter), 85 (21%) of these episodes were classified as relapses of *P. vivax* or recrudescence of an earlier *P. falciparum* infection. Hence, 59 (32%) of *P. vivax* cases, 22 (11%) of *P. falciparum* cases and 4 (36%) of mixed (*P. vivax* and *P. falciparum*) infections were considered recrudescence or relapse and excluded from the final analysis. No significant differences between study arms was found in the mean relapse and/or recrudescence rates using a paired *t*-test (*t*=0.82, d.f.=8, *P*=0.4) (Table 5.4).
Table 5.4 Number and percentage of relapses or recrudescences by study arm.

<table>
<thead>
<tr>
<th>Village</th>
<th>Malaria episodes</th>
<th>Relapses or recrudescences (%)</th>
<th>New malaria episodes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Motorema (Control)</td>
<td>29</td>
<td>3 (10)</td>
<td>26</td>
</tr>
<tr>
<td>Hatakao (ITHN)</td>
<td>31</td>
<td>9 (29)</td>
<td>22</td>
</tr>
<tr>
<td>Mosho (Control)</td>
<td>24</td>
<td>4 (17)</td>
<td>20</td>
</tr>
<tr>
<td>Purima (ITHN)</td>
<td>14</td>
<td>1 (7)</td>
<td>13</td>
</tr>
<tr>
<td>Warapana (Control)</td>
<td>107</td>
<td>25 (23)</td>
<td>82</td>
</tr>
<tr>
<td>Shakita (ITHN)</td>
<td>109</td>
<td>38 (35)</td>
<td>71</td>
</tr>
<tr>
<td>Piegrita (Control)</td>
<td>20</td>
<td>1 (3)</td>
<td>19</td>
</tr>
<tr>
<td>Kopariwe (ITHN)</td>
<td>17</td>
<td>2 (12)</td>
<td>15</td>
</tr>
<tr>
<td>Kashorawe (Control)</td>
<td>4</td>
<td>0 (0)</td>
<td>4</td>
</tr>
<tr>
<td>Lechoza (ITHN)</td>
<td>1</td>
<td>0 (0)</td>
<td>1</td>
</tr>
<tr>
<td>Yohoope (Control)</td>
<td>10</td>
<td>0 (0)</td>
<td>10</td>
</tr>
<tr>
<td>Iyewe-therl (ITHN)</td>
<td>4</td>
<td>0 (0)</td>
<td>4</td>
</tr>
<tr>
<td>Sta. Ma. Gualcas (Control)</td>
<td>33</td>
<td>2 (6)</td>
<td>31</td>
</tr>
<tr>
<td>Shashanawe (ITHN)</td>
<td>3</td>
<td>0 (0)</td>
<td>3</td>
</tr>
<tr>
<td>Carlitos (Control)</td>
<td>1</td>
<td>0 (0)</td>
<td>1</td>
</tr>
<tr>
<td>Clavotherl (ITHN)</td>
<td>1</td>
<td>0 (0)</td>
<td>1</td>
</tr>
<tr>
<td>Hishinowell-therl (Control)</td>
<td>3</td>
<td>0 (0)</td>
<td>3</td>
</tr>
<tr>
<td>Mario (ITHN)</td>
<td>1</td>
<td>0 (0)</td>
<td>1</td>
</tr>
<tr>
<td><strong>Overall</strong></td>
<td><strong>231</strong></td>
<td><strong>35 (6.8)</strong></td>
<td><strong>196</strong></td>
</tr>
<tr>
<td><strong>Control</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ITHN</td>
<td>181</td>
<td>50 (9.2)</td>
<td>131</td>
</tr>
</tbody>
</table>

* Insecticide Treated Hammock Nest (ITHN), control correspond to Placebo Treated Hammock Nets
b Number of individuals with malaria symptoms and positive slide
c Number of individuals with malaria considered relapses or recrudescences following the case definition

327 cases of malaria were considered as new episodes and included in the final analysis. 131 (40%) occurred in persons living in ITHNs villages and 196 (60%) in persons living in villages with PTHNs.
5.3.3 Malaria incidence rate

The malaria incidence rate was calculated per 1000 person years at risk. The mean time of observation per individuals was 698.5 days (range: 107-738 days) and 88.3% of the subjects were followed during the entire study period of 24 months. The village of Kashora, consisting of 39 people, was followed only for 380 days because at the end of 1999 the entire village decided to migrate out of the study area.

Figure 5.1 shows the malaria incidence rates, by age-group and sex, for each of the study arms. The incidence rate was substantially and significantly higher among children below 9 years old than among other age groups. Incidence rates also differed by sex and by study arm. Therefore, age group and sex were considered as confounders in the adjusted analysis.
Figure 5.1 Malaria incidence rate by age group, sex and study arm

Column shows malaria incidence rate divided by intervention group (Placebo Treated Hammock Net (PTHN) and Insecticide Treated Hammock Net group (ITHN)); age-group in years and sex. White columns correspond to female and grey columns correspond to male. Error bar indicate, 95% CI.
Figure 5.2 shows the spread of village malaria incidence rates in each intervention group. The variability of the incidence between villages and within the groups is high. In particular in the ITHNs group, there is one outlier that corresponds to the incidence rate in Shakita. It is thought that more than 80% of the people in this village during the second year of intervention failed to use the ITHNs they had been given, while in the other villages, usage rates of both PTHNs and ITHNs were very high.

Figure 5.2 Distribution of malaria incidence rate among villages, by study group

Diamonds correspond to malaria incidence rate in the villages that received Placebo Treated Hammock Nets (PTHN) and squares correspond to villages that received Insecticide Treated Hammock Nets (ITHN).
5.3.4 Effect of the intervention on malaria incidence rate

The intervention effect on malaria incidence rate was estimated first for all Plasmodium species and then for P. falciparum and P. vivax separately.

Mean cluster and overall incidence rates as well as results of the unadjusted and adjusted analyses are presented in Table 5.5 combining all species, in Table 5.6 for episodes due to P. falciparum and in Table 5.7 for P. vivax episodes. In all three cases the intervention effect was adjusted for village pair, age and sex, using a negative binomial regression model.

The mean malaria incidence was 114.6 per 1000 persons-years at risk in the ITHNs and 186.9 per 1000 persons-years at risk in the control group. The adjusted geometric mean Incidence Rate Ratio (IRR) for all Plasmodium species combined was significantly lower (IRR: 0.44, 95% CI: 0.41-0.48, \( P=0.02 \)) in the ITHNs group compared to the control group (PTHNs) (Table 5.5). The adjusted protective efficacy of ITHNs in reducing the mean malaria incidence rate was 55% (CI:52-59%), which is similar to that estimated in the unadjusted analysis.

A total number of 181 new cases of P. falciparum were found during the follow-up, representing 55% of the overall new cases. The mean P. falciparum incidence was lower in the ITHNs than in the control group, 63.8 and 106.6 per 1000 persons-years at risk respectively. Similar results to the all species incidence rate were obtained for P. falciparum. A significant difference (IRR: 0.46, 95% CI: 0.40-0.53, \( P=0.03 \))
between the ITHNs and PTHNs group was found, and the adjusted protective efficacy was 54\% (CI: 47-60\%) (Table 5.6).

To analyze the effect of the intervention on malaria due to *P. vivax*, village pairs 5, 8 and 9 were excluded from the analysis because no cases due to this species were diagnosed during the study period, in either the ITHN or control group. Also the incidence rate in pair 7 was zero, therefore it was not possible to calculate the geometric mean IRR, hence the analysis was carried out using Wilcoxon rank test on the IRR under the null hypothesis that IRR=1.

A total number of 123 cases were considered new cases of *P. vivax*, 38\% of the total number of malaria cases. The *P. vivax* incidence was lower in the ITHNs group than in the control group, 64.5 and 87.6 per 1000 persons-years at risk, respectively. However, no significant (IRR:0.62, 95\% CI: 0 – 1.72, z=-0.52, P=0.6) effect of ITHNs on the *P. vivax* malaria incidence was implied by the analysis (Table 5.7).
Table 5.5 Malaria (all species) incidence and results of the unadjusted and adjusted analysis of the intervention effect comparing Insecticide Treated Hammock Nets (ITHNs) vs. Placebo Treated Hammocks Nets (control).

<table>
<thead>
<tr>
<th>Pair</th>
<th>Village</th>
<th>n a</th>
<th>Observed episode (O)</th>
<th>Persons-years at risk b</th>
<th>Incidence rate c</th>
<th>IRR c</th>
<th>ln (IR)</th>
<th>Expected episode (E)</th>
<th>O/E</th>
<th>ln(O/E)</th>
<th>Unadjusted ln(IRR) d</th>
<th>Adjusted ln(IRR) d</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Motorema (Control)</td>
<td>50</td>
<td>26</td>
<td>89.4</td>
<td>298.8</td>
<td>0.81</td>
<td>5.67</td>
<td>26.9</td>
<td>0.97</td>
<td>-0.03</td>
<td>-0.21</td>
<td>-0.68</td>
</tr>
<tr>
<td></td>
<td>Hatakoas (ITHN)</td>
<td>51</td>
<td>22</td>
<td>92.9</td>
<td>236.9</td>
<td>0.81</td>
<td>5.47</td>
<td>24.5</td>
<td>0.90</td>
<td>-0.11</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Mosho (Control)</td>
<td>57</td>
<td>20</td>
<td>102.7</td>
<td>194.8</td>
<td>0.65</td>
<td>5.27</td>
<td>15.9</td>
<td>1.26</td>
<td>0.23</td>
<td>-0.43</td>
<td>-0.61</td>
</tr>
<tr>
<td></td>
<td>Purima (ITHN)</td>
<td>54</td>
<td>13</td>
<td>103.0</td>
<td>126.2</td>
<td>0.65</td>
<td>4.84</td>
<td>18.9</td>
<td>0.69</td>
<td>-0.38</td>
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<td>0.01</td>
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<td>-1.18</td>
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<td>0.11</td>
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<td>-1.18</td>
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<td>6</td>
<td>Vhoopee (Control)</td>
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<tr>
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<td>Iyewel-leri (ITHN)</td>
<td>38</td>
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<td>-1.06</td>
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<td>Sta. Ma. Guacmas (Control)</td>
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<td>31</td>
<td>223.1</td>
<td>139.0</td>
<td>0.39</td>
<td>4.93</td>
<td>27.0</td>
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<td>0.14</td>
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<td>-1.19</td>
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<td>53.7</td>
<td>0.39</td>
<td>3.98</td>
<td>8.6</td>
<td>0.35</td>
<td>-1.05</td>
<td>-1.18</td>
<td>-1.06</td>
</tr>
<tr>
<td>8</td>
<td>Carlitos (Control)</td>
<td>15</td>
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<td>1.34</td>
<td>3.51</td>
<td>1.2</td>
<td>0.83</td>
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<td>0.28</td>
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<tr>
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<td>0.09</td>
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<td>0.20</td>
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<td>1.91</td>
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<td>-1.65</td>
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<td></td>
<td>Mario (ITHN)</td>
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<td>2.7</td>
<td>0.37</td>
<td>-1.00</td>
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<tr>
<td>Overall</td>
<td>Control (ITHN)</td>
<td>495</td>
<td>196</td>
<td>875.3</td>
<td>186.8</td>
<td>0.61</td>
<td>5.04</td>
<td>186.2</td>
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<td>0.28</td>
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<td>-0.81</td>
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<td></td>
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<td>345.3</td>
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<td>-0.53</td>
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</tr>
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</table>

Unadjusted analysis: IRR = 0.46; 95% CI: 0.34 - 0.62; t = -2.91; P = 0.02
Adjusted analysis: IRR = 0.44; 95% CI: 0.41 - 0.48; t = -2.79; P = 0.02

a n, the study population in each village; (O) number of malaria cases observed in each village; b Time at risk expressed in years; c Incidence rate estimated as the number of observed malaria cases per 1000 person-years at risk; d IRR, Incidence rate ratio estimate as the malaria incidence rate in the ITHNs divided by the malaria incidence rate in the PTHNs group; (E) The expected number of episodes estimated from a negative binomial regression model adjusted for age, sex and pair, excluding the intervention effect. Then the expected numbers of event were aggregated by village and log transformed; e A paired t-test on the adjusted and unadjusted (ln IRR) was applied to test the null hypothesis IRR = 1, where unadjusted ln(IRR)=ln(TTHN)-ln(Tcontrol) and the adjusted ln(IRR)=ln (O/EITHN)-ln(O/Econtrol).
Table 5.6. *Plasmodium falciparum* incidence and results of the unadjusted and adjusted analysis of the intervention effect comparing Insecticide Treated Hammock Nets (ITHNs) vs. Placebo Treated Hammocks Nets (control).

<table>
<thead>
<tr>
<th>Pair</th>
<th>Village</th>
<th>n</th>
<th>Observed episode (O)</th>
<th>Persons-years at risk</th>
<th>Incidence rate</th>
<th>IRR 4</th>
<th>ln(IIR)</th>
<th>Expected episode (E)</th>
<th>O/E</th>
<th>ln(O/E)</th>
<th>Unadjusted ln(IIR) 4</th>
<th>Adjusted ln(IIR) 4</th>
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<tr>
<td>1</td>
<td>Motorema (Control)</td>
<td>50</td>
<td>14</td>
<td>94.3</td>
<td>148.5</td>
<td>0.60</td>
<td>5.00</td>
<td>11.4</td>
<td>1.22</td>
<td>0.20</td>
<td>-0.51</td>
<td>-0.49</td>
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<td>Hatako (ITHN)</td>
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<td>101.1</td>
<td>89.0</td>
<td>4.49</td>
<td>12.0</td>
<td>0.75</td>
<td>-0.29</td>
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<td>107.7</td>
<td>92.8</td>
<td>0.82</td>
<td>5.33</td>
<td>9.3</td>
<td>0.86</td>
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<td>-0.20</td>
<td>-0.31</td>
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<td>Purima (ITHN)</td>
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<td>4.33</td>
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<td>1.12</td>
<td>0.23</td>
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<tr>
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<td>Warapana (Control)</td>
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<td>43.6</td>
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<td>Shakita (ITHN)</td>
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<td>39</td>
<td>223.4</td>
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<td>5.16</td>
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<td>0.23</td>
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<tr>
<td>4</td>
<td>Piegrita (Control)</td>
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<td>4.87</td>
<td>7.5</td>
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<td>Leboza (ITHN)</td>
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<td>Yohoope (Control)</td>
<td>29</td>
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<td>4.80</td>
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<td>0.23</td>
<td>-1.47</td>
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<tr>
<td>7</td>
<td>Sta. Ma. Guacias (Control)</td>
<td>119</td>
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<td>227.4</td>
<td>96.7</td>
<td>0.56</td>
<td>4.57</td>
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<td>1.13</td>
<td>0.12</td>
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<tr>
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<td>5.4</td>
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<td>-0.60</td>
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<tr>
<td>8</td>
<td>Carlitos (Control)</td>
<td>15</td>
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<td>33.4</td>
<td>1.34</td>
<td>3.51</td>
<td>1.2</td>
<td>0.83</td>
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<td>1.17</td>
<td>0.16</td>
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<td>25.8</td>
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<td>-1.57</td>
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<td>-0.93</td>
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<tr>
<td>Overall</td>
<td>Control (ITHN)</td>
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<td>0.70</td>
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</table>

Unadjusted analysis IRR=0.46; 95% CI: 0.32 - 0.66; \( t=2.53; P=0.04 \)

Adjusted analysis IRR=0.46; 95% CI: 0.40 - 0.53; \( t=2.54; P=0.03 \)

* n, the study population in each village; (O) number of malaria cases observed in each village; \( ^a \) Time at risk expressed in years; \( ^b \) Incidence rate estimated as the number of observed malaria cases per 1000 person-years at risk; \( ^c \) IRR, Incidence rate ratio estimate as the malaria incidence rate in the ITHNs divided by the malaria incidence rate in the PTHNs group; \( ^d \) The expected number of episodes estimated from a negative binomial regression model adjusted for age, sex and pair, excluding the intervention effect. Then the expected numbers of event were aggregated by village and log transformed; \( ^e \) a paired \( t \)-test on the adjusted and unadjusted (ln IRR) was applied to test the null hypothesis IRR=1, where unadjusted ln(IWR)=ln(IWR-ITHN)-ln(IWR-Control) and the adjusted ln(IWR)=ln ((O/E)-ITHN)-ln(O/E-Control).
Table 5.7 *Plasmodium vivax* incidence and results of the unadjusted and adjusted analysis of the intervention effect comparing Insecticide Treated Hammock Nets (ITHNs) vs. Placebo Treated Hammocks Nets (control).

<table>
<thead>
<tr>
<th>Pair</th>
<th>Village</th>
<th>n</th>
<th>Observed episode (O)</th>
<th>Persons-years at risk</th>
<th>Incidence rate</th>
<th>Expected episode (E)</th>
<th>O/E</th>
<th>Unadjusted RR</th>
<th>Adjusted RR</th>
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<td>Motorema (Control)</td>
<td>50</td>
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<td>98.7</td>
<td>12.4</td>
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<td>1.72</td>
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<td>138.8</td>
<td>10.4</td>
<td>1.25</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Mosho (Control)</td>
<td>57</td>
<td>8</td>
<td>103.7</td>
<td>77.1</td>
<td>5.5</td>
<td>1.46</td>
<td>0.50</td>
<td>0.39</td>
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<tr>
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<td>7.0</td>
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<tr>
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<td>Warapana (Control)</td>
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<td>37</td>
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<td>0.88</td>
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<td>210.8</td>
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<td>29.6</td>
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<tr>
<td>4</td>
<td>Piegrita (Control)</td>
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<td>9</td>
<td>65.2</td>
<td>138.0</td>
<td>5.0</td>
<td>1.80</td>
<td>0.26</td>
<td>0.28</td>
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<tr>
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<td>Kopariwe (ITHN)</td>
<td>59</td>
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<td>111.0</td>
<td>36.0</td>
<td>8.0</td>
<td>0.50</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>Yohoope (Control)</td>
<td>29</td>
<td>2</td>
<td>57.1</td>
<td>35.0</td>
<td>2.5</td>
<td>0.82</td>
<td>1.17</td>
<td>1.37</td>
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<td>Iyewel-theri (ITHN)</td>
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<td>73.5</td>
<td>40.8</td>
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<td>Sta. Ma. Guacas (Control)</td>
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<td>226.3</td>
<td>26.5</td>
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<td>1.5</td>
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<tr>
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<td>Overall Control</td>
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<td>0.78</td>
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<td>Overall ITHN</td>
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<td>64.5</td>
<td>59.2</td>
<td>0.49</td>
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</tbody>
</table>

Unadjusted analysis IRR=0.74; 95% CI: 0 - 1.40; \( z = -1.15 \); \( P=0.24 \)

Adjusted analysis IRR=0.62; 95% CI: 0 - 1.72; \( z = -0.52 \); \( P=0.60 \)

---

*a* n, the study population in each village; (O) number of *P. vivax* cases observed in each village; *b* Time at risk expressed in years; *c* Incidence rate estimated as the number of observed *P. vivax* cases per 1000 person-years at risk; (E) The expected number of episodes estimated from a negative binomial regression model adjusted for age, sex and pair, excluding the intervention effect. Then the expected numbers of event were aggregated by village; *d* a Wilcoxon rank test on the adjusted and unadjusted IRR was applied to test the null hypothesis RR=1.
5.3.5 Results of cross-sectional surveys

Cross sectional surveys for malaria parasitaemia, haemoglobin level and splenomegaly were carried out in the study villages before the intervention (December 1998), six months after intervention (July 1999) and at the end of the study period (December 2000). The duration of each mass survey was approximately one month, starting in the villages of Ocamo locality, followed by villages in Mavaca locality.

Children less than six months were excluded from the analysis because children in this age-group often have high levels of haemoglobin for physiological reasons and also often have a palpable spleen. In the analysis of the last cross-sectional survey, two villages were excluded. Pair 5 was excluded because, at the end of 1999, an entire village (Kashora) of this pair migrated to another area, far away from the study area. Pair 8 was excluded because at the moment of the cross-sectional survey, in the village of Clavo-theri, a death occurred and all villagers attended the funeral, and it was decided not to interfere with the ritual.

702 (76%) persons from the study population were included in the analysis of the first cross-sectional survey carried out one month before the intervention (December 1998), 624 (68%) in the second (six months after intervention) and 516 (55%) in the third (two years after intervention). Table 5.8 shows the general characteristics of the study population during each cross-sectional survey, in terms of the study variables.
Details of prevalence of parasitaemia and splenomegaly, and mean haemoglobin level by village at the baseline level are shown in Table 5.9, page 186.

**Table 5.8 Coverage and general characteristics of the study population of each cross-sectional survey**

<table>
<thead>
<tr>
<th></th>
<th>Pre-intervention</th>
<th>Post-intervention</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Dec-1998</td>
<td>Jul-99</td>
</tr>
<tr>
<td>Population</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ITHNs</td>
<td>328</td>
<td>287</td>
</tr>
<tr>
<td>PTHNs</td>
<td>374</td>
<td>337</td>
</tr>
<tr>
<td>Overall</td>
<td>702</td>
<td>624</td>
</tr>
<tr>
<td>Parasite prevalence&lt;sup&gt;a&lt;/sup&gt;</td>
<td>6.0 (42/679)</td>
<td>8.5 (48/564)</td>
</tr>
<tr>
<td>Splenomegaly prevalence&lt;sup&gt;b&lt;/sup&gt;</td>
<td>12.3 (80/652)</td>
<td>12.3 (72/586)</td>
</tr>
<tr>
<td>2-9 years</td>
<td>6 (8/135)</td>
<td>2 (3/138)</td>
</tr>
<tr>
<td>Overall</td>
<td>12.3 (80/652)</td>
<td>12.3 (72/586)</td>
</tr>
<tr>
<td>Haemoglobin level (g/dl)&lt;sup&gt;c&lt;/sup&gt;</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Females</td>
<td>11.3 (1.4)</td>
<td>11.0 (1.6)</td>
</tr>
<tr>
<td>Males</td>
<td>12.4 (1.7)</td>
<td>12.0 (1.8)</td>
</tr>
<tr>
<td>Hb less than 9g/dl (%)</td>
<td>5% (33/666)</td>
<td>7% (46/618)</td>
</tr>
</tbody>
</table>

<sup>a</sup> Percentage (number of persons with malaria parasites/ number of persons examined);  
<sup>b</sup> Percentage (number of persons with palpable spleen/ number of persons examined);  
<sup>c</sup> Mean of the haemoglobin concentration (standard deviation)
Table 5.9 Mean haemoglobin concentration (g/dl), prevalence of parasitaemia and splenomegaly in the villages that were going to be ascribed to Insecticide Treated Hammock Net group (ITHNs) or Placebo Treated Hammock net group as control (baseline cross-sectional survey)

<table>
<thead>
<tr>
<th>Pair</th>
<th>Village*</th>
<th>Mean Hb (g/dl) (n)</th>
<th>Parasitaemia prevalence (n)</th>
<th>Splenomegaly prevalence (n)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Motorema (Control)</td>
<td>11.6 (15)</td>
<td>6.7 (15)</td>
<td>6.7 (15)</td>
</tr>
<tr>
<td></td>
<td>Hatakoa (ITHN)</td>
<td>12.1 (40)</td>
<td>4.7 (43)</td>
<td>17.5 (40)</td>
</tr>
<tr>
<td>2</td>
<td>Mosho (Control)</td>
<td>11.8 (49)</td>
<td>22.0 (50)</td>
<td>18.4 (49)</td>
</tr>
<tr>
<td></td>
<td>Purima (ITHN)</td>
<td>11.8 (46)</td>
<td>2.0 (49)</td>
<td>10.6 (47)</td>
</tr>
<tr>
<td>3</td>
<td>Warapana (Control)</td>
<td>11.8 (102)</td>
<td>2.9 (105)</td>
<td>18.6 (102)</td>
</tr>
<tr>
<td></td>
<td>Shakita (ITHN)</td>
<td>12.0 (61)</td>
<td>3.1 (64)</td>
<td>8.2 (61)</td>
</tr>
<tr>
<td>4</td>
<td>Piegrita (Control)</td>
<td>11.3 (29)</td>
<td>10.0 (30)</td>
<td>17.2 (29)</td>
</tr>
<tr>
<td></td>
<td>Kopariwe (ITHN)</td>
<td>11.7 (49)</td>
<td>13.5 (52)</td>
<td>19.6 (51)</td>
</tr>
<tr>
<td>5</td>
<td>Kashorawe (Control)</td>
<td>11.4 (34)</td>
<td>0 (37)</td>
<td>26.5 (34)</td>
</tr>
<tr>
<td></td>
<td>Lechoza (ITHN)</td>
<td>11.8 (41)</td>
<td>4.8 (42)</td>
<td>2.4 (41)</td>
</tr>
<tr>
<td>6</td>
<td>Yohoope (Control)</td>
<td>12.3 (23)</td>
<td>8.3 (24)</td>
<td>4.3 (23)</td>
</tr>
<tr>
<td></td>
<td>Iyewei-theri (ITHN)</td>
<td>11.7 (29)</td>
<td>9.4 (32)</td>
<td>6.9 (29)</td>
</tr>
<tr>
<td>7</td>
<td>Sta. Ma. Gusacas (Control)</td>
<td>12.5 (90)</td>
<td>3.2 (95)</td>
<td>3.3 (90)</td>
</tr>
<tr>
<td></td>
<td>Shashanawe (ITHN)</td>
<td>11.8 (21)</td>
<td>0 (21)</td>
<td>4.8 (21)</td>
</tr>
<tr>
<td>8</td>
<td>Carlitos (Control)</td>
<td>11.6 (10)</td>
<td>10.0 (10)</td>
<td>10.0 (10)</td>
</tr>
<tr>
<td></td>
<td>Clavotheri (ITHN)</td>
<td>12.4 (10)</td>
<td>10.0 (10)</td>
<td>10.0 (10)</td>
</tr>
<tr>
<td>9</td>
<td>Hishinowi-theri (Control)</td>
<td>11.7 (8)</td>
<td>0 (8)</td>
<td>0 (8)</td>
</tr>
<tr>
<td></td>
<td>Mario (ITHN)</td>
<td>11.8 (15)</td>
<td>0 (15)</td>
<td>0 (15)</td>
</tr>
<tr>
<td></td>
<td>Overall Control</td>
<td>11.8 (360)</td>
<td>7.0 (374)</td>
<td>11.7 (360)</td>
</tr>
<tr>
<td></td>
<td>ITHN</td>
<td>11.9 (312)</td>
<td>5.3 (328)</td>
<td>9.0 (315)</td>
</tr>
</tbody>
</table>

*Village
5.3.5.1 Effect of the intervention on mean haemoglobin level

Table 5.10 and Table 5.11 present the mean haemoglobin concentration and the results of the unadjusted and adjusted analysis of the second (six months after the intervention) and third cross-sectional survey (two years after intervention) respectively. Haemoglobin concentration was adjusted for age, sex, presence or absence of palpable spleen and pair using a regression model. The mean haemoglobin level, during the second cross-sectional survey was 11.6 and 11.9 g/dl in the ITHNs and control, respectively and 11.6 and 11.8 g/dl, during the third cross-sectional survey. No significant difference in the adjusted geometric mean haemoglobin concentration between study groups was found during the second (mean haemoglobin ratio: 1.01, $t=0.53$, df=8, $P=0.61$) or third cross-sectional survey (mean haemoglobin ratio: 1.03, $t=1.67$, df=6, $P=0.15$).
Table 5.10 Mean haemoglobin level (g/dl) and results of the unadjusted and adjusted analysis of the intervention effect comparing Insecticide Treated Hammock Nets (ITHNs) vs. Placebo Treated Hammocks Nets (control) after six months of intervention (second cross-sectional survey)

<table>
<thead>
<tr>
<th>Pair</th>
<th>Village</th>
<th>n</th>
<th>Observed Mean Hb (O)</th>
<th>ln(O)</th>
<th>Expected Mean Hb (E)</th>
<th>ln(E)</th>
<th>O/E</th>
<th>ln(O/E)</th>
<th>Unadjusted ln (MHR)</th>
<th>Adjusted ln (MHR)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Motorema (Control)</td>
<td>28</td>
<td>10.4</td>
<td>2.35</td>
<td>11.0</td>
<td>1.00</td>
<td>0.95</td>
<td>-0.05</td>
<td>0.13</td>
<td>0.08</td>
</tr>
<tr>
<td></td>
<td>Hatako (ITHN)</td>
<td>42</td>
<td>11.9</td>
<td>2.47</td>
<td>11.5</td>
<td>1.03</td>
<td>1.00</td>
<td>0.03</td>
<td>0.03</td>
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<td>2</td>
<td>Mosho (Control)</td>
<td>42</td>
<td>11.5</td>
<td>2.44</td>
<td>11.5</td>
<td>0.99</td>
<td>-0.01</td>
<td>-0.05</td>
<td>-0.05</td>
<td>0.01</td>
</tr>
<tr>
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<td>Purima (ITHN)</td>
<td>31</td>
<td>11.4</td>
<td>2.44</td>
<td>11.3</td>
<td>1.01</td>
<td>0.01</td>
<td>-0.05</td>
<td>-0.05</td>
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<tr>
<td>3</td>
<td>Warapana (Control)</td>
<td>88</td>
<td>11.2</td>
<td>2.42</td>
<td>10.9</td>
<td>0.96</td>
<td>-0.04</td>
<td>-0.05</td>
<td>-0.05</td>
<td>-0.07</td>
</tr>
<tr>
<td></td>
<td>Shakita (ITHN)</td>
<td>70</td>
<td>10.7</td>
<td>2.37</td>
<td>11.1</td>
<td>0.97</td>
<td>-0.02</td>
<td>-0.01</td>
<td>-0.02</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>Plegrita (Control)</td>
<td>23</td>
<td>11.6</td>
<td>2.46</td>
<td>11.4</td>
<td>1.02</td>
<td>0.02</td>
<td>0.02</td>
<td>0.02</td>
<td>0.00</td>
</tr>
<tr>
<td></td>
<td>Kopariwe (ITHN)</td>
<td>44</td>
<td>11.6</td>
<td>2.45</td>
<td>11.7</td>
<td>0.99</td>
<td>-0.01</td>
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<td>-0.01</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>Kashorawe (Control)</td>
<td>29</td>
<td>11.7</td>
<td>2.46</td>
<td>11.7</td>
<td>1.00</td>
<td>0.00</td>
<td>0.02</td>
<td>0.02</td>
<td>0.00</td>
</tr>
<tr>
<td></td>
<td>Lechoza (ITHN)</td>
<td>25</td>
<td>12.0</td>
<td>2.48</td>
<td>12.0</td>
<td>1.00</td>
<td>0.00</td>
<td>0.05</td>
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</tr>
<tr>
<td>6</td>
<td>Yoboope (Control)</td>
<td>24</td>
<td>11.5</td>
<td>2.44</td>
<td>11.8</td>
<td>0.97</td>
<td>-0.03</td>
<td>-0.03</td>
<td>-0.03</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Iyewel-theri (ITHN)</td>
<td>28</td>
<td>12.1</td>
<td>2.49</td>
<td>11.8</td>
<td>1.02</td>
<td>0.02</td>
<td>0.05</td>
<td>0.05</td>
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</tr>
<tr>
<td>7</td>
<td>Sta. Ma. Guacas (Control)</td>
<td>70</td>
<td>11.7</td>
<td>2.46</td>
<td>11.9</td>
<td>0.98</td>
<td>-0.02</td>
<td>-0.03</td>
<td>-0.03</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Shashanawe (ITHN)</td>
<td>12</td>
<td>13.3</td>
<td>2.59</td>
<td>12.2</td>
<td>1.09</td>
<td>0.09</td>
<td>-0.03</td>
<td>-0.03</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>Carlitos (Control)</td>
<td>13</td>
<td>12.9</td>
<td>2.55</td>
<td>12.5</td>
<td>1.03</td>
<td>0.03</td>
<td>0.05</td>
<td>0.05</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Clavotheri (ITHN)</td>
<td>7</td>
<td>12.5</td>
<td>2.52</td>
<td>13.1</td>
<td>0.95</td>
<td>-0.05</td>
<td>-0.05</td>
<td>-0.05</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>Hishinowel-theri (Control)</td>
<td>10</td>
<td>11.7</td>
<td>2.46</td>
<td>11.9</td>
<td>0.98</td>
<td>-0.02</td>
<td>-0.03</td>
<td>-0.03</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Mario (ITHN)</td>
<td>14</td>
<td>12.4</td>
<td>2.52</td>
<td>12.2</td>
<td>1.01</td>
<td>0.01</td>
<td>0.01</td>
<td>0.01</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Overall Control</td>
<td>327</td>
<td>11.6</td>
<td>2.45</td>
<td>11.6</td>
<td>1.00</td>
<td>0.00</td>
<td>0.04</td>
<td>0.03</td>
<td>0.01</td>
</tr>
<tr>
<td></td>
<td>ITTN</td>
<td>273</td>
<td>12.0</td>
<td>2.48</td>
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<td>1.01</td>
<td>0.01</td>
<td>0.01</td>
<td>0.01</td>
<td></td>
</tr>
</tbody>
</table>

Unadjusted analysis: Mean haemoglobin ratio=1.03; 95% CI: 1.02 - 1.04; t=1.49; P=0.17

Adjusted analysis: Mean haemoglobin ratio=1.01; 95% CI: 1.00 - 1.02; t=0.23; P=0.81

* n, number of persons examined during the cross-sectional survey; (O) Haemoglobin mean observed in each village; (E) The expected haemoglobin mean estimated from a regression model adjusted for age, sex, present or absent of palpable spleen and pair, excluding the intervention effect. Then the expected mean haemoglobin level by village were log transformed; 4 * a paired t-test on the adjusted and unadjusted ln of the mean haemoglobin ratio was applied to test the null hypothesis RR=1, where unadjusted ln RR=ln (O/ITHN)-ln(O/control) and the adjusted ln(RR)=ln (O/EITHN-O/Econtrol).
### Table 5.11 Mean haemoglobin level (g/dl) and results of the unadjusted and adjusted analysis of the intervention effect comparing Insecticide Treated Hammock Nets (ITHNs) vs. Placebo Treated Hammocks Nets (control) after two years of intervention (third cross sectional survey)

<table>
<thead>
<tr>
<th>Pair</th>
<th>Village</th>
<th>n&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Observed Mean Hb (O)</th>
<th>ln(O)</th>
<th>Expected Mean Hb (E)</th>
<th>O/E</th>
<th>ln(O/E)</th>
<th>Unadjusted ln (MHR) &lt;sup&gt;d&lt;/sup&gt;</th>
<th>Adjusted ln (MHR) &lt;sup&gt;e&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Motorema (Control)</td>
<td>28</td>
<td>10.7</td>
<td>2.37</td>
<td>10.8</td>
<td>0.99</td>
<td>-0.01</td>
<td>0.07</td>
<td>0.02</td>
</tr>
<tr>
<td></td>
<td>Hatako (ITHN)</td>
<td>37</td>
<td>11.4</td>
<td>2.43</td>
<td>11.3</td>
<td>1.01</td>
<td>0.01</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Mosho (Control)</td>
<td>37</td>
<td>10.9</td>
<td>2.39</td>
<td>11.1</td>
<td>0.98</td>
<td>-0.02</td>
<td>0.03</td>
<td>0.04</td>
</tr>
<tr>
<td></td>
<td>Purina (ITHN)</td>
<td>39</td>
<td>11.2</td>
<td>2.41</td>
<td>11.0</td>
<td>1.02</td>
<td>0.02</td>
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<td></td>
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<tr>
<td>3</td>
<td>Warapana (Control)</td>
<td>79</td>
<td>11.6</td>
<td>2.45</td>
<td>11.4</td>
<td>1.01</td>
<td>0.01</td>
<td>-0.02</td>
<td>-0.04</td>
</tr>
<tr>
<td></td>
<td>Shakita (ITHN)</td>
<td>40</td>
<td>11.4</td>
<td>2.43</td>
<td>11.7</td>
<td>0.97</td>
<td>-0.03</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>Piegrita (Control)</td>
<td>28</td>
<td>11.3</td>
<td>2.42</td>
<td>11.6</td>
<td>0.97</td>
<td>-0.03</td>
<td>0.06</td>
<td>0.05</td>
</tr>
<tr>
<td></td>
<td>Kopariwe (ITHN)</td>
<td>44</td>
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<td>2.48</td>
<td>11.8</td>
<td>1.02</td>
<td>0.02</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>Yehoope (Control)</td>
<td>21</td>
<td>11.5</td>
<td>2.44</td>
<td>11.9</td>
<td>0.97</td>
<td>-0.04</td>
<td>0.07</td>
<td>0.06</td>
</tr>
<tr>
<td></td>
<td>Iywei-theri (ITHN)</td>
<td>31</td>
<td>12.3</td>
<td>2.51</td>
<td>12.0</td>
<td>1.02</td>
<td>0.02</td>
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<td></td>
</tr>
<tr>
<td>7</td>
<td>Sta. Ma. Gualcas (Control)</td>
<td>75</td>
<td>12.4</td>
<td>2.52</td>
<td>12.4</td>
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<td>0.00</td>
<td>-0.02</td>
<td>-0.02</td>
</tr>
<tr>
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<td>Shashanawe (ITHN)</td>
<td>15</td>
<td>12.1</td>
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<td>12.3</td>
<td>0.98</td>
<td>-0.02</td>
<td></td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>Hishinowel-theri (Control)</td>
<td>10</td>
<td>11.6</td>
<td>2.45</td>
<td>12.2</td>
<td>0.95</td>
<td>-0.05</td>
<td>0.10</td>
<td>0.08</td>
</tr>
<tr>
<td></td>
<td>Mario (ITHN)</td>
<td>15</td>
<td>12.9</td>
<td>2.56</td>
<td>12.5</td>
<td>1.03</td>
<td>0.03</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Overall</strong></td>
<td><strong>Control</strong></td>
<td>278</td>
<td>11.4</td>
<td>2.4</td>
<td>11.6</td>
<td>0.76</td>
<td>-0.02</td>
<td>0.04</td>
<td>0.03</td>
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<td></td>
<td><strong>ITHN</strong></td>
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<td>11.9</td>
<td>2.5</td>
<td>11.8</td>
<td>0.78</td>
<td>0.01</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Unadjusted analysis**
Mean haemoglobin ratio = 1.041; 95% CI: 1.038 - 1.044; **t** = 2.25; **P** = 0.07

**Adjusted analysis**
Mean haemoglobin ratio = 1.027; 95% CI: 1.027 - 1.027; **t** = 1.67; **P** = 0.15

<sup>a</sup> n, number of persons examined during the cross-sectional survey; (O) Haemoglobin mean observed in each village; (E) The expected haemoglobin mean estimated from a regression model adjusted for age, sex, present or absent of palpable spleen and pair, excluding the intervention effect. Then the expected mean haemoglobin level by village were log transformed; <sup>d</sup> a paired **t**-test on the adjusted and unadjusted ln of the mean haemoglobin ratio was applied to test the null hypothesis RR=1, where unadjusted lnRR= ln(R<sub>ITHN</sub>) - ln(R<sub>control</sub>) and the adjusted ln(RR)= ln (O/E<sub>ITHN</sub>) - ln(O/E<sub>control</sub>).
5.3.5.2 Effect of the intervention on parasite prevalence

During the second cross-sectional study, carried out during the highest transmission season, the total number of parasite positive was 42 (6%) persons. Equal proportions (39.5%) of *P. vivax* and *P. falciparum* were found. *P. malariae* represented 17% of the parasite positive slides and mixed infection, *P. vivax* and *P. malariae*, represented 4%. The parasite prevalence in the ITHNs group was lower in the ITHNs than in the control group (2.8 and 10.4% respectively). A significant difference was found in prevalence of parasitaemia between study arms (relative risk: 0.17, 95% CI: 0–0.53, \( z=-2.55, P=0.01 \)). The mean reduction in malaria cases amounted to 83% (95% CI: 47-100%) (Table 5.12). The third cross-sectional survey was not analysed because only seven out of 516 people had a positive slide.
Table 5.12 Parasite prevalence and results of the unadjusted and adjusted analysis of the intervention effect comparing Insecticide Treated Hammock Nets (ITHNs) vs. Placebo Treated Hammocks Nets (control) after six months of intervention (second cross-sectional survey)

<table>
<thead>
<tr>
<th>Pair</th>
<th>Village</th>
<th>n</th>
<th>Number of positive slides (O)</th>
<th>Parasitaemia prevalence</th>
<th>Expected parasitaemia (E)</th>
<th>O/E</th>
<th>Unadjusted RR</th>
<th>Adjusted RR</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Motorema (Control)</td>
<td>28</td>
<td>4</td>
<td>14.3</td>
<td>2.2</td>
<td>1.78</td>
<td>0.33</td>
<td>0.30</td>
</tr>
<tr>
<td></td>
<td>Hatakos (ITHN)</td>
<td>42</td>
<td>2</td>
<td>4.8</td>
<td>3.8</td>
<td>0.53</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Mosho (Control)</td>
<td>44</td>
<td>4</td>
<td>9.1</td>
<td>2.9</td>
<td>1.40</td>
<td>0.31</td>
<td>0.33</td>
</tr>
<tr>
<td></td>
<td>Purima (ITHN)</td>
<td>35</td>
<td>1</td>
<td>2.9</td>
<td>2.1</td>
<td>0.47</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Warapana (Control)</td>
<td>90</td>
<td>13</td>
<td>14.4</td>
<td>12.1</td>
<td>1.08</td>
<td>0.85</td>
<td>0.84</td>
</tr>
<tr>
<td></td>
<td>Shakita (ITHN)</td>
<td>73</td>
<td>9</td>
<td>12.3</td>
<td>9.9</td>
<td>0.91</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>Piegira (Control)</td>
<td>23</td>
<td>2</td>
<td>8.7</td>
<td>0.9</td>
<td>2.18</td>
<td>0.24</td>
<td>0.22</td>
</tr>
<tr>
<td></td>
<td>Kopariwe (ITHN)</td>
<td>47</td>
<td>1</td>
<td>2.1</td>
<td>2.1</td>
<td>0.48</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>Yehoope (Control)</td>
<td>24</td>
<td>2</td>
<td>8.3</td>
<td>0.8</td>
<td>2.36</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Isewet-theri (ITHN)</td>
<td>29</td>
<td>0</td>
<td>0</td>
<td>1.2</td>
<td>0.00</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>Sta. Ma. Gualcas (Control)</td>
<td>73</td>
<td>8</td>
<td>11.0</td>
<td>6.7</td>
<td>1.19</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Shashanawe (ITHN)</td>
<td>13</td>
<td>0</td>
<td>0</td>
<td>1.3</td>
<td>0.00</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>Carlitos (Control)</td>
<td>13</td>
<td>1</td>
<td>7.7</td>
<td>0.6</td>
<td>1.61</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Clavotheri (ITHN)</td>
<td>8</td>
<td>0</td>
<td>0</td>
<td>0.4</td>
<td>0.00</td>
<td></td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>Hisbonew-theri (Control)</td>
<td>10</td>
<td>1</td>
<td>10.0</td>
<td>0.4</td>
<td>2.39</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Mario (ITHN)</td>
<td>15</td>
<td>0</td>
<td>0</td>
<td>0.6</td>
<td>0.00</td>
<td></td>
<td></td>
</tr>
<tr>
<td>9</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Overall</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Unadjusted</td>
<td>Adjusted</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>305</td>
<td>35</td>
<td>10.4</td>
<td>26.7</td>
<td>1.75</td>
<td>RR=0.26</td>
<td>RR=0.17</td>
</tr>
<tr>
<td></td>
<td>ITHN</td>
<td>262</td>
<td>13</td>
<td>2.8</td>
<td>21.3</td>
<td>0.30</td>
<td>RR=0.53</td>
<td></td>
</tr>
</tbody>
</table>

Unadjusted analysis  RR=0.26; 95% CI: 0 - 0.55; z=2.55; P=0.01
Adjusted analysis RR=0.17; 95% CI: 0 - 0.53; z=2.55; P=0.01

a n, the study population in each village; (O) number of positive slides in each village; b Parasite prevalence, number of malaria positive slide/number of slides examined per 100; (E) The expected number of episodes estimated from a logistic regression model adjusted for age, sex and pair, excluding the intervention effect. Then the expected numbers of event were aggregated by village; c a Wilcoxon rank test on the adjusted and unadjusted RR was applied to test the null hypothesis RR=1
5.3.5.3 Effect of the intervention on splenomegaly prevalence

Table 5.13 and 5.14 shows the mean prevalence of splenomegaly by village at each cross-sectional survey. In general the prevalence was low in all cross-sectional surveys. In the second cross-sectional survey pair 8 was excluded from the analysis because no palpable spleen was found in either study arm. No significant differences were found between study arms in any of the two post-intervention surveys, as analysed using a rank test on the adjusted ratio between ITHNs and PTHNs groups. However, the splenomegaly prevalence was lower in the ITHN villages than in the control group at baseline level as well as during the second and third cross-sectional surveys.
Table 5.13 Mean splenomegaly prevalence and results of the unadjusted and adjusted analysis of the intervention effect comparing Insecticide Treated Hammock Nets (ITHNs) vs. Placebo Treated Hammocks Nets (control) after six months of intervention (second cross-sectional survey)

<table>
<thead>
<tr>
<th>Pair</th>
<th>Village</th>
<th>n*</th>
<th>Observed splenomegaly (O)</th>
<th>Splenomegaly prevalence b</th>
<th>Expected splenomegaly (E)</th>
<th>O/E</th>
<th>Unadjusted RR c</th>
<th>Adjusted RR d</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Motorema (Control)</td>
<td>28</td>
<td>5</td>
<td>17.9</td>
<td>3.6</td>
<td>1.40</td>
<td>0.80</td>
<td>0.58</td>
</tr>
<tr>
<td></td>
<td>Hatako (ITHN)</td>
<td>42</td>
<td>6</td>
<td>14.3</td>
<td>7.4</td>
<td>0.81</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Mosho (Control)</td>
<td>42</td>
<td>4</td>
<td>9.5</td>
<td>3.4</td>
<td>1.18</td>
<td>0.66</td>
<td>0.65</td>
</tr>
<tr>
<td></td>
<td>Purima (ITHN)</td>
<td>32</td>
<td>2</td>
<td>6.3</td>
<td>2.6</td>
<td>0.77</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Warapana (Control)</td>
<td>89</td>
<td>14</td>
<td>15.7</td>
<td>11.8</td>
<td>1.18</td>
<td>0.63</td>
<td>0.65</td>
</tr>
<tr>
<td></td>
<td>Shakia (ITHN)</td>
<td>71</td>
<td>7</td>
<td>9.9</td>
<td>9.2</td>
<td>0.76</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>Piegrita (Control)</td>
<td>23</td>
<td>1</td>
<td>4.3</td>
<td>1.6</td>
<td>0.63</td>
<td>2.04</td>
<td>1.86</td>
</tr>
<tr>
<td></td>
<td>Kopariwe (ITHN)</td>
<td>45</td>
<td>4</td>
<td>8.9</td>
<td>3.4</td>
<td>1.17</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>Kashorawe (Control)</td>
<td>29</td>
<td>7</td>
<td>24.1</td>
<td>5.1</td>
<td>1.38</td>
<td>0.33</td>
<td>0.37</td>
</tr>
<tr>
<td></td>
<td>Lechoza (ITHN)</td>
<td>25</td>
<td>2</td>
<td>8.0</td>
<td>3.9</td>
<td>0.51</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>Yohoope (Control)</td>
<td>24</td>
<td>2</td>
<td>8.3</td>
<td>3.1</td>
<td>0.65</td>
<td>2.14</td>
<td>1.97</td>
</tr>
<tr>
<td></td>
<td>Iyewei-theri (ITHN)</td>
<td>28</td>
<td>5</td>
<td>17.9</td>
<td>3.9</td>
<td>1.28</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>Sta. Ma. Guacas (Control)</td>
<td>71</td>
<td>10</td>
<td>14.1</td>
<td>8.5</td>
<td>1.17</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Shashawae (ITHN)</td>
<td>12</td>
<td>0</td>
<td>0</td>
<td>1.5</td>
<td>0.00</td>
<td></td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>Hihlaowel-theri (Control)</td>
<td>10</td>
<td>2</td>
<td>20.0</td>
<td>1.3</td>
<td>1.55</td>
<td>0.33</td>
<td>0.38</td>
</tr>
<tr>
<td></td>
<td>Mario (ITHN)</td>
<td>15</td>
<td>1</td>
<td>6.7</td>
<td>1.7</td>
<td>0.58</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Overall</td>
<td>316</td>
<td>45</td>
<td>14.3</td>
<td>38.4</td>
<td>1.14</td>
<td>0.63</td>
<td>0.64</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>270</td>
<td>27</td>
<td>9.0</td>
<td>33.6</td>
<td>0.74</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>ITHN</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Unadjusted analysis  RR=0.63; 95% CI: 0.31 - 1.47; z = -0.42; P = 0.67  
Adjusted analysis  RR=0.64; 95% CI: 0.29 - 1.31; z = -0.70; P = 0.48

* n, the study population in each village; (O) number persons with palpable spleen in each village;  
  b Splenomegaly prevalence, number with palpable spleen/number of examined persons per 100; (E) The expected number of episodes estimated from a logistic regression model adjusted for age, sex and pair, excluding the intervention effect. Then the expected numbers of event were aggregated by village; 
  c, d a Wilcoxon rank test on the adjusted and unadjusted IRR was applied to test the null hypothesis RR=1
Table 5.14 Mean splenomegaly prevalence and results of the unadjusted and adjusted analysis of the intervention effect comparing Insecticide Treated Hammock Nets (ITHNs) vs. Placebo Treated Hammocks Nets (control) after two years of intervention (third cross-sectional survey)

<table>
<thead>
<tr>
<th>Pair</th>
<th>Village</th>
<th>n</th>
<th>Observed splenomegaly (O)</th>
<th>Splenomegaly prevalence</th>
<th>Expected splenomegaly (E)</th>
<th>O/E</th>
<th>Unadjusted RR</th>
<th>Adjusted RR</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Motorema (Control)</td>
<td>29</td>
<td>0</td>
<td>0</td>
<td>1.3</td>
<td>0</td>
<td>∞</td>
<td>∞</td>
</tr>
<tr>
<td></td>
<td>Hatako (ITHN)</td>
<td>37</td>
<td>4</td>
<td>10.8</td>
<td>2.7</td>
<td>1.49</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Mosho (Control)</td>
<td>37</td>
<td>5</td>
<td>13.5</td>
<td>2.9</td>
<td>1.71</td>
<td>0.19</td>
<td>0.19</td>
</tr>
<tr>
<td></td>
<td>Purima (ITHN)</td>
<td>39</td>
<td>1</td>
<td>2.6</td>
<td>3.1</td>
<td>0.33</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Warapana (Control)</td>
<td>79</td>
<td>4</td>
<td>5.1</td>
<td>4.2</td>
<td>0.95</td>
<td>1.48</td>
<td>1.13</td>
</tr>
<tr>
<td></td>
<td>Shakita (ITHN)</td>
<td>40</td>
<td>3</td>
<td>7.5</td>
<td>2.8</td>
<td>1.07</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>Piegrita (Control)</td>
<td>28</td>
<td>1</td>
<td>3.6</td>
<td>0.8</td>
<td>1.32</td>
<td>0.62</td>
<td>0.61</td>
</tr>
<tr>
<td></td>
<td>Kopariwe (ITHN)</td>
<td>45</td>
<td>1</td>
<td>2.2</td>
<td>1.2</td>
<td>0.80</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>Yohoope (Control)</td>
<td>21</td>
<td>3</td>
<td>14.3</td>
<td>2.9</td>
<td>1.05</td>
<td>0.90</td>
<td>0.92</td>
</tr>
<tr>
<td></td>
<td>Iyewi-theri (ITHN)</td>
<td>31</td>
<td>4</td>
<td>12.9</td>
<td>4.1</td>
<td>0.96</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>Sta. Ma. Gualcas (Control)</td>
<td>77</td>
<td>6</td>
<td>7.8</td>
<td>4.9</td>
<td>1.21</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Shashanawe (ITHN)</td>
<td>15</td>
<td>0</td>
<td>0</td>
<td>1.1</td>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>Hlishinovel-theri (Control)</td>
<td>10</td>
<td>2</td>
<td>20.0</td>
<td>1.4</td>
<td>1.45</td>
<td>0.31</td>
<td>0.42</td>
</tr>
<tr>
<td></td>
<td>Mario (ITHN)</td>
<td>16</td>
<td>1</td>
<td>6.3</td>
<td>1.6</td>
<td>0.62</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td><strong>Overall</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td><strong>Unadjusted</strong></td>
<td><strong>Adjusted</strong></td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>281</td>
<td>21</td>
<td>9.2</td>
<td>18.4</td>
<td>1.10</td>
<td>0.66</td>
<td>0.69</td>
</tr>
<tr>
<td></td>
<td>ITHN</td>
<td>223</td>
<td>14</td>
<td>6.0</td>
<td>16.6</td>
<td>0.75</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Unadjusted analysis: RR = 0.66; z = -0.68; P = 0.49
Adjusted analysis: RR = 0.69; z = -0.84; P = 0.39

* n, the study population in each village; (O) number persons with palpable spleen in each village; b Splenomegaly prevalence, number with palpable spleen/number of examined persons per 100; (E) The expected number of episodes estimated from a logistic regression model adjusted for age, sex and pair, excluding the intervention effect. Then the expected numbers of event were aggregated by village; cd a Wilcoxon rank test on the adjusted and unadjusted IRR was applied to test the null hypothesis RR = 1, e it is not possible to estimate 95% CI of the RR owing to the infinite value of pair 1.
5.4 Discussion

The epidemiological results presented in this chapter give clear evidence that ITHNs had a statistically significant effect in reducing malaria incidence rate, compared with PTHNs, when considering all species together and also considering \textit{P. falciparum} alone. The estimated overall protective efficacy (all species) was 55\%. A reduction was also found in the incidence rate of malaria caused by \textit{P. vivax}, although this was not statistically significant. The cross-sectional surveys showed that ITHNs had significant impact on parasite prevalence during the high transmission seasons. It was not possible to show any impact of ITHNs on splenomegaly prevalence or mean haemoglobin level, probably because splenomegaly prevalence was low and the mean haemoglobin level was high in the study area.

The observed reduction in the overall incidence rate of malaria is consistent with the overall reduction in the parasitaemia prevalence shown by the cross-sectional study. The lack of significance of the impact of ITHNs on \textit{P. vivax} incidence could be explained by the small sample size obtained when only this species is taken into account.

As was highlighted in the introduction of this chapter, recrudescences and relapses can complicate estimates of the effect of interventions on the malaria incidence. This issue was addressed by adopting a conservative case definition, which minimised the chance of including relapses or recrudescences as new cases.
Initially, it was considered important to assess the effect of the intervention on the prevalence of splenomegaly and haemoglobin level, because data available from previous studies, carried out in the same villages, had shown consistently high prevalence of splenomegaly and low levels of haemoglobin (Torres et al., 1988; Marcano, 1991; Villegas, 1997; Perez-Mato, 1998). Details of these studies are presented in Chapter 1, page 39. In contrast, when the first cross-sectional survey of this study was carried out in December 1998, the mean haemoglobin level of the population was found to be considerably higher, and the overall splenomegaly prevalence rate lower, compared to these past studies. The absence of a detectable effect of the intervention on splenomegaly and anaemia was probably due to low initial levels of these parameters. This apparent general decline in splenomegaly and anaemia could be due to different factors. First — as was mentioned in Chapter 1, page 39 — the improvement in the malaria control programme at the local level may have had an impact. Secondly, in 1998 the incidence reported in the area was lower than previous years (see Chapter 1, page 39), a prolonged drought spell between October 1997 to December 1998 caused forest fires near the villages resulting in smoky air, and known breeding sites had dried up. More generally, it could be that the decline represents part of a consistent and secular trend toward lower levels of malaria endemicity (and/or lower levels of the other causes of splenomegaly and anaemia). Alternatively, it could be that the decline was temporary, reflecting year-to-year fluctuations in malaria transmission.
A protective efficacy on malaria incidence similar to the one estimated in the present study has been reported in other trials carried out in South America. The overall result in a large study that included multiple study areas in Colombia, Ecuador and Peru, with a range of different epidemiological conditions, implied that ITNs significantly reduced the month malaria incidence rate\(^8\) over four months by 40% compared to untreated nets (Kroeger et al., 1995). However when considering the results reported in that study separately by area, it is notable that significant differences between the study arms were found only in the Pacific Coast of Colombia and not on the north coast of Ecuador and Peru and on the Amazon region of Peru. In the Colombia Pacific coast study area, the most prevalent parasite was \textit{P. falciparum} (69%) and the most frequent mosquito caught in HBCs was \textit{An. neivai}, which in this particular place has a nocturnal biting pattern. In the rest of the study areas involved in this work, the most prevalent parasite was \textit{P. vivax} and the main vectors (or at least the predominant human-biting anophelines) were \textit{An. evanse} in the Amazon region of Peru and \textit{An. albimanus} in the coast of Peru and Ecuador, which has a biting peak in the early evening. In these areas, no significant differences between treated and untreated nets were found.

Another study was carried out in Nicaragua, where there is a high prevalence of \textit{P. vivax} (99%), the protective efficacy of ITNs varied according to the coverage achieved: the protective efficacy was 68% in communities with an average ITNs

\(^8\) The four months malaria incidence rate was measured based on self-diagnosed cases of malaria reported to researchers.
coverage of 50%; 31% in communities with an ITNs coverage of 16-30%; and no significant protective efficacy in communities with ITN coverage below 16%. In the latter study, the author pointed out that the observed protective impact on malaria could be related to the fact that in this part of Nicaragua, An. albimanus tends to bite more often indoors, and later in the evening, compared to others areas of Latin America (Kroeger et al., 1999).

The studies by Kroeger et al. (1995, 1999) on ITNs against malaria transmitted by An. albimanus have some methodological limitations. For instance for the estimation of the malaria incidence they relied on resident's reports of self-diagnosed cases, recalled over the last four months which could be inaccurate.

Richards et al. (1993) in Guatemala, where the prevalent parasite was P. vivax (90%) and the main vector is An. albimanus, found significant difference in the malaria incidence (measured by careful case detection with exclusion of possible recrudescence/relapse) between communities with untreated and treated nets and those without nets. Although it was not possible to show any benefit from the treatment given the limited power of the study. They concluded that both treated and untreated nets might have been reducing exposure.

The present study also adds information to the limited data available on the impact of ITNs on prevalence of parasitaemia in areas of low and moderate transmission. The observed 83% reduction in parasite prevalence found in the present study is similar to
that reported in Pakistan, where the transmission is considered low (less than 1 infective bite per year) and the prevalent parasite is *P. vivax* (77%) (Rowland *et al.*, 1996). In that study it was reported that ITNs with permethrin 500mg/m² significantly reduced the prevalence of *P. falciparum* infection in the ITNs group compared to the control group (no nets), giving a protective efficacy of 43% (RR:0.57, 95% CI 14-63%) (*P*=0.009). However the prevalence of *P. vivax* infection was not significantly different between the ITNs and control groups (*P*=0.633). The authors suggested that one factor contributing to this was that the prevalence survey was carried out three months after the peak transmission season of *P. vivax* (Rowland *et al.*, 1996). In India, Misra (1999) compared the effect on malaria of ITNs and residual spraying with a control group with no nets. ITNs reduced the prevalence of parasitaemia by 61% in a cross-sectional survey in the high transmission season.

A reduction of 33% in parasite prevalence was reported in areas of high transmission as in Tanzania (Maxwell *et al.*, 2002). In areas of stable malaria a reduction of 8% was found when the control group had no nets (such as Burkina Faso, Cameroon, Ghana, Kenya) and 10% when the control group had untreated nets (such as in Gambia and Thailand) (Lengeler, 2003).

Clear evidence of the effect of ITNs on haemoglobin level and prevalence of splenomegaly has not been collected in previous trials carried out in Central and South America. Regarding haemoglobin level, similar results were reported in a randomised controlled study carried out in Pakistan, in an area of low transmission
(Rowland et al., 1996). In that trial, as in the present one, baseline anaemia was very low, and there was no evidence for a reduction due to ITNs.

In general, in the reviewed literature, it was found that the impact of ITNs is substantially higher when the control group had no nets compared to trials of ITNs versus untreated nets. For instance, in areas with stable malaria (defined as EIR more than 1 infective bite per person per year), an overall protective efficacy of 46% on the incidence of mild malaria episodes was found when the control group did not have nets, while a protective efficacy of 35% was found when the controls had untreated nets. In areas with low transmission (defined as EIR less than 1 infective bite per person per year <1), the protective efficacy on the incidence of *P. falciparum* cases was 60% compared with a protective efficacy of 33% when the control group had untreated nets (Lengeler, 2003).

Regarding haemoglobin level, in studies carried out in Africa in malaria holoendemic areas, higher differences between study arms were found when the control group did not have nets at all, while when the control group had untreated nets, the differences were smaller. Lengeler in his systematic review of randomised studies (2003) included six studies: three trials where ITNs were compared to no nets (Marbiah et al., 1998; Habluetzel et al., 1999) (unpublished results of Binka in Ghana, see in Lengeler (2003) and three studies which compared ITNs with untreated nets (Snow et al., 1987, 1988; D'Alessandro et al., 1995b). The results indicate that the PCV of children in the treated nets group was higher by 1.4 absolute PCV percent compared
to children not using nets at all. When the control group used untreated nets, the differences were smaller, and amounted to 0.4 absolute PCV percent.

Concerning the splenomegaly prevalence, bigger effects were also found in areas of higher transmission and when the control group had no nets. Lengeler's review showed that the prevalence of splenomegaly was only 2% lower overall in users of treated net when compared to users of untreated nets, while an overall reduction of 30% was found when the comparison was between users of treated nets and a control group that did not use nets at all (Snow et al., 1987, 1988; D'Alessandro et al., 1995b; Moyou-Somo et al., 1995).

During the present study it was decided to compare ITNs with untreated nets because it was considered potentially unethical to have a control group with no nets. It was observed that the majority of Yanomami, except those from Shakita village, showed a high acceptance of the nets, as well as conscientious use and good care of them. They put sticks or stones on the bottom of the hammock to keep the nets far from the skin, therefore reducing contact with the body.

It is possible that the placebo treated nets could have been an effective barrier between humans and mosquitoes and hence have had an impact on malaria incidence and on the other parameters tested. This possibility — that the untreated nets also reduced malaria, to a lesser degree than the treated ones — has a important public health implications, especially for very remote areas, where transmission is relatively
intense and where access to both diagnosis and treatment, and to insecticide for re-
treatment, is very poor. This possible effect of untreated nets, and its implications, is
discussed in more detail in the next and final chapter.

5.4.1 Conclusions

This study showed that an intervention with ITHNs, in an area where the main vector
is An. darlingi with a nocturnal biting pattern, caused a significant and substantial
reduction in the incidence of malaria cases due to P. falciparum, compared with
PTHNs. Incidence of P. vivax malaria was also lower in the ITHN group, but the
difference was not significant. There was also a significant reduction (85%) in the
prevalence of parasitaemia (all species) but the prevalence of anaemia and
splenomegaly was low in both groups, and there was no significant difference
between them.
CHAPTER 6. General discussion and conclusions

Since the start of the malaria eradication campaign in 1946, the malaria control programme in Venezuela has been based on a standardized set of control methods: early diagnosis and treatment of cases, regular Indoor Residual Insecticide Spraying (IRS) and fogging during outbreaks. The same methods have been applied in the Amazon region, but experience has shown that they do not yield the expected results and cannot be regularly and reliably delivered where the population is scattered, where some ethnic groups have semi-nomadic habits or where the houses do not have solid walls, as is the case of the Yanomami people and other ethnic groups in the tropical rain forest.

The aim of the present study was to evaluate the efficacy of ITHNs as a mean of malaria control in the context of the forest environment and Yanomami culture. This chapter summarizes the main conclusions of the study and its limitations. It also considers whether and how ITHNs might be used on a larger scale in the Amazon region and in particular in the Yanomami area.

The study results give clear evidence that ITHNs reduced the incidence of *P. falciparum* malaria episodes, and the prevalence of infection. Entomological surveillance showed a reduction of the anopheline density associated with ITHNs, but the evidence for a mass killing effect was not conclusive. More careful studies need to be done to determine whether or not ITHNs have a mass killing effect on *An. darlingi* populations.
The study also compared different procedures for impregnating the nets. Analysis of pieces of nets dried in different ways showed that nets impregnated with lambdacyhalothrin and dried vertically in the sun contained the target dose (10mg/m²), as did nets dried in the traditional recommended way, flat and in the shade. Moreover, bioassays showed impregnation at village level and drying nets vertically in the sun to be effective in killing an average of 89% of mosquitoes after six months of regular use and exposure to dust. It is therefore concluded that at village level, in this particular context, it is more practical to dry the nets in the sun and vertically, permitting them to be used just a few hours after the impregnation, and the usual recommendation to dry flat and in the shade is unnecessarily restrictive.

Regarding the impact of ITNs on splenomegaly and anaemia, the present study did not show a significant effect of the intervention. This is probably due to the fact that the mean population haemoglobin level was already high and the prevalence of splenomegaly was low before the beginning of the intervention.

Previous studies have shown that larger and more statistically significant differences in most epidemiological outcomes can be found when the ITNs intervention is compared with a group with no nets rather than with a group with untreated nets (Abdulla et al., 2001; Lengeler, 2003). During the present study in the two years of the intervention, a decrease in malaria incidence and other indices was observed in both groups of study villages when compared with the same communities in previous years. Similarly, malariological indices in both groups of
study villages were generally lower than in other parts of the Amazon state of Venezuela (MSDS, 1999-2000). These facts suggest that the un-impregnated nets may also have reduced the incidence of malaria, as has been suggested in other studies (Choi et al., 1995; D'Alessandro et al., 1995b; Jana-Kara et al., 1995; Maxwell et al., 1999; Schellenberg et al., 2001). In order to clarify whether or not PTHNs also reduced malaria incidence, further retrospective analysis of data already gathered has been planned. Time series analysis on the incidence of malaria in the Ocamo and Mavaca clinics, during the study and previous years, will be used to build a model of relationship between incidence and seasonal rainfall and river height in the years before intervention. This will be used to generate estimates of the incidence expected after intervention, if the intervention had not taken place. The observed incidence in PTHN communities will then be compared with these expected values.

Guyatt and Snow (2002), recently reviewed available data relating to the efficacy of untreated nets and treated nets in Africa. They concluded that the efficacy of untreated nets could be at least half of that obtained by treated nets. On the other hand, as already suggested by Lines et al. (1987) and Curtis (1992), the presence of the insecticide on the nets could increase the level of personal protection over untreated nets even if these are not properly used or are damaged.

In the present study it was observed that after two years of regular use the hammock nets were worn out, with a large number of holes while a reduction of the incidence of malaria was still being observed. This supports the idea that
treatment with insecticide provides improved personal protection to a person sleeping under a torn net. It is generally agreed that the beneficial effects of net programmes depend upon the treatment of nets with insecticides which serve both as a repellent and as a killer of the local malaria vector population.

A review of the literature related to the implementation of ITNs on a large scale emphasises two issues that cannot be addressed in short-term efficacy trials such as the present study. The first relates to the short and long term effects of an artificially induced reduction in the intensity of malaria transmission on the immune response and child mortality. The second, is whether the impact of ITNs in the context of well randomized controlled trials can be replicated under malaria control programme conditions (Lengeler, 2003).

Regarding the effect on immunity, it has been hypothesized that in areas with relatively intense transmission, reducing the parasite exposure using ITNs could interfere with the natural acquisition of functional immunity to malaria and therefore shift the mortality and morbidity to older age groups (Trape and Rogier, 1996). It is even possible that the beneficial impact seen immediately after the intervention could fade and disappear in the longer term, when levels of immunity have adjusted to the new, lower, level of exposure. However, it is generally agreed that this is a potential problem only for hyper and holo-endemic malaria areas, and not for those areas with low levels of malaria prevalence as observed in the present study and in general in Central and South America. The fact that malaria incidence in the study areas varies widely from year to year, apparently in
response to inter-annual variations in seasonal rainfall, suggests that the burden of malaria in this area is sensitive to natural variation in transmission intensity. This in turn suggests that artificial reductions in transmission (and/or exposure) would also produce a reduction in malaria morbidity and mortality.

Different strategies have been considered for the implementation of ITNs within the framework of malaria control programmes. In order to guarantee sustainability, these approaches must allow easy acquisition, delivery and regular treatment/re-treatment of nets. The insecticides currently in use lose potency between 6-12 month after the impregnation, whilst more frequent re-impregnation may be necessary where nets are washed often (Lines, 1996).

As part of a general discussion on health sector reform, there is a current debate about the economic sustainability of this and other health interventions, whether they should be delivered free of charge, subsidised and/or fully charged to the user.

In Africa different approaches have been applied and evaluated. For instance, in The Gambia and Kenya, when impregnated nets and/or re-treatment were given free of charge, the coverage of ITNs as a control measure was high and an improvement in child survival was registered. However, when a cost-recovery programme was introduced afterwards, people were unwilling to pay for the services that had once been available for free. In Kenya the result of the transition from free-treatment to a cost-retrieval approach was that the coverage declined from 61-67% to 7% (Cham et al., 1997; Snow et al., 1999). In The Gambia in
villages where insecticide was provided free, 77% of nets were treated with
insecticide and in villages where charges were made coverage was only 14%. The
mortality in children was significantly lower in villages where insecticide was
provided free and the introduction of a charge for insecticide into the first group
of villages and the provision of free insecticide in the latter abolished this
difference (Cham et al., 1997).

In 1996 in Tanzania, a social marketing programme (the KINET project) was
started, with the aim of achieving substantial and sustainable use of ITNs in young
children and pregnant woman. Within this programme social marketing was
defined “as a form of public-private partnership that uses a commercial
marketing approach but without the motive of financial profit”, emphasizing the
health benefits of its products and aiming to keep prices as low as possible. This
usually involves subsidies to make insecticide-treated nets affordable and
accessible (Schellenberg et al., 2001). This project reported that ownership of
ITNs as well as untreated nets increased considerably in three years, from 10 to
60% and 58% to 83% respectively (Abdulla et al., 2001). The epidemiological
evaluation showed an increase in child survival (Schellenberg et al., 2001), and
reduction of parasitaemia and anaemia in children aged under 2 years (Abdulla et
al., 2001). The studies also found that ITNs reduced the prevalence of high
parasitaemia and severe anaemia by more than one third in pregnant woman
(Marchant et al., 2002). However, as the same authors stated, the level of re-
treatment was low and, despite intense promotional activity, only a third of the
insecticide-treated nets users retreated their nets after 6 months (Schellenberg et
al., 2001). It remains to be seen now whether the epidemiological effects will be maintained. In The Gambia, Kenya, and Tanzania, the main reason identified by the villagers for reduced acquisition and/or re-treatment of nets after the introduction of fees was the lack of money (Cham et al., 1997; Snow et al., 1999; Schellenberg et al., 2001; Guyatt et al., 2002). In studies carried out in a highly endemic area of Tanzania where the distribution of nets and treatment/re-treatment was given free of charge over 4 years, more that 90% of re-treatment was achieved. These studies also showed reduction in infective bites and a highly significant reduction in malarial morbidity in children aged 6 months to 2 years (Maxwell et al., 2002).

Curtis et al. (2003) based on the above study in Tanzania and on a much larger study in Kenya (Wiseman et al., 2003), consider that ITNs should be viewed as a public good, -like vaccines-, and should be provided via the public sector with generous assistance from donors. The authors concluded “that teams distributing free ITNs, replacing them after about 4 years when they are torn and retreating them annually, have high productivity and provide more comprehensive and equitable coverage than has been reported for marketing systems” (Curtis et al., 2003). In this debate about whether or not ITNs “should” be sold, some authors argue that in Africa, current levels of funding are not enough to provide “free ITNs for all persons at risk of malaria” and to sustain this indefinitely. Whether or not this is true in African countries where the great majority of the rural population is exposed to intense transmission, it is certainly not true in Venezuela. “Free nets to all 13,000 Yanomami” is certainly affordable. The isolation of
Yanomami communities may be a key advantage in this respect. This isolation makes feasible to provide ITNs effectively free in villages of the Upper Orinoco, with little or no danger that they will be traded and sold back in the nearest city, Puerto Ayacucho. It is different in Africa, where the people most at risk are part of, and are definitely not isolated, from the general population. In other words, the isolation and difficulty of access, which are normally major problems, offer an important opportunity to deliver subsidised goods without the danger of "leakage" to non-target groups.

How can the current debate be applied to the particular context of this study? It is relevant to emphasise that the Venezuelan Constitution states that "Health" is a social right (Contitución de la República Bolivariana de Venezuela, 1999), and that equity and being free of charge are basic principles of the national health system.

It is also important to highlight that in the particular context of this study, in some indigenous peoples such as the Yanomami, the introduction of money is a relatively recent event. The majority of the people still do not have regular access to it. Only a few of them – e.g. community health workers, school teachers, and other local or regional government employees, who live in the so-called nearby villages, have regular access to money. Therefore a sustainable implementation of ITHNs for malaria control in this region cannot rely on purchasing/selling at community level. Other approaches must be developed. In the first instance, in order to obtain high coverage, the government should provide nets to Yanomami
free of charge. At the same time the local cooperatives could be supported to manufacture nets with provision of netting free of charge. Once the nets are produced they will be exchanged for other goods, as is the usual practice in the Yanomami culture. As mentioned in Chapter 2 page 65, the Rotary Club in 1996 donated netting to the Yanomami cooperatives. In Ocamo and Mavaca localities approximately 1000 hammock nets were manufactured over a period of two years and exchanged for other commodities.

Regarding the distribution of nets, different approaches should be used. As previously mentioned, the shabonos have been arbitrarily divided, according to the level of difficulty of access, into three categories: nearby, intermediate and faraway shabonos. The distribution can be carried out quite effortlessly in the first two categories, through the regular visits of medical doctors or local health worker to these villages. Conversely in the faraway villages, where the Yanomami population is scattered in a wide area of rather difficult access, the distribution is more complicated. To overcome this problem, nets could be distributed when the periodic mass immunisation activities are carried out with the support of military helicopters and/or taking advantage of the trading networks and exchanges between the Yanomami communities, and their periodic reciprocal visits to each other.

At the beginning of this study it was already hypothesised that nets could be distributed to faraway villages through the usual Yanomami network of exchanges, and in order to gather evidence on this, all personnel working in the
area were asked to record the number of nets found in villages that were not included in the study. As shown in Figure 6.1, page 216, hammock nets distributed or made in the cooperatives were found in faraway villages of Ocamo and Mavaca localities. When Yanomami from faraway villages were asked how they had obtained the nets the answer was that they had exchanged them for local products such as tobacco, *yopo* (a hallucinogenic natural substance) and plantains. In a few cases they had received them as a gift from visitors. Therefore, if there is reasonably good availability of nets in the nearby villages it is expected that their use will spread relatively effortlessly to the intermediate and faraway villages (i.e. from Ocamo and Mavaca localities toward villages upstream on the Orinoco).

Other issues related to sustainability involving community participation include the re-impregnation of nets. During this study young villagers were trained and coordinated by health-workers for the insecticide impregnation of nets at a village level, and this proved to be effective and practicable. Regular re-impregnation of the existing nets can easily be carried out in the nearby and intermediate villages. However, in the faraway villages regular re-impregnation may be somewhat more difficult. In this case, the use of long lasting impregnated nets, which are now or will soon be available in the market, could be a valid alternative. However, the exact longevity of the insecticide in these long lasting nets remains to be established under real-life conditions (Guillet *et al.*, 2001).

Taking into account the evidence produced and the experience gained during the present study, it is proposed that, for eventual implementation of ITNs as a control
measure in the area, the community could and should be involved in manufacturing, (re)impregnating and distributing the nets. This involvement not only improves the prospect for sustainability of the control measure, but also the acquisition of the skills to use technologies that can improve the communities' own health status and quality of life. This is valid for any community in endemic areas, and is particularly relevant in indigenous communities in the process of rapid acculturation.

Some issues arose during the present research that are worth considering if the measure is to be scaled up as part of a regional control programme in Amazonas. The issues are not exclusively related to this research. Rather, they are particular expressions of a general problem in the process of getting research results into practice, problems that could hinder the effectiveness of the control measure if more extensively implemented.

At the beginning of this study, rumours circulated in the municipality referring to the “poison” contained in the nets, and the use of Yanomami people as “guinea-pigs”. Rumours were progressively transformed into a systematic campaign that reached regional and national levels of government, involving politicians, health officers and academics. This campaign was never based on research evidence but produced great concern in the village populations as well as among regional and central officers of the Ministry of Health (see Annex 11). At the local level the Yanomami were afraid of the possibility that there was “poison” in the nets, but at the same time they were pleased (according to their comments to study staff) with
the nets, because they were sleeping better. There were no mosquito bites and no side effects. The villagers also showed they valued the nets by the way they were maintaining them – repairing holes– and by exchanging them as goods, as noted above.

After a prolonged process of dissemination of information, during the year 2000, the Ministry of Health decided to buy long lasting impregnated nets (Permanet®) to be used as part of the malaria control in Amazonas and Bolívar states, southern Venezuela. This decision was based on the Roll Back Malaria guidelines and the preliminary data of this thesis (Magris et al., 2000; PAHO/WHO, 2000). In 2001, however the health authorities were changed and the newly appointed officers decided not to distribute the Permanet® nets in the Yanomami population in order to avoid political problems, even though the same ITNs were being distributed in other areas of Venezuela, including the rest of the Amazonas State.

The rumours and systematic campaign coincided with the launching of the book “Darkness in El Dorado” where it was suggested that scientific biomedical and anthropological research carried out since 1960 in the Yanomami population of Brazil and Venezuela may have included violation of ethical codes and human rights. This book was not a rigorous analysis of the information, more a piece of journalism, but it had an impact on the research activities with the indigenous population.

Finally, Insecticide Treated Hammock Nets as a vector control measure can be integrated into the Primary Health Care system with community participation.
This malaria vector control measure avoids the major logistical problems associated with residual spraying and therefore allows protection from malaria to be sustained in relatively inaccessible areas.

In summary, this thesis considered a widespread problem of malaria in a context where particular ethical, political, and scientific factors were in play. It supports the notion that ITN coverage for malaria control in indigenous areas is scientifically sound, affordable and a culturally acceptable technology that can contribute to placing public health practice in the hands of the people.
Figure 6.1 Study area and map of nets “migration” from the study area. Red arrows indicated the areas where nets from Ocamo and Mavaca were found.
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APPENDIX
Annex 1. Approval of the ethical committee

Caracas 4 de septiembre de 1998

Dr. José Félix Oletta
Ministro de Sanidad y Asistencia Social
Su Despacho.

Apreciado Dr. Oletta:

Tengo a bien dirigirme a Ud en esta oportunidad a fin de comunicarle que en el día de hoy se reunió por segunda vez la Comisión de Ética “Ad hoc” que Ud, con fecha 23 de julio de 1998, nombró para evaluar el trabajo de investigación de la Dra. Magda Magría, titulado “Evaluación de los Mosquitos Impregnados con Insecticida Lambdaclorpirina para el Control de la Malaria”

A dicha reunión asistieron los Drs. Dora Piñero, Nina Incani y el suscrito La Dra. María Riera y el Dr. Antonio Rondón no pudieron asistir

Se revisaron los recados recibidos en respuesta a nuestra solicitud de fecha 14 de agosto de 1998 y consideramos que los mismos aclaran las dudas que nos planteamos en la primera reunión.

Basados en esta información, consideramos que no existen impedimentos éticos para la realización del mencionado trabajo.

Dr. Gabriela y Empirle
Coordinador.

Dr. Marta Rodríguez, Directora General Sectorial de Investigación y Educación
Annex 2. Informed consent form

PROYECTO MOSQUITEROS

CONSENTIMIENTO INFORMADO

El PROYECTO MOSQUITEROS es un proyecto del Ministerio de Salud y Asistencia Social (CAICET-Malaria) bajo la responsabilidad de las doctoras Magda Magris y Yasmin Rubio. Tiene por objeto evaluar el uso de mosquiteros tratados con y sin insecticida para el control de la malaria (prisi-prisi) en los shabonos de Ocama y Mavaca del Alto Orinoco. El uso del mosquitero lo protege de las picadas de mosquitos (ukushi), jejes (pares) y de otros insectos, por lo que si usarlo todos los días lo protege de enfermar de malaria. El insecticida utilizado para impregnar los mosquiteros no hace daño a las personas ni otros animales domésticos como perros y monos. Sólo mata a los ukushi, pares y otros insectos que se paran sobre el mosquitero.

Se le entregará a cada persona del shabono un mosquitero para que lo use todos los días. Si se va de viaje, debe llevarse el mosquitero y utilizarlo. Entendemos que no debemos cambiar ni regalar el mosquitero.

Durante el estudio los médicos buscarán los casos de malaria, para lo cual deben tomar una gota de sangre para el diagnóstico y les darán tratamiento a las personas que resulten positivas.

Entendemos que si en algún momento no deseamos continuar participando en el estudio, nos podemos retirar libremente y no comprometernos a devolver el mosquitero.

Los abajo firmantes, responsables del shabono integrado por grupos familiares, declaramos que se nos ha explicado claramente, entendemos lo que se está haciendo y que estamos de acuerdo en participar.

Andes (116)  Miquelina (511)  Jesuita (522)
Amaunua (071)  
Verica (013)  
Naikiruma (143)  
Aida (061)  
Chirino (052)  
Hosouwa (252)

Shabono: Barrio Wituqan Nuevo
Fecha: 19/12/1998

3. Experimental Procedure

The following sections are quoted from details pointed by Dr. Peter Bouhg and Mohamed Ali of Salford University.

3.1 Chemicals and solvents

HPLC grade hexane, acetone, anhydrous sodium sulphate were obtained from Fisons, Lougborough, UK. Standard Lambdacyhalothrin (your standard I have no idea from where but our standard was purchased from Promochem, UK. Decachlorobiphenyl (DCBP) was obtained from British Greyhound.

3.2 Preparation of Lambdacyhalothrin Standard Solution

The stock solutions and calibration standards were stored at 40°C while working calibration standards were prepared fresh when required. A stock solution was prepared from certified Lambdacyhalothrin at 1000mg l-1 concentration by accurately weighing 0.1 mg directly into 100cm³ volumetric flask on a Oertling balance and making up with hexane. From the stock solution, working calibration standards were prepared at varying concentrations i.e., for medium range; 3, 2,1, 0.75, 0.5 and 0.25 g ml-1 (ppm range) in hexane with volumetric standard (DCBP) concentration held constant at 50 g l-1, for low range; 200-10 g l-1(ppb range). The 0.5 g ml-1 of standard was run after every 10 samples as a continuing check.
3.3 Extraction Procedure

In the initial experiments, an approximately 1cm² per piece of netting from each smaller pieces (each 5cm x 5cm) were cut off, accurately weighed and put into separate beakers containing 20 ml of, hexane, and then allowed to stand for 30 min. The supernatant was decanted through a glass sinter (Porosity 4) capped with anhydrous sodium sulphate (c.a. 5 g). The extraction procedure was repeated twice and the extracts combined. The extract was evaporated to about 5 ml by rotary evaporation under reduced pressure at 40°C, then transferred into 15 ml conical vial with a 1 ml mark, and evaporated just to dryness with a gentle stream of clean dry nitrogen. 1 ml of volumetric standard DCBP (50 g ml⁻¹) in hexane was added to re-dissolve the residue (or evaporated to about 0.5 ml before 50 l of 10 mg ml⁻¹ of DCBP was added). The final sample extract was transferred to an auto-sampler vial with a PTFE-lined crimp cap and could be stored at 4°C for at least two weeks.

3.4 GC-NICI-MS analysis

The analyses of the standards and extracts obtained from nets were performed on Hewlett-Packard 5890A GC interfaced to VG Trio 1000 quadruple mass spectrometer (Fisons Instruments, Wythenshaw, Manchester, UK) and equipped with a Hewlett-Packard auto-sampler, operating in electron impact (EI), and negative ion chemical ionization (NICI) modes employing full scan and selected ion monitoring or recording (SIM/SIR). 25 m x 0.25 mm id x 0.25m film thickness DB-5 capillary column with helium head pressure of 5 psi, was used to achieve separation using the following temperature programme; initial column temperature, 100°C, hold for 1
min, increase at 35°C min to 240°C, then increase at 8°C min to 300°C min, final
temperature hold for 2 min; the total cycle time was 15 min. All spectra were
acquired in the NICI (full scan and SIR). The general mass spectrometer conditions
were; ion source 250°C; electron voltage 70eV; photomultiplier voltage 450V; and
the filament and the source currents 4.4 and 345 A respectively. When operating in
scanning mode, the scanned mass range was 50-550 u in 0.9s while in SIR mode, the
mass span was 0.02 u in 0.02s. The voltages of the filter parameters for NICI were
periodically optimized using the ion at m/z 452 generated from the calibration
compound, perfluorotributylamine (PFTBA). The linearity and dynamic range of the
GC-MS using the SIR or Full Scan mode was demonstrated by generation of
standard curves for each analyte containing four, five or six levels of concentration
that were analyzed in duplicate. Standard deviations were calculated from five
replicate injections of the daily calibration standard at 0.5 ng/ l, using response
factors generated from the regression statistics. The limit of detection (LOD) was
calculated as three times the standard deviation and this was used to calculate the
MDLs for each analyte. A stock solution was prepared from certified
Lambdacyhalothrin at 100 mg/l concentration. From the stock solution, working
calibration standards were prepared at varying concentrations in hexane with
volumetric standard (DCBP) concentration held constant at 50 gl-1. GC-NICI-MS
calibration curves were based on the peak area using 1-3 of the most intense product
ions (205,241,243) for the compound (SIR mode). Peak areas were obtained from the
mass chromatograms generated for the quantitation ion of the analyte (205).
Calibration curves were obtained from plots of response factor (Lambdacyhalothrin
peak area/DCBP peak area) against ratio analyte/internal concentration. All the calibration curves either in medium or low concentrations were linear over the entire range with correlation coefficients between 0.997 and 1.0.6-9.
Annex 4. Washing surveillance

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Annex 5. Chart used to measure the holes in the nets
Annex 6. Form used to record the number and position of holes in the nets

**HUECOS Y REPARACIONES DE LOS MOSQUITEROS**

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Annex 7. CDC light traps record form

**CAPTURAS CON TRAMPA DE LUZ**

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<th>Número de tubo</th>
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Luna: N: Nueva, C: Creciente, LL: Llena, M: Menguante


244
Annex 8. Active case detection record form

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**Busqueda Activa de Casos**

ID: código de la persona


**Sexo:** F: femenino, M: Masculino
Annex 9. Passive case detection record form

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ID: código de la persona


Sexo: F: femenino, M: Masculino
Annex 10. Cross-sectional survey record form

CORTES TRANSVERSALES

FECHA: ____________________________

1. IDENTIFICACION

COMUNIDAD: 1. OCAMO 2. MAVACA

SHABONO:
1. Ocamo 9. Warapana
2. Tumba 10. Shakita
3. Clavotheri 11. Hatako
4. Carlito 12. Purima
7. Shashana 15. Keparima
8. Yohoope 16. Mosho
17. Mario
18. Ishinowei-theri

NOMBRE: ____________________________

SEXO: 1. Femenino 2. Masculino

EDAD: ____________________________

VISITANTE: 1. SI 2. NO

2. CLINICA

SINTOMAS:

FIEBRE: 1. SI 2. NO

ESCALOFRIOS: 1. SI 2. NO

CEFALEA: 1. SI 2. NO

DOLOR ESPLÉNICO: 1. SI 2. NO

VOMITO: 1. SI 2. NO

DIARREA: 1. SI 2. NO

OTROS: ____________________________
SIGNOS:

TEMPERATURA:

HACKETT:

OBSERVACIONES:

EMBARAZO

HEPATITIS CRONOCA

OTRAS:

3. LABORATORIO

HEMOGLOBINA (gms/dl)

LAMINA: 1. Positiva  2. Negativa

ESPECIE:


5. *Pv+pm*  6. *Pf+Pm*  Pf gametos 1. SI  2. NO

DENSIDAD *Pf* TROFOZOITOS 200GB

DENSIDAD PARASITARIA p/ul

MICROSCOPISTA
Annex 11. Review of articles from the national Venezuelan press related to the present study
Como ratones de laboratorio utilizan a indios del Amazonas

Puerto Ayacucho especial por Andrés García. Tradicionalmente los grupos yanomamí vienen utilizando la lucha contra el mejor sistema de protección contra los mosquitos y enfermedades del territorio. En la actualidad, las actividades de lucha contra la malaria se están realizando con un foco especial en el Amazonas, donde se han identificado casos de malaria en comunidades indígenas. En el municipio de Puerto Ayacucho, se han llevado a cabo campañas de vacunación y tratamiento para prevenir la transmisión del mosquito anopheles, uno de los responsables de la enfermedad. Las actividades han sido financiadas por el Gobierno Nacional y la Organización de Estados Americanos, quien fomenta la colaboración entre países para la prevención y control de enfermedades. Las actividades de control de enfermedades se realizan en colaboración con la Universidad de Nueva York, que contribuye con la investigación y el desarrollo de nuevas estrategias de prevención. Los resultados de estas actividades han sidopositivos, con una disminución significativa de los casos de malaria en la comunidad. Sin embargo, se sigue trabajando para fortificar los esfuerzos de prevención y control, especialmente en los lugares donde la enfermedad sigue siendo una amenaza.

Baruta: Proyecto y plan estratégico de servicios

El proyecto de la Fundación Plan Estratégico Caracas busca fortalecer los servicios de salud en la ciudad de Caracas. El objetivo principal es mejorar la atención médica y el acceso a los servicios de salud para la población. El proyecto se desarrolla a través de la implementación de estrategias de mejora continua en los sistemas de salud, con el fin de garantizar la calidad y eficiencia de los servicios. Se han identificado áreas de mejora en la atención primaria, la gestión y la coordinación de los servicios de salud. El proyecto involucra a diferentes actores, incluyendo a los servicios de salud, las instituciones educativas y las organizaciones de la sociedad civil. Se ha establecido un comité de seguimiento para monitorear los resultados y hacer ajustes en caso necesario. El proyecto está financiado por el Ministerio de Salud y el Fondo para la Vivienda y los Servicios Públicos. Se espera que el proyecto tenga un impacto significativo en la mejora de la atención médica en la ciudad de Caracas.

Recortan en más de 50% presupuesto de Alcaldías de los Valles del Tuy

Por: Emer P. Carrillo en Cúcuta, septiembre. Los municipios de los Valles del Tuy, localizados en la región de la Frontera oriental, están enfrentando una crisis económica debido a la caída de los precios del petróleo. Aproximadamente 50% del presupuesto de los alcaldes ha sido recortado, lo que representa una disminución significativa en sus capacidades para llevar a cabo proyectos y programas municipales. La medida ha sido tomada en el marco de una política nacional de ajuste fiscal. Los alcaldes han expresado su preocupación por el impacto que este recorte tendrá en la prestación de servicios a la población, ya que muchos proyectos están en etapa de implementación. Los alcaldes han solicitado al Gobierno Nacional que considere la situación y tome acciones para mitigate el impacto de estos recortes. A pesar de la crisis económica, los alcaldes han expresado su compromiso con la gestión eficiente de los recursos y la mejora continua de los servicios municipales.
Intentan probar con yanomamis
efectividad de fármaco inglés

Enero Ayséchou, (Es-
pecial, por Andrés García).—Los máximos representantes de la etnia yanomami, los shamenas del Alto Orinoco, buscan en la orientación del
humo y del viento, los con-
juntos para sacar de la selva
a los "chupadores de san-
gre", quienes aparecen en el
rocejo de investigaciones, quien es conocido como re-
tores de experimentación para
probar un fármaco de
origen inglés.

Para los pueblos de la etnia
más ancestral del planeta, la
lucha que se les presenta, no es una lucha, además cuan-
tan con sus rituales y sus bra-
vejas, para "separar" de sus
tierras a toda una cantidad de investigadores que son
comprometidos con el Cen-
tro de Investigaciones de
Enfermedades Tropicales,
Simón Bolívar (CAICET), en-
trar además, que se llama
el fármaco el Proyecto Modulati-
no, planean contemplar as-
tar a algunas comunidades

En Charallave se unifican
esfuerzos para la seguridad
Charallave, septiembre (Especial Bea-
triz Martínez).—Los in-
dustriales de Charallave
antiguaron a Alcaldía de esta localidad, Antonio
José Hernández dos molineros e equipos de transmisión como yanomami, como son Mavas-
ca y Ocáno, de los con-
octos vecinales a la pro-
bar el insecicida típico, tra-
vés de la importación de los
remedios, para sacar los
los "chupadores".

El problema radica en que el Caicet y el paje se-
lesiano José Bortol, han ve-
rado sosteniendo que los ya-
nomamis rachan al pro-
grama tradicional de Mias-
mología, considerándolo ins-
eficiente, pero resulta ser
que los mismos yanomamis de las
comunidades indígenas pe-
gan esta posición y afir-
man que nunca han sido con-
lados para ninguno de los
programas de salud en
Alto Orinoco.

Jornadas preventivas de medicina
en casos de desastres en Petare

En Charallave se unifican esfuerzos para la seguridad
Charallave, septiembre (Especial Beat-
riz Martínez).—Los indus-
triales de Charallave entregarón al Alcaldía de
esta localidad, Antonio José Hernández dos
molineros e equipos de transmisión, como yanomami, como son Mavasca y Ocáno, de los cont-
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gan esta posición y afir-
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Jornadas preventivas de medicina
en casos de desastres en Petare

Panorama de la situación en la sala de emergencias de Petare

En Charallave se unifican esfuerzos para la seguridad
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Jornadas preventivas de medicina
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eficiente, pero resulta ser
que los mismos yanomamis de las
comunidades indígenas pe-
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Jornadas preventivas de medicina
en casos de desastres en Petare

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mología, considerándolo in-
eficiente, pero resulta ser
que los mismos yanomamis de las
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Alto Orinoco.

Jornadas preventivas de medicina
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Min-Sanidad niega que se estén utilizando indígenas en pruebas de laboratorio

El Ministro de Sanidad, José Félix Oletta aseguró que con el "Proyecto de Evaluación de Mosquiteros Impregnados con el Insecticida Lambda-Chrialothrin para el control de la Malaria en el Alto Orinoco", no se pretende utilizar a los indígenas Yanomamis como "ratones de laboratorio", tal como lo han venido señalando algunas personas, ya que "las más elementales normas morales y éticas niñan esa posibilidad".

En un documento enviado por el doctor Pietta, se explique que "este proyecto, financiado por el programa de control de enfermedades endémicas, busca desarrollar tecnologías alternativas para el control de la Malaria en el Alto Orinoco; donde las medidas tradicionales de control, no han tenido la cobertura y eficacia deseada, debido a peculiaridades del medio y de la cultura de la población Yanomami que la habita. Esta situación se ha reflejado en las últimas décadas en elevados índices parasitarios y altas tasas de prevalencia de complicaciones severas de la malaria, a pesar del esfuerzo desarrollado por médico y personal de malarialogía que desarrollan actividades en el área."

En el documento se establecen las causas que han conspirado contra la efectividad de esas medidas tradicionales de control, entre las que resaltan: 1) Viviendas no adecuadas para ser rociadas, 2) Población seminómada, no protegida por los rociamientos durante una buena parte del año, 3) Comportamiento del vector que no reposa en las viviendas rociadas sino en el monte y que pida en horas nocturnas cuando la nebulización ya no tiene efecto, 4) Serias dificultades logísticas para llegar a la mayor parte de la población y 5) la falta de participación comunitaria en el control y escasa aceptación o franco rechazo de estas medidas.

ULTIMAS NOTICIAS
5 OCTUBRE 1998
El Proyecto Mosquitero: Alternativa Antimalárica
Por Luis Fuenmayor Toro

Recientemente fue invitado a participar en una mesa redonda que se realizó en el Centro Amazónico para la Investigación y Control de Enfermedades Tropicales (CAICET), dentro de las actividades científicas que se realizaron con motivo de su XVI aniversario. La mesa trató el tema: "Investigación, Ética y Comunicación", y estuvo motivada por una denuncia irresponsable que circuló en la prensa nacional hace poco, en la cual se acusaba a los investigadores del Centro de utilizar como "conejillos de indias" a la población indígena del Estado Amazonas. En la denuncia se mezclaban informaciones de un proyecto de investigación que se lleva a cabo en la región y otras derivadas de programas de control de enfermedades y epidemias que realiza el Ministerio de Sanidad y Asistencia Social, las cuales son intervenciones del Estado venezolano dirigidas a proteger a la población del país de determinados peligros: en el caso del Amazonas de enfermedades como el paludismo, la fiebre amarilla, la oncocercosis, entre otras.

Se criticaba en las informaciones referidas la realización del "Proyecto Mosquitero" llevado a cabo por la doctora Magda Magris, el cual es financiado por el Proyecto Control de Enfermedades Endémicas (PCEE) del SAS y que es aprobado por la Dirección de Investigaciones de la Escuela de Malanología "Arnaldo Gabaldón" del SAS, por el comité evaluador del PCEE, por el comité evaluador de la Dirección de Investigación y Educación del SAS, por la Dirección Regional de Salud del Amazonas y por un comité de ética "ad hoc" nombrado por el propio Ministerio de Sanidad.

Pero lo más importante es que desde que se estén ejecutando este proyecto, la morbimobility y mortalidad por paludismo en el Alto Orinoco se ha reducido en forma muy importante. Eso debiera acabar con cualquier duda y debería hacer rectificar a quienes por intereses bastantes agredan al CAICET y sus investigadores...
Puerto Ayacucho (Especial por Andrés García).- Las diferencias entre los médicos investigadores del Centro Amazonónico de Investigaciones Científicas de Enfermedades Tropicales, Caicet, y las comunidades yanomamis del Alto Orinoco parecen acentuarse, una vez que en la selva corrió como el viento la noticia sobre la aprobación del proyecto mosquitero por parte de la Comisión de Ética de Sanidad, presidida por la doctora Marta Rodríguez, directora de Investigación Nacional, tras considerar que "no existen contradicciones" sobre este proyecto que se aplicará en las comunidades, sin antes haberlas consultado, o lo que es lo mismo se impondrá sin la participación comunitaria, hecho que obligó a Malariaología a no asistir a la referida reunión por abrigar reservas sobre una medida alternativa que fue probada con un estudio preliminar en 1986 en Las Majadas, estado Bolívar con resultados dudosos.

Aunque el ministro José Félix Oletta luego que a los yanomamis se les fome como ratones de laboratorio, no abandó en detalles sobre este trabajo que consiste en impregnar mosquitos con el insecticida Icon y obligar a un grupo de yanomamis a dormir dentro de éstos, para probar su eficacia, ensayo que no ha sido apoyado por los médicos de la región tras conocer el fracaso de este experimento en varios países de Latinoamérica, entre estos Perú, donde se obtuvo un resultado sin diferencias significativas entre las comunidades de intervención y control.

Por otro lado el ministro aceptó la ejecución de un proyecto de esta naturaleza en una etnia que jamás ha utilizado mosquero y cuyo uso provocará un fuerte choque cultural, si es que se aplica, ya que los yanomamis lanzaron advertencias respecto a no permitir el referido ensayo, tal como ocurrió este fin de semana cuando en varias comunidades se impidió la toma de muestra para los estudios de oncosecrosis.

El ministro habla de la ineludible de los controles vectoriales que viene desarrollando Malariaología con malathion y deltametrina, "si él conoce perfectamente los resultados, entonces por qué no prueba con otra medida alterna nontia distinta al mosquitero impregnado que no ha dado ningún resultado positivo en los países donde se ha aplicado", advirtió el Alcalde del Alto Orinoco Jaime Turón quien aseguró que no permitirá que a lo yanomamis se les continue utilizando en ensayos que chocan contra su cultura.

"Si los médicos del Caicet quieren probar el Icon que tomen espas de paludismo y las niegan en sus casas o en las oficinas de la empresa inglesa que esté detrás de este proyecto para que ellos demuestren si su veneno es bueno o es malo, o, que pongan a sus familiares a dormir en estos mosquitos donde seguramente no soportarán la hediondez", dijo Turón.

Trascendió por otro lado, que la mayoría de los médicos que vienen prestando asistencia en Amazonas, al conocer la intención del Caicet, mostraron sus reservas en cuanto al proyecto, al considerar que el grupo humano formado por los yanomamis es muy sensible a cualquier proceso de intervención, lo que podría acarrear consecuencias impredecibles.

CARACAS, MIERCOLES 7 DE OCTUBRE DE 1998

Yanomamis rechazan visita de investigadores de Caicet
Hacia la Certificación ISO 9002

Caracas - Maracaibo - Valencia - Maracay
Barquisimeto - Puerto La Cruz - Punto Fijo
Amazonas
Los yanomamis como negocio

En la Amazonas venezolana, hay un poco más de 10 mil indígenas de la etnia más ancestrales del planeta: los yanomamis.

El mimosamis salvaje, en el mejor uso del término, permitió a los descendientes de los indígenas, mantener ocultas sus tradiciones y formas de vida, puestas en peligro a partir de la penetración por el sur, del imperio brasileño, portugués, y por el norte, por la llamada misión evangelizadora, que intereses internacionales patrocinaron para dominar y "civilizar" a los indígenas.

Pero no ha sido fácil, la pelea entre curaré y metralla, parece desigual, sin embargo, los yanomamis perviven en el Amazonas, contando, dos, pero con mucha dignidad.

Hoy, cuando todo el país olvidó los intereses de la conquista, los indígenas del sur del país, libran batallas por la subsistencia. Hay muchos indígenas y científicos interesados en el problema indio, pero, la mayor oculta, como en el pasado, sus verdaderos intereses. Son miles de millones de dólares que mucha gente consigue pensando en los yanomamis como inmata, colocando de seguidas proyectos para "salvar indio" al lado, tal como ocurre por estos días en el sector salud.

Proyecto Mosquitero, avalado por la siguiente a nivel nacional y refrendado por un Instituto de Investigación regional, que consiste en impregnar mosquiteros con un insecticida inglés, llamado Icon, para luego colocar a los yanomamis dentro y probar su efectividad y el tipo de mosquitero que resulta más beneficioso.

Este experimento científico en la vida de los yanomamis, en más que un crimen de lesa humanidad; sin embargo, como los intereses son muchos, los promotores del Proyecto Mosquitero, dicen contar con el aval a interés del propio ministro de sanidad y como jugada mágica, se han asociado con un sector de la iglesia católica, donde estos últimos tendrán la exclusiva de vender los mosquiteros, tal como lo revela una carta del procurador de la zona, José Bertoldi.