PROTECTIVE EFFICACY OF LAMBDA-CYHALOTHrin TREATED NETS IN
ANOPHELES GAMBIAE PYRETHROID RESISTANCE AREAS OF CÔTE D’IVOIRE

MARIE-CLAIRE HENRY,* SERGE-BRICE ASSI, CHRISTOPHE ROGIER, JOËL DOSSOU-YOVO, FABRICE CHANDRE,
PIERRE GUILLET, AND PIERRE CARNEVALE
Institut Pierre Richet, Bouaké, Côte d’Ivoire; Institut de Médecine Tropicale du Service de Santé des Armées, Marseille, France;
Parasitic Diseases and Vector Control, Control, Prevention and Eradication, WHO, Geneva, Switzerland

Abstract. The efficacy of nets treated with lambda-cyhalothrin, a pyrethroid insecticide, on malaria infection and
disease was assessed for the first time at the community level in Anopheles gambiae pyrethroid resistance areas. The study
was carried out in northern Côte d’Ivoire, which is an area of kdr resistance. Four pairs of villages were selected
and matched according to demographic, sociological, and ecological criteria. Among each pair, a village was randomly
allocated to receive mosquito nets. More than 80% of beds were covered with nets treated with lambda-cyhalothrin and
retreated after 6 months. In each village, 54 children aged 0–59 months were randomly selected and clinically monitored
for 8 periods of 7 days throughout the year. Results showed that the efficacy of treated nets was maintained with a
reduction of the prevalence of asymptomatic malaria infection by 12% and an estimated protective efficacy against
malaria disease of 56%.

INTRODUCTION

Many studies have shown the efficacy of insecticide-treated
bed nets in reducing malaria transmission and morbidity and
all-cause child mortality.1 The only insecticides used for the
impregnation of bed nets are pyrethroid insecticides, such as
lambda-cyhalothrin, deltamethrin, or permethrin.

In the sub-Saharan savannah region of Côte d’Ivoire, inten-
sive cotton cultivation has involved large-scale insecticide
spraying for several years. It is strongly suspected that this has
been a source of selection for DDT and pyrethroid resistance
on mosquito populations including those of Anopheles gam-
biae, which breed in the lowlands where insecticide residues
concentrate after being washed down by the rains. This has
led to the selection of a resistance mechanism called knock
down resistance (kdr) with a high frequency of kdr allele.2 In
the course of phase II trials for the WHO Pesticide Evalua-
tion Scheme on evaluation of insecticides, tests carried out in
experimental huts on the efficacy of nets treated with per-
methrin and deltamethrin showed that these nets reduced the
rates of hut entry, increased rates of hut exit, and caused some
mortality in the local populations of An. gambiae despite their
resistance to pyrethroids.3 In addition, a trial in the village of
Kafiné in Côte d’Ivoire, where An. gambiae is very resistant
to pyrethroids, showed that Olyset nets into which permethrin
is industrially incorporated conferred personal protection
against malaria attacks.4

The current trial was carried out in the sub-Saharan savannah
zone of Côte d’Ivoire where the association of malaria risk
with different systems of rice cultivation has been previously
measured.5 The main malaria vectors of this area were An.
gambiae s.s. and Anopheles funestus, but An. gambiae was
predominant in villages close to rice fields (Dossou-Yovo J,
unpublished data). An. gambiae was strongly resistant to
pyrethroids with a kdr allelic frequency of around 90%. An.
funestus was still susceptible to these insecticides (Koffi AA,
unpublished data). The current study aimed to evaluate the
epidemiologic impact of nets treated with lambda-cyhalothrin
in a region with intense transmission due to An. gambiae
highly resistant to pyrethroids. A comparison was made be-
tween eight villages, four provided with treated nets and the
other four without any nets. The malaria burden on children
less than 5 years old has been studied in terms of prevalence
and density of malaria infection, of clinical malaria attacks,
and level of anemia. The entomological impact of the treated
nets was evaluated simultaneously in the same villages (Dos-
sou-Yovo J, unpublished data).

MATERIALS AND METHODS

Site and sampling. A longitudinal study was conducted in
the Department of Korhogo, in the North of Côte d’Ivoire,
from July 1999 to June 2000. The required numbers of com-

munities and children for epidemiologic evaluation of the in-
tervention were estimated to detect a 50% protective efficacy
with a power of 80%, a significance level of 5%, a design
effect of 0.25, an average of 2.6 clinical malaria attacks per
child-year in the reference group,5 and 20% of the children
lost to follow-up.6 Thus, the required number of communities
was calculated to be 4 villages for the intervention group
and 4 villages for the control group, and the required number
of children was 54 subjects per village followed during 56 days
distributed over 8 periods of 7 consecutive days scheduled at
6 week intervals throughout the year. Among the 12 villages
where malaria transmission and morbidity were previously
measured, 4 pairs of villages were selected and matched ac-
cording to the lowland rice cultivation system, the social and
behavioral characteristics and the size of village (Table 1).

Among each pair of matched villages, one was randomly al-
located to receive impregnated mosquito nets, and the other
remained as control. After updating the census of each vil-
lage, including children born since 1997, samples of children
aged 0–59 months were selected from randomly sampled
compounds as in an earlier study.5 Each head of family, or the
guardian of the child, gave informed consent. The Ministry of
Public Health of Côte d’Ivoire granted ethical approval for
this study. During the monitoring periods, children of villages,
whether participating in the study, were treated free of charge
by the medical team.
Before impregnation, a comprehensive census was done in each village to identify every bed and sleeping mat in every house to be covered by an insecticide-treated net (ITN). At the start of the trial in June 1999, every mosquito net was treated, in the village, with lambda-cyhalothrin formulated as a capsule suspension (15 mg a.i./m²) and marked with both wash-resistant and washable markers to check for washing or movement of nets to different houses from those for which they were intended. Mosquito nets were dipped again after 6 months in December 1999. In each village, the correct use of ITNs was regularly checked by two village health workers trained for this trial. The four villages without mosquito nets received ITNs after the trial. All bed nets were given free of charge.

**Data collection.** Active case detection (ACD) for malaria episodes was done during 8 periods of 7 days at 6 weekly intervals. Each day during these surveys a nurse, assisted by two health workers from the village trained for this study, visited the households of each child of the sample, and a physician supervised the field work. The presence or absence and state of health of each of the children were thus recorded daily on a specially prepared sheet (one sheet per household). The nurse examined and recorded data on every detected case of sickness at home. A thick blood film was taken from every sick child. Children were treated according to the clinical diagnosis made by the nurse. When a malaria attack was suspected, the patient was treated with chloroquine at 25 mg/kg body weight for 3 days according to the recommendations of the National Program for Malaria Control. Cross-sectional surveys (CSS) were carried out on every asymptomatic child (confirmed by an axillary temperature < 37.5°C) included in the study. During each survey, a blood sample was taken on the sixth day to confirm that those classified as asymptomatic were free of malaria infection in the days before the blood sample was taken. In November 1999 (end of the rainy season), capillary blood was also taken from each asymptomatic child, 0–2 years old, to measure packed cell volume.

**Laboratory examination.** Thick smears were Giemsa stained in the field and examined at the Institut Pierre Richet in Bouaké to identify *Plasmodium* species. Asexual stages of *Plasmodium falciparum* were counted in the blood volume occupied by 200 leukocytes, and parasite density was calculated by assuming 8,000 leukocytes/µL of blood. Thick smears from each village were read by the same experienced technician, under the supervision of a parasitologist. The technicians were also compared on the same set of blood samples. Their rate of parasite detection and parasite density estimates did not differ significantly. Cross-check quality control was regularly done on a randomly selected sample representing 10% of all thick smears.

**Data analysis.** Demographic, clinical, parasitological, and attendance data were double entered independently in an Access database (version 7, 1995). Data were analyzed using Epinfo (version 6, 1995), STATA® statistical package (StataCorp. 2001) and Egret (version 2, 1999) software programs. For each person, only one blood sample per monitoring period was considered for the analysis. When a pathological condition was detected, the blood sample taken during the clinical episode was retained for analysis. When many blood samples were available in an asymptomatic period, one of them was randomly selected for the analysis. The association between the parasite density and the occurrence of clinical episodes was tested using a random-effect logistic regression model, taking clinical status (pathological episode versus asymptomatic state) as the dependent variable, and parasite density and age as the independent variables. In this type of model, a random intercept variable is allowed to vary with subjects, and this random subject-specific intercept allows one to take into account the interdependence of the observations made on the same person. The independent variables and their interaction terms were tested and kept in the model when their effects were significant likelihood ratio statistic, *P* < 0.05. For each pathological period, the probability that it was caused by malaria was estimated by the attributable fraction calculated from the odd ratios associated with the estimated parasite density in the logistic model. The pathological episodes were clinically defined by a high axillary temperature (≥37.5°C), a body hot to the touch, sweats, shivers, headaches, nausea or vomiting, or by a history of fever during the 48 hours preceding the first day of ACD or, for infants, anorexia or any pathological condition described by the mother. For individuals and given periods, the number of malaria attacks was estimated by the sum of probabilities that pathological episodes were due to malaria, depending on the parasite density. Malaria incidence density was calculated by the ratio of pathological episodes attributable to malaria divided by the child-days under survey during the monitoring periods. Clinical malaria incidence data were gathered by villages, surveys, and age groups (0–23, 24–59 months). The effect of the intervention was tested using the likelihood ratio statistic in a Poisson regression model taking into account the effect of the design (matching), survey, and age, with the estimated number of malaria attacks as dependent variable and the cumulative number of monitoring days as exposure variable. A goodness-of-fit test was used to check the adequacy of the model. Protective Efficacy was calculated as:

\[
PE = \left(1 - \frac{\text{Adjusted incidence density ratio}}{100}\right) \times 100.
\]

The confidence interval for the clinical protective efficacy was calculated from the confidence interval of the adjusted incidence density ratio. Parasitological data were analyzed separately in terms of prevalence and density of *P. falciparum* asexual blood forms. A generalized estimating equation (GEE) approach was used for statistical analysis of repeated measures, which can be used with normal distributions and discrete data. To take into account the interdependence of observations made on the same person, an exchangeable correlation structure was used in which the correlation be-

<table>
<thead>
<tr>
<th>Agrosystem</th>
<th>With ITN</th>
<th>Without ITN</th>
</tr>
</thead>
<tbody>
<tr>
<td>Double cropping rice/year</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Single cropping rice/year</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>No rice cultivation</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Senufo subethnical group</td>
<td>Tiembaraka</td>
<td>Tiembaraka</td>
</tr>
<tr>
<td>Size of village (N inhabitants)</td>
<td>605</td>
<td>1305</td>
</tr>
</tbody>
</table>

ITN, insecticide-treated net.
between these observations made on one person at different times is assumed to be the same. The differences were tested by the Wald test and 95% confidence intervals were calculated. The prevalence of asymptomatic malaria infections was analyzed as a binomial response. The positive asymptomatic parasite density was log transformed and analyzed with a link function for a normally distributed response. The GEE approach allows some departure from the hypothesis about the distribution of the dependent variable and gives robust estimates of regression coefficients taking into account the interdependence of observations made on the same person. Comparisons between prevalences and between parasite densities and packed cell volumes were performed by chi-square test and analysis of variance taking into account the study design. Statistical tests were considered as significant when \( P < 0.05 \).

RESULTS

Population description. For one year, 426 children in 8 villages (216 in the 4 villages furnished with lambda-cyhalothrin–impregnated mosquito nets and 210 in the 4 untreated villages) were parasitologically and clinically monitored. Children born during the study were not included. Mean age was comparable (27 months) in the two groups. Sex ratio was well balanced: range of M/F sex ratio was 0.8 and 1.0, respectively, in the treated and control groups. Children’s participation in clinical monitoring was high in both groups (Figure 1). A total of 16% were lost to follow up of which 2% had died. Each child in the control and treated groups was visited on average on 43 days (± 8) of the 56 active detection days scheduled. A total of 2,128 blood films were made with an average of 5 per child. The distribution of blood samples is shown in Figure 2. One fever episode where no thick smear was taken was excluded from the study. Each fever syndrome corresponds to one episode of illness in a child at one survey. Average coverage rate with ITNs was regularly checked and ranged from 76.7% to 84.0% in different villages.

Parasitological and hematological indexes of asymptomatic children observed by CSS. The mean annual rates of prevalence and parasite density in asymptomatic infections with \( P. falciparum \) observed in the baseline study in 1997 were comparable in the two groups of villages (\( P > 0.30 \)). The rates of infection were about 80% and the densities averaged 143 to 199 asexual stages of \( P. falciparum \) per \( \mu L \) (Table 2). In 1999, after introduction of the ITNs, the observed rate of asymptomatic infections was higher in the untreated group than the treated one: 68.5 (95% CI, 64.9–72.1) versus 56.6%, (53.0–60.2) (\( P < 0.001 \)). The parasite density was significantly higher in the untreated group: 69 (95% CI, 53–91) versus 29 (22–38) asexual \( P. falciparum \) stages per microliter (\( P < 0.001 \)) (Table 3). The effect of the ITNs on anemia in the children aged 0 to 2 years was significantly positive in November 1999 (Table 3) with a mean hematocrit 2.0% higher in the treated group than in the control: 32.8 (95% CI, 31.9–33.7) versus 30.8%, (29.6–31.9) (\( P = 0.007 \)).

Clinical malaria observed by ACD. We considered only one fever episode per patient per survey. In 1997, the frequency of clinical malaria attacks per child per year was comparable in the 2 groups of villages (2.3 versus 2.9, \( P = 0.36 \)) (Table 2). In 1999, in the ITN villages, the incidence was significantly lower than in the control villages: 0.8 versus 1.8 clinical malaria attacks per child per year (\( P < 0.001 \)). The protective efficacy of treated nets was 56% (95% CI, 25–75%). The impact was comparable in the two age classes.
Furthermore, the resistance to pyrethroids suggested that prevalence was lower in areas where the vectors were susceptible to pyrethroids. Various trials in experimental huts in Côte d'Ivoire and other countries in West Africa have shown that insecticide-treated nets can still provide personal protection from biting as well as a high mortality of mosquitoes resistant to pyrethroids. These data obtained in Phase II trials called into question the continued effectiveness of treated nets.

### DISCUSSION

Studies conducted during the past 20 years with insecticide-treated nets in various epidemiologic conditions have confirmed their effectiveness in reduction of the burden of malaria. The extension of the range of resistance to pyrethroids in Côte d'Ivoire and other countries in West Africa has called into question the continued effectiveness of treated nets and of the current strategy for malaria prevention based on promotion of the use of these nets. A trial in a village where *An. gambiae* is resistant to pyrethroids suggested that *Olyset* nets, which incorporate permethrin, are still effective in reducing by 50% the incidence of malaria attacks. Various trials in experimental huts in Côte d'Ivoire have shown that insecticide-treated nets can still provide personal protection from biting as well as a high mortality of mosquitoes resistant to pyrethroids. These data obtained in Phase II trials according to WHOPES protocol were the starting point of the current study, which corresponds to a Phase III WHOPES protocol.

In 1997, a study on the relationship of malaria to rice cultivation in 12 villages in the Department of Korhogo led to the selection of 2 groups of 4 villages, in which the annual incidence of infection (80%) and of malaria morbidity (about 2.6 malaria episodes per child per year) were similar and where the annual entomological inoculation rate was around 113 infective bites per person (Dossou-Yovo J, unpublished data). The implementation of ITNs in 1999 led to the coverage in 4 villages of more than 80% of beds with nets treated with lambda-cyhalothrin (15 mg a.i./m²). Re-impregnation was done after 6 months, and coverage was sustained at about 80%. Longitudinal surveys on representative samples of children aged less than 5 clearly showed that the children in the treated communities were healthier than in the untreated communities for all parameters measured. In terms of standard malaria indices, the prevalence of infection with *P. falciparum* appeared to be reduced by 12% in the netted community, compared with the untreated. It lies in the range of 5–12% based on numerous studies that compared treated nets with no nets in areas where the vectors are susceptible to pyrethroids. It should be noted that in the current study, the prevalence of infection was recorded after ascertaining that the child was asymptomatic, whereas in most other studies the symptomatic/asymptomatic status of the child has not been taken into account. Regarding clinical status, the mean hematocrit was higher in the young children in the netted villages than in the controls. The difference found was comparable to that observed in other studies. Furthermore, the treated nets reduced the risk of clinical malaria attacks by 56% (95% confidence limits 25 to 75) among the children. This protection factor was comparable to the range of 46–48%. It was also similar to the 49% reported in the study of the clinical impact of nets treated with lambda-cyhalothrin in Sierra Leone where the vectors were susceptible to this insecticide.

Although the reduction in clinical cases observed in Korhogo in the both groups of villages from January to May was natural, following reduction in transmission during the dry season (Dossou-Yovo J, unpublished data), and although the low malaria incidence continued throughout June and July 2000 because of the late start of the rainy season in that year, the percentage impact of the treated nets on clinical incidence remained constant during both the rainy and the dry seasons. The reduction in all investigated parasitological as well as clinical parameters was correlated with a sharp reduction in malaria transmission (26 infective bites per man per year in

### Table 2

Malarialmetric indices and clinical malaria in the study groups in 1997 before implementation of ITN

<table>
<thead>
<tr>
<th>Methodology</th>
<th>Parameters</th>
<th>Group of not treated villages in 1999</th>
<th>Group of treated villages in 1999</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cross-sectional surveys</td>
<td>Mean <em>P. falciparum</em> prevalence %</td>
<td>1,048 (84.0 (77.7–90.3))</td>
<td>1,032 (80.0 (72.8–87.2))</td>
</tr>
<tr>
<td>Cross-sectional surveys</td>
<td>Geometric mean parasite density (falciparum trophozoites/µL)</td>
<td>1,048 (199 (122–325))</td>
<td>1,032 (143 (83–246))</td>
</tr>
<tr>
<td>Active case detection</td>
<td>Incidence of clinical malaria episodes per child-year</td>
<td>1,209 (2.3 (1.6–3.2))</td>
<td>1,136 (2.9 (2.1–4.0))</td>
</tr>
</tbody>
</table>

### Table 3

Effect of ITN on malarialmetric and haematological indices in asymptomatic children observed in 1999 during cross-sectional surveys in an area of pyrethroid resistant vectors.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Age of children</th>
<th>Control group</th>
<th>Treated group</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean <em>P. falciparum</em> prevalence %</td>
<td>0–4 yrs</td>
<td>911 (68.5 (64.9–72.1))</td>
<td>970 (56.6 (53.0–60.2))</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Geometric mean parasite density (falciparum assexual forms/µL)</td>
<td>0–4 yrs</td>
<td>911 (69 (53–91))</td>
<td>970 (29 (22–38))</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Packed cell volume %</td>
<td>0–2 yrs</td>
<td>72 (30.8 (29.6–31.9))</td>
<td>83 (32.8 (31.9–33.7))</td>
<td>0.007</td>
</tr>
</tbody>
</table>
netted villages versus 55 in the controls) (Dossou-Yovo J, unpublished data).

The parasitological and clinical parameters observed in 1999 were lower than those of 1997. Those reductions may be attributed to the health education that we have carried out, provision of medical supplies, and recruitment of medical auxiliaries without any specific antimalarial campaign.

One hypothesis that has been proposed to explain the success of pyrethroid-treated nets in areas with pyrethroid resistance is based on the fact that the irritability of resistant mosquitoes due to pyrethroids is less than that of susceptible insects. Additionnal studies have confirmed that the resistant mosquitoes remained a longer time on treated surfaces. Thus, the time of contact of the resistant insects with a pyrethroid deposit may be so long that enough insecticide would be absorbed to reach the relatively high dose required to kill them.

The current study carried out at the community level has demonstrated that the An. gambiae resistance due to the kdr gene did not have an influence on the efficacy of malaria vector control in Northern Côte d’Ivoire, when treated nets were used. If such results are encouraging in areas of kdr resistance, one does not know what could happen in areas of metabolic resistance. Indeed, involvement of metabolic resistance mechanisms have been described in An. gambiae in North Cameroon and An. funestus in KwaZulu Natal. However, the efficacy of pyrethroid-treated nets has never been evaluated in these conditions. It should be done in the future. We may be reasonably optimistic but we have to continue searching for alternative methods or insecticides to counter insecticide resistance. Careful monitoring of resistance in malaria vectors and its impact on disease control is clearly a priority in Africa where most countries rely on vector control to reduce malaria transmission and morbidity.

Received February 14, 2005. Accepted for publication June 9, 2005.

Acknowledgments: The authors thank Dr. F. Simondon of Institut de Recherche pour le Développement for advice on the protocol and Prof. C. F. Curtis for relevant comments and help in the translation of this paper. They would like to thank very much Dr. M. Zaim and WHO/PES for their permanent support and crucial fund raising for this study. The authors also thank the inhabitants of the villages for participating in the study.

Authors’ addresses: Marie-Claire Henry, Serge-Brice Assi, Joel Dossou-Yovo, Fabrice Chandre, Pierre Carnevale, Institut Pierre Richet, BP 1500, Bouaké 01, Côte d’Ivoire, Telephone: +225 31 63 37 46, Fax: +225 31 63 27 38, (Because of war, the Institut Pierre Richet is currently closed.) Christophe Rogier, Unité de Parasitologie, Institut de Médecine Tropicale du Service de Santé des Armées (IMTSSA), BP 46, Parc du Pharo, 13998 Marseille-Armées, France, Telephone: +33 4 91 15 01 50/52, Fax:+ 33 4 91 15 01 64, E-mail: christophe.rogier@wanadoo.fr. Pierre Guillet, Parasitic Diseases and Vector Control, Control, Prevention and Eradication, WHO, 1211 Geneva 27, Switzerland, E-mail: guilletp@who.int.

Reprint requests: Marie-Claire Henry, Centre Muraz, 01 BP 390 Bobo-Dioulasso 01, Burkina Faso, Telephone: +226 20 97 29 44, Fax: +226 20 98 15 51, E-mail: depauw.henry@fasonet.bf.

REFERENCES

8. Rogier C, Ly AB, Tall A, Cisse B, Trape JF, 1999. Plasmodium falciparum clinical malaria in Dielmo, a holoendemic area in Senegal: no influence of acquired immunity on initial symp-