Epidemiol. Infect. (1999), **122**, 461–469. Printed in the United Kingdom

Risk factors for *Plasmodium vivax* infection in the Lacandon forest, southern Mexico

R. DANIS-LOZANO[1], M. H. RODRIGUEZ[2]*, L. GONZALEZ-CERON[1] AND M. HERNANDEZ-AVILA[3]

[1] *Centro de Investigación de Paludismo, Instituto Nacional de Salud Pública, Tapachula, Mexico*
[2] *Centro de Investigaciones Sobre Enfermedades Infecciosas, Instituto Nacional de Salud Pública, Av. Universidad 655, Col. Sta. Maríá Ahuacatitlan, Cuernavaca, Mexico 62508*
[3] *Centro de Investigaciones en Salud Poblacional, Instituto Nacional de Salud Pública, Cuernavaca, Mexico*

(*Accepted 18 February 1999*)

SUMMARY

A study was conducted to characterize the risk of *Plasmodium vivax* infection in the Lacandon forest, southern Mexico. Blood samples and questionnaire data were collected in 1992. Malaria cases ($n = 137$) were identified by the presence of symptoms and a positive thick blood smear. The control group included individuals with negative antibody titres and no history of malaria ($n = 4994$). From 7628 individuals studied, 1006 had anti-*P. vivax* antibodies. Seroprevalence increased with age. Risk factors associated with infection included: place of birth outside the village of residence (odds ratio, OR 11·67; 95% CI 5·21–26·11); no use of medical services (OR 4·69, 95% CI 3·01–7·29), never using bed-nets (OR 3·98, 95% CI 1·23–12·86) and poor knowledge of malaria transmission, prevention and treatment (OR 2·30, 95% CI 1·30–4·07). Health education represents the best recommendation for controlling the disease in the area.

INTRODUCTION

Plasmodium vivax is responsible for 98% of all malaria cases in Mexico, 20% of which are reported by the state of Chiapas every year [1]. Half of the infections in Chiapas occur in the Lacandon forest, bordering with Guatemala (annual *P. vivax*-parasite index of 85·71 per 1000 inhabitants in 1993) [1]. *Anopheles albimanus*, *A. pseudopunctipennis* and *A. vestitipennis* are the principal malaria vectors in this area [2, 3], where epidemic outbreaks, associated with refugee migrations from Central America, began in 1981 [2]. The instability of human settlements and rapid ecological changes, caused by the clearing of the forest for agriculture and cattle raising, contribute to a persistent but unstable malaria transmission that is difficult to control.

* Author for correspondence.

To better improve malaria control in this region this study was designed to identify the determining risk factors of *P. vivax* infection among inhabitants in the area [4].

MATERIALS AND METHODS

Site selection

The study was carried out in eight villages located in the Lacandon tropical rain forest, at a mean altitude of 500 m. The mean annual temperature in the area is 26 °C, with relative humidity of 85% and a mean annual rainfall of 3000 mm [5], where precipitation extends from May to November. Several rivers cross the region, of which, the Lacantún and Usumacinta are the most important. The study selected villages according to their localization (Fig. 1). One group of four villages was located along the Lacantún River:

https://doi.org/10.1017/S0950268899002319 Published online by Cambridge University Press

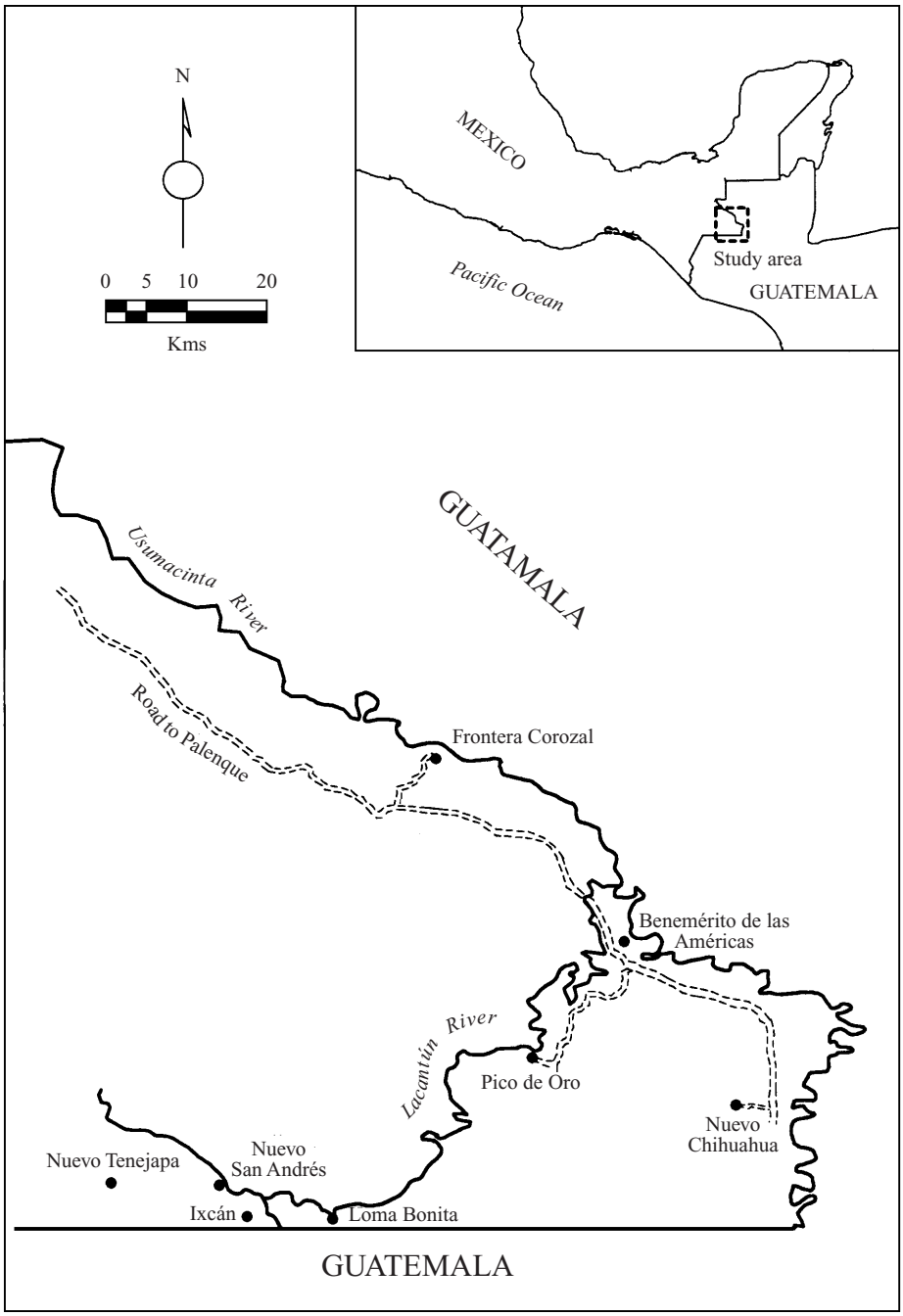

Fig. 1. Map of the study area.

Nuevo San Andrés, Nuevo Tenejapa, Ixcán and Loma Bonita. These villages are geographically isolated with difficult access to medical care services. The other group of four villages, situated along the road to Palenque City (Frontera Corozal, Benemérito de las Américas, Nuevo Chichuahua and Pico de Oro) is more dispersed, with a high influx of Central American migrants, but with better access to medical care services. In both areas, most houses are made of mud or bamboo, thatch or corrugated-sheet roofs, while very few are made of brick, with concrete walls. The population in the villages includes diverse combinations of Mexican-Indian (mestizo) groups speaking Mayan dialects (Chool, Tzeltal and Tzotzil) and Spanish. The majority of the population subsists from farming corn, beans, and peppers, while a smaller proportion cultivates coffee and raises cattle.

Survey methods.

From May to September 1992 interviewers conducted a census in each village and collected in-

https://doi.org/10.1017/S0950268899002319 Published online by Cambridge University Press

formation from household members regarding demographic characteristics, time of residence in the study area, occupation, history of malaria, access to antimalaria treatment, and personal protection against mosquitoes. Interviewers also examined the condition of bed nets, type of housing and construction materials used, and the presence of animals and vegetation near the dwellings. Information on children less than 10 years of age was obtained from one or both parents. Local interpreters aided in interviewing non-Spanish speakers. At the time of the interview, field workers also obtained a blood sample by fingerprick. Before administering the questionnaire and blood sampling, informed consent was obtained from all adult participants and from parents of minors.

To study risk factors for malaria two groups were identified. One (cases, $n = 137$) consisted of individuals who reported symptoms of malaria during the last 6 months before the interview and who were diagnosed with *P. vivax* infection (thick film smear) at local health facilities or through health visitors of the Chiapas State Malaria Control Program. The second group (controls, $n = 4994$) included individuals with no fever episodes during the previous 6 months and with anti-*P. vivax* antibody titres (absorbency detected by ELISA) below the positive cut-off point. Individuals with positive serology but no diagnostic record in the control program, most probably have had contact with *P. vivax*, but because an active infection (within 6 months) could not be documented they were excluded from the study.

Measurement of anti-*P. vivax* antibodies

Dried blood samples collected on filter papers (Whatman No. 2) [6] were stored with silica gel and transported to the laboratory at the Centre for Malaria Research (CIP) in Tapachula, where they were stored at -20 °C until processed. The presence of antibodies against *P. vivax* in the samples were investigated using an ELISA [7]. A detergent-soluble *P. vivax* antigen, an extract of a mixture of several batches of enriched parasite preparations obtained from infected patients was used as antigen. Blood samples were eluted in PBS (corresponding to a dilution of 1:100 of original sera) and were added to individual *P. vivax*-sensitized wells, followed by peroxide-conjugated goat anti-human gamma globulin (against IgM, IgG, IgA; Miles Sci.) and 2,2 azinobis-(3 ethylbenz-thiazoline-6 sulphonic acid) (Sigma Chemical Co.) as a substrate.

All samples were analysed in duplicate; positive controls, obtained from subjects that suffered several malaria episodes, and negative controls, obtained from people that never suffered malaria nor visited malaria endemic areas, were included in each plate. Plates were read using a spectrophotometer (Bio-Kinetics Reader EL 312) at 405 nm. Optical density values greater than three standard deviations above the mean absorbency (0·25) value obtained in 20 negative control sera were considered potential cases.

Data analysis

Malaria knowledge index

Beliefs and practices related to malaria were evaluated using a questionnaire based on three main questions: how is malaria transmitted? how is malaria treated? and how can malaria be prevented? According to the number of correct answers an index with three categories was constructed: low (no correct answers), medium (two correct answers) and high (three correct answers).

An exploratory analysis was conducted to obtain the frequencies and descriptive statistics of each variable. We examined the relationship of malaria infection with: age (in 5-year intervals), place of residence, place of birth (the village of residence *vs.* other), occupation, history of malaria, access to antimalaria treatment, bed net usage and condition of nets (good condition *vs.* with holes), use of smoke, mosquito coils or commercial insecticides, type of housing, presence of animals and vegetation (0–20, 21–29, 30–50 and 60–100%) and knowledge/attitude index. Bivariate models were used to estimate the association between malaria infection and each variable. We also estimated the age and community adjusted odds ratios. In a final analysis, all variables significant at $P > 0{\cdot}10$ were included simultaneously in a multivariate logistic regression model. Analysis were performed using the statistical packages, EGRET (version 5.0, Statistics and Epidemiology Res. Co. Seattle WA) and STATA (version 3.1, Computer Resource Centre, Santa Monica, CA).

RESULTS

Seroprevalence

Of the 7628 individuals included in the census, 1006 had positive anti-*P. vivax* antibody titres. The mean absorbency value was 0·41, ranging from 0·25 to 2·82.

https://doi.org/10.1017/S0950268899002319 Published online by Cambridge University Press

Table 1. *Seroprevalence of* Plasmodium vivax *in eight villages in the Lacandon Forest Chiapas, Mexico, 1992*

Villages	Seropositives (Total no. tested)*	Seroprevalences 95% CI†
Villages along the road		
Nuevo Chihuahua	84 (519)	16·1 (13·1–19·6)
Frontera Corozal	385 (2580)	14·9 (13·5–16·3)
Benemétito de las Américas	264 (2229)	11·8 (10·5–13·2)
Pico de Oro	92 (1147)	8·0 (6·5–9·7)
Sub total	825 (6475)	12·7 (11·9–13·5)
Villages along the Lacantún river		
Ixcan	86 (396)	21·7 (17·7–26·1)
Nuevo San Andrés	35 (196)	17·8 (12·7–23·9)
Nuevo Lenejapa	35 (218)	16·0 (11·4–21·6)
Loma Bonita	25 (191)	13·1 (8·6–18·7)
Sub total	181 (1001)	18·0 (15·7–20·6)
Total	1006 (7476)	13·4 (12·6–14·2)

* Total of individuals tested.
† CI, confidence interval.

The distribution of antibody titres (absorbency) in the population was skewed to the right in both sexes. In all villages, the seroprevalence increased with age, peaking in the over 60 years age group. Seroprevalence was highest among individuals working in agriculture and cattle raising (27·4%), followed by those involved in domestic activities (21·1%), schoolchildren (5·8%) and merchants (12·9%).

The villages located along the Lacantun River had higher seroprevalence rates (18·0%) than those located along the road (12·8%). Prevalence rates higher than 10% were found in seven villages (Table 1), where Ixcan was the highest (22·2%). The mean age for cases and controls were 29·6 and 24·4 years, respectively. The highest number of cases occurred in the 10–19 years age group (35·04%) and the lowest in the age group of $>$ 60 years (5·84%).

Risk factors significantly associated with *P. vivax* infection according to the bivariate and multivariate analysis are presented in Table 2. Place of birth other than the village of residence was associated with a higher risk of infection. After adjusting for age and community of residency, it was estimated that immigrant individuals had an 11-fold greater risk of malaria infection. When adjusted in the multivariate model for other important predictors, the observed association was attenuated but remained significant (OR 8·48, 95% CI 3·48–20·63).

The use of medical services had an important effect on the risk of infection. The percentage of cases and controls that preferred not to use the health services were 21·9 and 5·09% respectively. Not using medical services was associated with an increased risk of malaria (multivariate adjusted OR 3·68, 95% CI 2·16–6·29).

Bed net usage had an important protective effect; 15·3 and 8·6% of cases and controls, respectively, never used them. In the bivariate analysis, participants who reported an infrequent use of bed nets, compared to those who reported continual usage, had a 30% increased risk of infection and those who never used bed nets had a twofold risk increase. However, this association did not remain when analysed in the multivariate model. When the analysis of this variable was restricted to women and children, no protection was documented for those using bed nets infrequently. However, never using bed nets resulted in a 3·4-fold increase in the risk. In the multivariate model for women and children, never using bed nets was the only factor that remained significant, with an OR 3·98 (95% CI 1·23–1286).

Bed nets with holes greater than 2 cm were observed in 17·52% of the cases and 9·7% of the controls. There was no association between the bed net condition and the risk of infection in the multivariate analysis. When analysed strictly for women and children, only those with bed-net holes larger than 2 cm had an increased risk of 3·5-fold. In the multivariate model, the increase in the risk was more apparent (OR 7·44, 95% CI 1·34–41·34). Individuals not using indoor insecticide spraying had an OR of 2·67, but this association was not significant in the

https://doi.org/10.1017/S0950268899002319 Published online by Cambridge University Press

Table 2. *Risk factors associated with infection with* Plasmodium vivax *in the Lacandon forest, Chiapas, Mexico. 1992, thought bivariate, logistic regression and multiple logistic regression analysis*

Risk factors	Cases* No. (%)	Controls* No. (%)	Bivariate unadjusted OR (95% CI)	Logistic regression adjusted† OR (95% CI)‡	Multiple regression OR (95% CI)
Place of birth outside the Study area	9 (6·57)	45 (1·0)	7·7 (3·72–15·80)	11·67 (5·21–26·11)§	8·48 (3·48–20·63)§
Study area	129 (93·43)	4949 (99·0)	1·0	1·0	1·0
Use of medical care					
No	30 (21·90)	254 (5·09)	5·23 (3·43–7·97)	4·69 (3·01–7·29)§	3·68 (2·16–6·29)§
Yes	107 (78·10)	4740 (94·41)	1·0	1·0	1·0
Use of bed-nets					
Never	21 (15·33)	432 (8·65)	2·05 (1·25–3·36)	1·67 (0·75–3·71)	1·35 (0·35–5·19)
Sometimes	45 (32·85)	1496 (29·96)	1·30 (0·87–1·85)	1·07 (0·72–1·58)	1·18 (0·79–1·75)
Always	71 (51·82)	3006 (61·39)	1·0	1·0	1·0
Use of bed-nets¶					
Never	19 (26·76)	324 (9·63)	3·40 (1·93–5·98)	3·98 (1·23–12·86)§	
Sometimes	17 (23·94)	1006 (29·93)	0·98 (0·55–1·74)	0·85 (0·46–1·54)	
Always	35 (49·29)	2031 (60·42)	1·0	1·0	
Condition of bed-nets					
Hole > 2 cm	24 (17·52)	488 (9·77)	1·95 (1·24–3·05)	1·46 (0·59–3·64)	1·28 (0·30–5·39)
Hole < 2 cm	4 (2·92)	188 (3·76)	0·84 (0·31–2·22)	0·71 (0·22–2·31)	0·72 (0·21–2·37)
Intact	109 (78·10)	4318 (86·46)	1·0	1·0	1·0
Condition of the bed-nets¶					
Hole > 2 cm	21 (29·75)	363 (10·80)	3·44 (2·05–5·79)	7·44 (1·34–41·43)§	
Hole < 2 cm	2 (2·81)	136 (4·04)	0·87 (0·01–3·30)	1·44 (0·27–7·65)	
Intact	48 (67·60)	2862 (85·15)	1·0	1·0	
Use of commercial coils					
No	132 (96·53)	4534 (90·79)	2·67 (1·12–6·39)	1·83 (0·73–4·60)	1·64 (0·65–4·15)
Yes	5 (3·65)	460 (9·21)	1·0	1·0	1·0
Use of smoke					
No	100 (72·99)	3584 (71·77)	1·06 (0·72–1·55)	1·06 (0·72–1·58)	
Yes	37 (27·10)	1410 (28·23)	1·0	1·0	
Use of blankets					
No	65 (47·45)	3058 (61·24)	0·56 (0·40–0·79)	0·62 (0·43–0·88)§	
Yes	72 (52·55)	1935 (38·76)	1·0	1·0	
Knowledge, attitudes related to malaria					
Low	34 (24·63)	896 (17·44)	2·15 (1·36–3·39)	2·30 (1·30–4·07)‖	2·13 (1·20–3·77)
Middle	61 (44·20)	1711 (34·96)	2·02 (1·36–3·00)	2·40 (1·57–3·67)§	2·20 (1·42–3·40)‖
High	42 (30·43)	2387 (47·49)	1·0	1·0	1·0
Vegetation around the house (%)					
60–100	75 (54·74)	2533 (50·72)	1·04 (0·71–1·53)	1·39 (0·82–3·08)	
30–59	21 (15·32)	1009 (20·20)	0·74 (0·44–1·24)	1·38 (0·66–286)	
0–29	41 (29·92)	1452 (29·07)	1·0	1·0	
Place where animals pass the night (chicken)					
Outside	82 (59·85)	2396 (47·98)	1·98 (1·35–2·91)	0·88 (0·56–1·40)	0·81 (0·51–1·47)
Other house	8 (5·84)	101 (2·02)	4·59 (2·12–9·91)	22·15 (2·23–219·8)§	0·92 (0·51–1·65)
In the house	8 (5·84)	236 (4·73)	1·97 (0·92–4·18)	1·64 (0·61–4·41)	0·90 (0·47–1·72)
No animals	39 (28·47)	2261 (45·27)	1·0	1·0	1·0

* Totals vary due to missing information.
† Adjusted for age and village.
‡ CI, confidence intervals.
§ $P < 0·001$ value for multivariated adjusted.
‖ $P < 0·05$ value for multivariated adjusted.
¶ Women and children only.

https://doi.org/10.1017/S0950268899002319 Published online by Cambridge University Press

multivariate model (Table 2). The use of other protection methods, such as smoke (OR 1·06) and blankets (OR 1·63) did not provide significant protection.

A significant associated between knowledge of malaria and risk of infection was documented in the bivariate analysis. Individuals who had a medium or low level knowledge (according to the index) presented higher risk of infection (OR 2·02 and 2·15, respectively). The observed association remained unchanged, even after controlling for other potential risk factors in the multivariate model (Table 2).

The amount of vegetation surrounding the house had little effect on the risk of infection according to the bivariate analysis. The presence of domestic animals such as cows, horses, pigs, dogs and cats had no effect on risk infection; however, the presence of chicken coops near human dwellings was associated with an increased risk of infection as detected in the bivariate (OR 4·59) but no significant association was documented in multivariate analyses (Table 2).

DISCUSSION

Anti-*P. vivax* seroprevalence rates in the eight villages under study reflected frequent contact of the population with this parasite. In seven villages the prevalence and antibody titres increased above the age of 10; this most likely reflects the cumulative effect of repetitive exposures to the parasite [8–10]. Higher seroprevalence rates in the villages located along the river, compared with prevalence rates in villages located along the road ($P = 0{\cdot}0001$, χ^2 test), could in part, be explained by a higher proportion of agricultural workers in riverine villages (27·41 *vs.* 23·60%). The geographic characteristics of the area, combined with difficult access to the villages tend to force residents to start work during the early morning, thus increasing the probability of contact with mosquito vectors [11–13].

Although *A. albimanus*, *A. pseudopuncipennis* and *A. vestitipennis* have been identified as the principal malaria vectors in the area [2], in a parallel study carried out in the same area, only *A. albimanus* and *A. vestitipennis* were found infected with *P. vivax* [14]. Both mosquito species are exophagic, but they also readily feed on humans indoors. The normal habitat of *A. vestitipennis* is undisturbed forest and *A. albimanus* breeding occurs in low lands associated with cattle corrals [3, 15]. Both ecological types are found in the study area, and it is possible that the antibody-prevalence pattern (high in working age groups) and its occupational distribution (agriculture and domestic activities) result from the combined contribution of these vectors to malaria transmission.

Malaria in the area, as well as in most parts of Mexico, is unstable with seasonal outbreaks [16]; this and other local conditions, including social unrest, made it very difficult to conduct a study on malaria risk factors using clinical cases. Restricted access to individuals diagnosed parasitically by the programme limited our study to risk factors for recent infections/reinfections. By excluding participants with high antibody titres who were not confirmed by the programme, we strengthened the case definition to include incident cases. The proportion of individuals with high antibody titres but no parasitic diagnosis was 15·83%. This group may represent a portion of the population exposed to the parasite, probably several times in the past, which has acquired resistance to the disease, and therefore showed no clinical symptoms [17, 18] or if symptomatic they self-medicated.

The cross-sectional nature of our study design [19] does not allow precise identification of the conditions at the time that infection occurred, however, it proved adequate to describe the general conditions that may influence disease transmission in the study area. This level of analysis is useful for malaria control programmes which, with few resources, have to target interventions and to make policy decisions.

As previously mentioned, residents born outside of the study area were at an increased risk of malaria infection; 12 times higher than that of those born in the village of residence. Pipiatt and Byass reported similar results in Africa [20]. In the case of the Lacandon forest, before social unrest began, migrating individuals, from non-endemic areas in northern Mexico, were attracted to the area, based on expectation of obtaining land and better opportunities. Other groups from endemic areas of Central America also arrived in the region escaping from violence in their homelands. The increased risk of infection with malaria parasites in individuals from the first group, could be due to a lack of immunity to the parasites [21, 22]; whereas, encountering new parasite strains unfamiliar to the immune system [23, 24] could also explain the high risk of infection in the second group.

Type of housing appears as a determining risk factor for malaria infection in other parts of the world [25–27]; however, 90% of the dwellings in the

Lacandon forest are made of wood and mud. Consequently, this study did not assess housing types to the risk of infection with *P. vivax* because of the limited variability in the construction of houses. House improvement has been recommended as a general strategy for malaria control [27, 28], but this measure is obviously restricted by the economy and general welfare of the community and can scarcely be incorporated into the activities of the local control programme.

The use of bed nets in malaria endemic areas offers high levels of protection against mosquito bites, in addition to a decrease in malaria incidence [29]. This study determined that non-use of bed nets resulted in a fourfold risk of infection when restricted to women and children in the area (most of the men over 15 years of age sleep in the fields outside of the villages for 1 or 2 weeks). Bed nets in poor condition also had an important association with higher risk of infection among women and children. These results are similar to those obtained by Fungladda and others [21] in Thailand and Banguero [22] in Colombia.

The levels of protection provided by even untreated bed nets in the Lacandon forest are encouraging to promote their use as part of an organized control programme. Other studies have shown that insecticide-treated bed nets reduce child mortality [13, 30] and severe morbidity [31] in *P. falciparum* malarious endemic areas, but to our knowledge no definitive studies on the use of bed nets against *P. vivax* are available. However, the results from our study and those of Richards and colleagues [32], demonstrating effective control of *A. albimanus* and *A. vestitipennis* with permethrin-treated bed nets in Guatemala, indicate their potential utility in prevention programmes.

Indoor insecticide spraying also protected from infection. It must be noted that the local malaria control programme is mainly based on indoor DDT spray, but some refusal of this measure occurs in the area. In many houses, occupants wash walls and floors soon after spraying, as this is believed to prevent mortality of domestic animals. However, residents spray other locally available commercial insecticides (mainly pyrethroids) with less residual effect than DDT. The protective effect of insecticide application, coupled with that obtained using bed nets indicates that indoor malaria transmission does occur in the area. On the other hand, it also indicates that appropriate education should be provided to the local population regarding the effects of the control measures. According to our results, the use of other types of protection, such as smoke to repel mosquitoes, were not effective as protection measures in the Lacandon forest, as also found by Snow and colleagues [33].

Not using state provided health services was associated with an increased risk of *P. vivax* infection, which was not related to the differences in health-service accessibility between the two village groups studied ($P < 0{\cdot}05$). An apparent contradiction exist between our definition of case (*P. vivax* infection diagnosed by a blood smear) and that not using health services was associated to higher risk of infection. It should be noted that malaria cases that did not attend local health facilities were diagnosed by health workers periodically visiting the villages. Blood smears collected by these personnel are diagnosed at a district central laboratory. Health service usage seems to be related to the people's perception of the sickness and its treatment, as in endemic areas of Africa [25, 34]. After one or more fever episodes, local people learn that vivax malaria is not lethal and that treatment with anti-malaria drugs available in pharmacies can be effective. In the study area, 4-aminoquinoline-based drugs brought from Central America are sold openly and used in insufficient doses as temporary palliatives.

Not using local health facilities was partly a result of poor information regarding malaria. Results from the application of the knowledge/attitude questionnaire indicted that only half of the population in the area knew how malaria is transmitted or of any form of prevention. The same proportion recognized only two or three symptoms of the disease and the lack of information about the disease resulted in a twofold greater risk of infection. Similar results were obtained in studies conducted in Guatemala [35].

In defining malaria incident cases as symptomatic individuals testing positive for *P. vivax* by blood smear, we found that this definition has the disadvantage of not including self-medicated cases or those not receiving attention in health facilities, and thereby had no blood smear record. To assess potential bias, we also compared the group with positive serology and no record of a *P. vivax* positive smear with seronegative controls. Significant differences between groups included age, place of origin, and non-bed net usage. However, use of health services was positively associated with serology. The latter suggests that our case definition mostly detected new cases and that the excluded group most likely

represented chronic or prevalent infections. However, follow-up data to further validate this observation have not yet been obtained.

Among the recommendations derived from this study for the Malaria Control Programme, health education seems to be the most important, in view of the effects it could have on the recognition, prevention and appropriate treatment of the disease. With increasing costs of insecticides and limited resources to control malaria, community participation with the control programmes efforts should be encouraged. This dependence on insecticides could be alleviated by modifications within the household to diminish attraction of the vectors (displacement of domestic animals and clearance of vegetation around human dwellings), as well as avoidance of human-vector contact (use of bed nets). Although the conditions for human-vector contact in agricultural fields were not investigated in this study; it is probable that the men who sleep in the fields should also use bed nets.

ACKNOWLEDGEMENTS

We thank Angel F. Betanzos-Reyes and technicians Luis Dominguez-Sibaja, Victor H. Lopez-Estrada, Pedro Garcia, Arturo Robledo-Perez, Fernando Cano Melendez, Crecencio Diaz-Espinoza, and Juan Ventura for their assistance in field work. This work was financially supported by the UNDP/World Bank/WHO Special Program for Research and Training in Tropical Diseases and the Ministry of Health of Mexico.

REFERENCES

1. Dirección General de Epidemiología, Secretaría de Salud. Situación del paludismo en México. Pal Enf Trans Vector 1994; **5**: 1–16.
2. Loyola EG, Arredondo-Jimenez JI, Rodriguez MH, Bown DN, Vaca-Marin MA. *Anopheles vestitipenis*, the probable vector of *Plasmodium vivax* in the Lacandon forest of Chiapas, Mexico. Trans R Soc Trop Med Hyg 1991; **85**: 171–4.
3. Arredondo-Jimenez JI, Gimnig J, Rodriguez MH, Washino RK. Genetic differences among *Anopheles vestitipennis* subpopulations collected using different methods in Chiapas state, southern Mexico. J Am Mosq Control Assoc 1996; **12**: 396–401.
4. Castillo-Salgado C. Epidemiological risk stratification of malaria in the Americas. Mem Inst Oswaldo Cruz 1992; **87** (suppl III): 115–20.
5. Mauricio JM, Valladares R, Garcia H. Lacandona. Una incorporación anárquica al desarrollo nacional. San Cristobal de las Casas, Mexico. Centro de Investigaciones Ecológicas del Sureste, 1984.
6. Thaver S, Droper CC. Serological survey in the tropics. Reliability of the method of collecting blood on absorbent paper. Trans R Soc Trop Med Hyg 1974; **68**: 1–8.
7. Gonzalez CL, Rodriguez MH. An enzyme-linked immunosorbent assay using detergent-soluble *Plasmodium vivax* antigen for seroepidemiological surveys. Trans R Soc Trop Med Hyg 1991; **85**: 358–61.
8. Nardin EH, Nussenzweig RS, MacGregor IA, Bryan JH. Antibodies to sporozoites. Their frequent occurrence in individuals living in an area of hyperendemic malaria. Science 1979; **206**: 597–9.
9. Druilhe P, Puebla RM, Miltgen F, Perrin L, Gentilini M. Species and stage specific antigens in exoerythrocytic stages of *Plasmodium falciparum*. Am J Trop Med Hyg 1984; **33**: 336–41.
10. Greenwood BM. Immune response to sporozoite antigens and their relationship to naturally acquired immunity to malaria. Bull WHO 1990; **68** (suppl): 184–90.
11. Bordas E, Navarro L, Dows W. Estudio Comparativo de los hábitos del adulto de tres especies de *Anopheles* mexicanos. Rev Inst Salubr Enferm Trop Mexico 1951; **12**:O 35–8.
12. Elliott R. The influence of vector behaviour on malaria transmission. Am J Trop Med Hyg 1972; **21**: 755–63.
13. Adiamah JH, Koram KA, Thomson MC, Lindsay SW, Todd J, Grenwood BM. Entomological risk factors for severe malaria in a per-urban area of the Gambia. Ann Trop Med Parasit 1993; **87**: 491–500.
14. Arredondo-Jimenez JI. Comparative ecology of alopatric populations of *Anopheles vestitipennis* (Diptera: Culicidae) [dissertation]. Davis, California: University of California, 1995.
15. Rodriguez AD, Rodriguez MH, Meza RA, et al. Dynamics of population densities and vegetation association of *Anopheles albimanus* larvae in a coastal area of Southern Chiapas, Mexico. J Am Mosq Control Assoc 1993; **9**: 48–56.
16. Rodriguez MH, Loyola EG. Malaria in Mexico. Proceedings of the 58th Annual Conference of the California Mosquito and Vector Control Association. Sparks, NV. Cal Mosq Vector Control Assoc, 1990: 49–52.
17. Smith T, Genton B, Baea KHP, et al. Relationship between *Plasmodium falciparum* infection and morbidity in a highly endemic area. Parasitol 1994; **109**: 539–49.
18. Molineaux L. *Plasmodium falciparum* malaria: some epidemiological implications of parasite and host diversity. Ann Trop Parasitol 1996; **90**: 379–93.
19. Schlesselman JJ. Case-control studies. Design, conduct, analysis. New York: Oxford University Press, 1982.
20. Pipiatt R, Byass P. Risk factors for malaria among British missionaries living in tropical countries. J Trop Med Hyg 1990; **93**: 397–402.
21. Funglada W, Sornmani S, Klongkamnuankaran K, Hungsapruek T. Sociodemographic and behavioral

factors associated with hospital malaria patients in Tanchanaburi, Thailand. J Trop Med Hyg 1987; **90**: 233–7.
22. Banguero H. Socioeconomic factors associated with malaria in Colombia. Soc Sci Med 1984; **19**: 1099–104.
23. Conway DJ, Grenwood BM, McBride JS. The epidemiology of multiple-clone *Plasmodium falciparum* infections in Gambian patients. Parasitol 1991; **103**: 1–6.
24. Marsh K. Malaria; a neglected disease. Parasitol 1992; **104**: 53–69.
25. Grenwood BM. The microepidemiology of malaria and its importance to malaria control. Trans R Soc Trop Med Hyg 1989; **83** (suppl): 25–9.
26. Bruce-Chwatt LJ. Essential malariology, 2nd ed. London: Heinemann, 1985.
27. Schofield CJ, White GB. House design and domestic vectors of disease. Trans R Soc Trop Med Hyg 1984; **78**: 285–92.
28. Osaka C, Gamage M, Richard C, et al. Clustering of malaria infections within an endemical populations: risk of malaria associated with the type of housing construction. Am J Trop Med Hyg 1991; **45**: 77–85.
29. Curtis CF, Lines JD, Carnevale P, et al. Impregnated bed-nets and curtains against malaria mosquitoes. Curtis CF. ed. Control of disease vectors in the community. Wolfe Publishing: CRC Press, 1991: 5–46.
30. Bradley AK, Grenwood BM, Grenwood AM, et al. Bed nets (mosquitoes nets) and morbidity from malaria. Lancet 1986; **ii**: 204–7.
31. Nevill CG, Some ES, Mung'ala VO, et al. Insecticide-treated bednets reduce mortality and severe morbidity from malaria among children on the Kenyan coast. Trop Med Int Health 1996; **1**: 139–46.
32. Richards Jr FO, Zea-Flores R, Sexton JD, et al. Efectos de los mosquiteros impregnados con permetrina sobre los vectores de la malaria en el norte de Guatemala. Bol Oficina Sanit Panam 1994; **117**: 1–11.
33. Snow RW, Bradley AK, Hayes R. Byass P, Greenwood BM. Does woodsmoke protect against malaria? Ann Trop Med Parasitol 1987; **81**: 449–51.
34. Cham K, MacCormack C, Touray A, Beldeh S. Social organization and political factionalism: PHC in the Gambia. Hlth Policy Plan 1987; **2**: 214–26.
35. Ruebush II TK, Weller SC, Klein RE. Knowledge and beliefs about malaria on the Pacific coastal plain of Guatemala. Am J Trop Med Hyg 1992; **46**: 451–9.