Individual risk factors for *Plasmodium vivax* infection in the residual malaria transmission focus of Oaxaca, Mexico

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Abstract

Objective. To identify individual risk factors for malaria infection of inhabitants in the residual transmission focus on the Pacific coast of Oaxaca, Mexico. **Materials and Methods.** A population-based, matched case-control study was conducted from January 2002 to July 2003 comparing the frequency of exposure to individual risk factors in subjects presenting clinical malaria and uninfected controls. A malaria case was defined as an individual living in the study area presenting malaria symptoms and a *Plasmodium vivax*-positive thick blood smear; controls were individuals negative to *P. vivax* parasites and antibodies of the same gender and with ± five years as the case. A standardized questionnaire was used to record information about the individual risk factors associated with malaria episodes in cases and two controls for each case. **Results**. In a multiple conditional logistic regression model analysis of data from 119 cases and 238 controls, 18 out of 99 variables were significantly associated ($p< 0.05$) with increased risk of malaria, including: being born in another locality (RM 3.16, 95% IC 1.16-6.13); speaking only an autochthonous language (RM= 2.48, 95% IC 1.19-3.77); having poor knowledge about malaria (RM= 2.26 95% IC 1.10-4.66 $P< 0.02$); the amount of vegetation around the house (RM= 20.43, 95% IC 5.98-70.87, $P< 0.000$; RM= 3.78, 95% IC 1.21-11.80, for 60-100% and 30-59%, respectively);

Danis-Lozano R, Rodríguez MH, Betanzos-Reyes AF, Hernández-Avilia JE, González-Cerón L, Méndez-Galván JF, Velázquez-Monroy OJ, Tapia-Conyer R. Factores individuales de riesgo para la infección con *Plasmodium vivax* en el foco residual de transmisión de paludismo de Oaxaca, México. Salud Publica Mex 2007;49:199-209.

Resumen

Objetivo. Identificar los factores de riesgo individuales determinantes para contraer paludismo en habitantes del foco residual de transmisión de paludismo localizado en la costa del Pacífico de Oaxaca. **Material y métodos.** Se realizó un estudio pareado de casos y controles, con base poblacional de enero de 2002 a julio de 2003, comparando la frecuencia de exposición a diversos factores de riesgo individuales en sujetos que presentaron un cuadro clínico de paludismo y controles no infectados. Un caso de paludismo fue definido como un individuo que vive en el área de estudio que presentó síntomas de paludismo y diagnosticado positivo a *P. vivax* en examen de gota gruesa de sangre, los controles fueron individuos negativos a parásitos y anticuerpos anti-*P. vivax* del mismo sexo y ± cinco años la edad del caso. Se usó un cuestionario estandarizado para registrar información de factores de riesgo individuales asociados a episodios de paludismo en casos y dos controles por caso. **Resultados**. El análisis en un modelo de regresión logística condicional múltiple, 18 de 99 variables fueron significativamente asociadas ($p< 0.05$) con el incremento en el riesgo de paludismo, incluyendo: nacer fuera de la localidad (RM 3.16, 95% IC 1.16-6.13); hablar sólo un idioma autóctono (RM= 2.48, 95% IC 1.19-3.77); pobre conocimiento de cómo se transmite y trata el paludismo (RM= 2.26 95% IC 1.10-4.66 $P< 0.02$);

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living in houses constructed with perishable materials (RM= 2.85, 95% IC 1.62-5.01); living on the periphery of the town (RM= 6.23, 95% IC 3.50-11.0); sleeping on a dirt floor (RM= 2.98, 95% IC 1.78-5.01) or with two or more people in the same bed (RM= 1.85, 95% CI 1.09-3.14); not using bed nets (RM= 2.39, 95% IC 1.18-4.83, *P*< 0.003) or using bed nets with holes (RM= 13.93, 95 IC 2.48-78.01); traveling outside of the village (RM= 9.16, 95% IC 1.98-42.2); and previous malaria cases in the house (RM= 5.84, 95% IC 3.33-10.22). **Conclusions**. Risk of malaria infection was associated with socio-cultural and environmental factors exposing individuals to mosquito bites. A higher risk of malaria infection occurred outside the locality and by intradomiciliar transmission probably as a result of relapsing asymptomatic relatives.

Key words: malaria; *Plasmodium vivax*; risk factors; Mexico

cobertura de vegetación alrededor de la casa (RM= 20.43, 95% IC 5.98-70.87, *P*< 0.000; RM= 3.78, 95% IC 1.21-11.80, para 60-100% y 30-59%, respectivamente); casas construidas con materiales perecederos (RM= 2.85, 95% IC 1.62-5.01); localización de la casa en la periferia de la localidad (RM= 6.23, 95% IC 3.50-11.0); dormir en el suelo (RM= 2.98, 95% IC 1.78-5.01); dormir con dos o más personas en la misma cama (RM= 1.85, 95% CI 1.09-3.14); no uso de mosquiteros (RM= 2.39, 95% IC 1.18-4.83, *P*< 0.003), uso de mosquiteros con agujeros (RM= 13.93, 95 IC 2.48-78.01); viajes fuera de la localidad de residencia (RM= 9.16, 95% IC 1.98-42.2); y casos previos de paludismo en la casa (RM= 5.84, 95% IC 3.33-10.22). **Conclusiones**. El riesgo para la infección de paludismo se asoció a factores socioculturales y ambientales que incrementan la exposición de los individuos a la picadura de mosquitos. Un riesgo mayor de infectarse por paludismo ocurrió en la periferia de la localidad y por transmisión intradomiciliaria probablemente a causa de los familiares asintomáticos con recaídas.

Palabras clave: malaria; *Plasmodium vivax*; factores de riesgo; México

Malaria transmission is under control in most regions of Mexico, but persists in residual foci located on the Pacific coast. In the past, these foci were the origin of outbreaks when malaria control activities declined.[1] The main residual focus of malaria transmission is located in the foothills and on the coast of Oaxaca state. The number of reported malaria cases in the area presented a progressive decrease between 1990 (9 112 cases) and 1997 (844 cases), but an outbreak occurred in 1998 (over 15 000 cases). All cases are caused by *Plasmodium vivax* and the main vectors are *Anopheles pseudopunctipennis* and *An. albimanus*.

Recent studies circumscribed the residual transmission focus around the city of Pochutla.[2] These studies demonstrated substantial variability in malaria transmission among localities and that the main factors determining malaria transmission in this focus are related to climate and geographic and hydrologic conditions that favor the nearby breeding of mosquito vectors. This exceptional combination of conditions supporting mosquito vector abundance could explain the malariogenic potential and resilience to control within the focus. Additionally, the proximity of malarious villages to the main roads suggested that short-range human movements were responsible for the dispersion of the transmission around Pochutla, but it was not possible to identify the individual risk factors for malaria infection. Understanding these factors may help to better identify key components perpetuating malaria transmission in the focus. Presented herein are the results of a case-control study conducted to identify determinant risk factors for clinical malaria in the residual malaria transmission focus of Oaxaca.

Material and Methods

Study area

The study protocol and the informed consent letter signed by participants prior to interviews and sampling was approved by the Ethics Committee of the National Institute of Public Health, Mexico. The study area occupies a corridor of 150 km along the Pacific Ocean coast of the state of Oaxaca, between 15° 35'N and 16° N and between 96° W and 97° W. Malaria cases peak during the dry season between October and May. The mean annual temperature in the area is 26 °C with relative humidity of 85%. This area reported the highest number of malaria cases in the country during the outbreak of 1998.

The study included 60 villages with 25 583 inhabitants. Localities were selected following a balanced sampling strategy[3] according to a 4-level malaria transmission intensity index based on the annual malaria incidence and persistence of transmission during the previous 10 years.[2] The localities were selected based on the distribution of this index by size of the village and its distribution according to elevation: coast (0-200 masl), foothills (200-750 masl) and mountain ranges (750-1500 masl). Accordingly, 15 villages from the coast, 29 from the foothills and 16 from the mountain ranges were

selected (figure 1). The inhabitants belong to Mexican ethnic groups that speak Spanish and Zapotecan. The majority of them subsist from cultivating corn, beans, and peppers, while a smaller proportion cultivates coffee and raises cattle. People live in small villages of around 60 to 250 houses, usually connected by unpaved roads. Houses are made of mud or bamboo, thatch or corrugated tin sheet roofs, and few are made of brick with concrete walls.

Epidemiological survey

A census, along with a cross-sectional prevalence survey to measure anti-*P. vivax* antibodies, were carried out during October and December 2001 in 23 of the villages where the case-control study was conducted (Danis R. *et al*. in preparation). Dried blood samples from finger pricks were collected on filter papers and the presence of antibodies against *P. vivax* in the samples was investigated using an ELISA.[4] Matched controls for the case-control study were selected in this survey among subjects who were negative to anti-*P. vivax* antibodies.

Study design and case definitions

A prospective case-control study was conducted[5] in the health jurisdictions of the coastal and central valleys of Oaxaca from January 2002 to July 2003. Malaria cases were detected in the community by the Malaria Control Program's active case detection system and by passive surveillance (febrile individuals seeking care from community health workers or health clinics). A case of malaria was defined as an individual currently living in the study area with no antecedents of previous

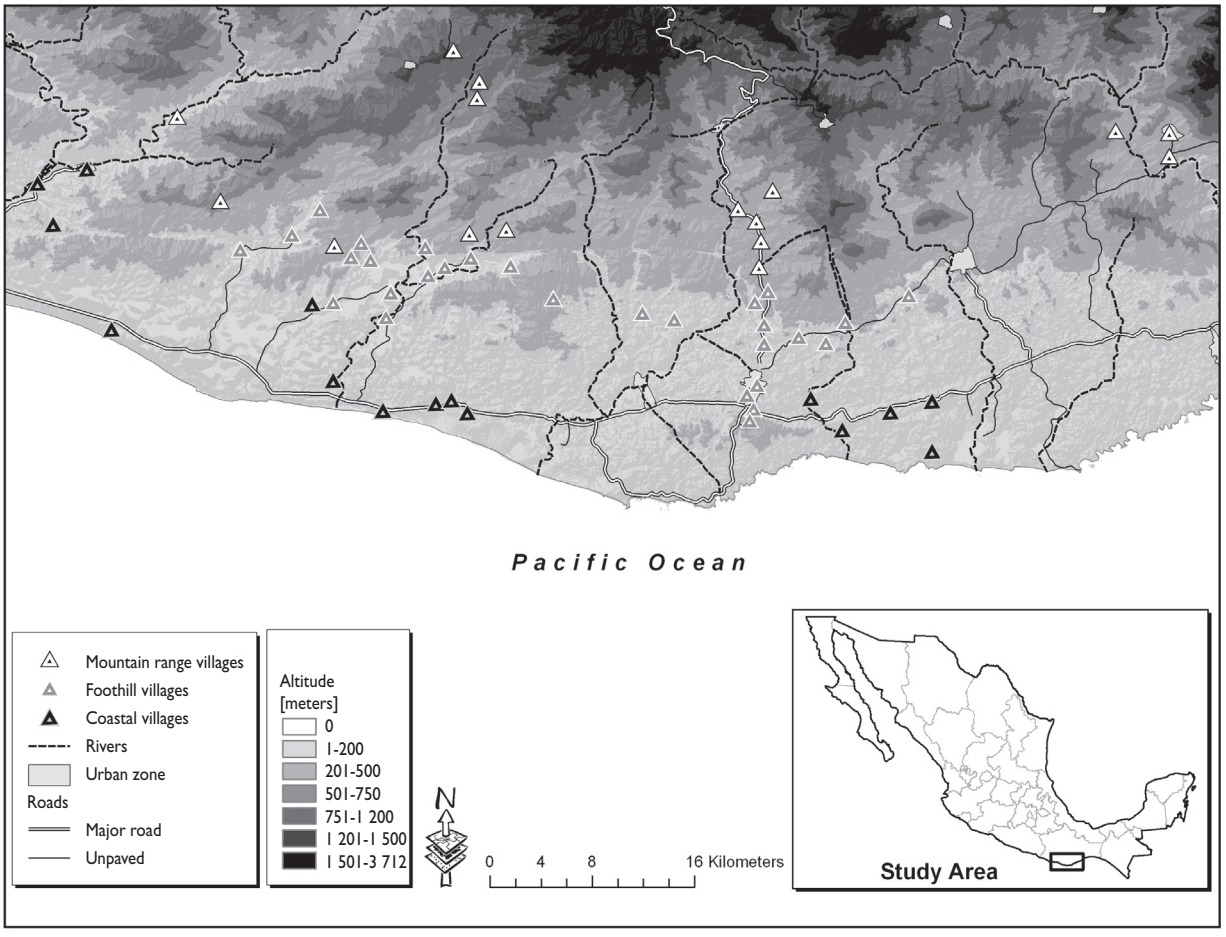

FIGURE 1. STUDY AREA WITHIN THE MALARIA RESIDUAL TRANSMISSION FOCUS ON THE PACIFIC COAST OF THE STATE OF OAXACA, MEXICO

malaria episodes, presenting symptoms compatible with malaria (febrile paroxysms, isolated intermittent fever, headache, malaise and sweating) that began in a period no more than two weeks before the interview, and who had a positive blood film for malaria (*P. vivax* parasites in Giemsa-stained thick blood smear). Each case was matched with two controls defined as individuals, with an age of ± five years as the case, living in the study area, with no symptoms of malaria and who were negative for *P. vivax* parasites by thick blood smear examination and negative for antibodies to *P. vivax* in the sero-epidemiological survey. Matched controls should have been present in the area on the day the case was diagnosed (at risk of becoming a case). For malaria cases recruited in villages not included in the census, controls provided blood samples for investigating *P. vivax* parasites using microscopy and antibodies to *P. vivax* using ELISA. Only subjects with negative results were included as controls.

Selection of cases and controls

Malaria incidence in the residing population was monitored from January 2002 to July 2003 and registered on a weekly basis, enabling the collection of the information during the entire study period. Malaria cases were detected by community health workers (active case detection) from patients seeking care from either health workers or local clinics, and registered on a weekly basis. Blood samples were taken from all suspected malaria cases and slides were examined by trained technicians in the laboratory closest to the Malaria Control Program (MCP). All positive cases received anti-malaria treatment according to the MCP treatment scheme (1 500 mg of chloroquine and 15 mg/kg of primaquine for adults).[6] Patients that fulfilled the inclusion criteria were recruited into the study and interviewed within a week. Controls were matched and interviewed within a week to adjust for the seasonality of transmission.

Sample-size calculations

According to our study design,[7] the sample size was calculated using Epi Info version 6.0, using sample size and power module for cohort studies. The population in the study area was 713 968. Assuming a frequency of disease in the non-exposed of 0.04 (approximately 3.9 per 100 000) based on malaria incidence reports by the MCP, a significance level of 0.95, a statistical power of 80% and a minimum detectable odds ratio difference of 0.5, the required sample size for the cohort was 621 714 people.

Exposure variables

A questionnaire previously validated in towns in the same state was used to collect the information. It recorded the activities of each individual during the two weeks before the onset of symptoms of the case. Trained technicians carried out the interviews and conducted observations and measurements. The questionnaire contained eight modules. The first module (identification section) included the subject's name, age, gender and birthplace. The second module included three questions on schooling, literacy and spoken languages. The third module had 40 questions on occupation and domestic, recreational and religious activities, including the time during the day or night when they were practiced and the frequency and location in which they were practiced. The fourth module, with nine questions, investigated activities and time expending at the work place, and contact with people coming from malaria endemic areas. The fifth module, with seven questions, investigated sleeping conditions and use of protection against mosquito bites, including: number of beds and bed nets per person, perception about mosquitoes and use of protective measures against the mosquitoes (mosquito coils, indoor insecticides, smoke and blankets). The sixth module, with six questions, investigated previous malaria episodes in the household, including: number of episodes, treatment and number of treatment days. The seventh module had 19 questions on movements outside the village, as well as the history of traveling outside town within the preceding 12 months before disease onset, including: sites visited, number and frequency of visits, duration, main reasons for the visits, activities during the stay, and use of protection measures in these sites. The eighth module, with three questions, investigated the use of health services and/or other types of health care and the availability and access to health services. If the interviewee reported using bed nets, the interviewer inspected the conditions of the bed net. Interviewers also recorded the characteristics and type of construction materials of the houses, their location within the village, the presence of animals and the amount of vegetation (0-20, 21-29, 30-50 and 60-100%) in a perimeter of 10 m around the house. Reproducibility among trained technicians in this activity was very good (K= 0.91).

A Malaria Knowledge Index (MKI) was constructed for each interviewed person according to three main questions: How is malaria transmitted? How is malaria treated? And how could malaria be prevented? This index allowed for combining the information from more than one question, increasing the power of these variables.[8] Each answer was graded according to how

close it came to the correct one. For instance, to the possible answers as to how malaria is transmitted, a value of two was given if the answer was "by mosquito bites" and a value of zero was given if the answer was "I don't know." The answer to the question as to how malaria is cured was given a value of two if the answer was "antimalarial drugs," but if the answer was "by analgesics" a value of zero was given; to how malaria is prevented, a value of two was given if the answer was "tablets," "insecticides," "bed nets" or "removal of algae"; if the answer was "smoke," a value of one was given. A value of zero was given if the answer was "I don't know." The index had three categories according to the number of correct answers: low (score of 0 to 1) medium (scores between 2 and 4) and high (scores of 5 or more).[9]

Statistical analysis

Coded data were double-entered into a database. The association of each risk factor with malaria cases was first analyzed with a bivariate model (odds ratios [OR] and 95% confidence intervals [95% CI]). The parameter with the lowest OR in each category was used as reference in the models. Variables associated with malaria cases in the bivariate analysis ($p< 0.2$) were selected and analyzed using multiple conditional logistic regression models.[10] A multivariate conditional logistic regression model was used to adjust for confounding variables. Models were fitted, including all the exploratory variables, and subsequently dropped one by one until only those that were statistically significant remained (p-value of the Likelihood Ratio Test > 0.05). Several interaction parameters were examined. The adjusted attributable risk was population-calculated from each of the final logistic regression models.[11] In order to avoid an excessive correlation (colinearity) among independent variables, associations were evaluated using a test of independence (chi square) for qualitative variables, the Cramer correlation coefficient for contingency charts with one or more degrees of freedom, and the Pearson coefficient of lineal correlation for quantitative variables. To test for dose response effect on multilevel categorical variables, a likelihood ratio test with the lineal combination of the parameters representing each level of the variable was conducted. All analyses were conducted using Stata version 8.0, Software (Stata Corporation, Collage Station, TX, USA).

Results

General characteristics of the cases

A total of 119 cases, out of 155 that occurred in the area during the study period, and 238 controls were included in the study, with a gender ratio of 1.44 men per woman (211/146). Nobody refused to be interviewed and the number of cases included in the study was determined solely by the opportunity for detection and the possibility of conducting the interview within two weeks after detection. The majority of cases, 50.7% (33 cases), were recruited from the foothills, 40% from localities on the mountain ranges (26 cases) and 9.2 (6 cases) from the coastal region (table I). Sixty-five cases (54.6% of the included cases) originated in villages where the anti-*P. vivax* seroprevalence study was carried out. The number of malaria cases was highest in the 5 to 10 year old population group (table II), but the percentage distribution of cases among age strata was not statistically different ($p= 0.57$) and the age distribution was not different ($p= 0.082$) from that of the whole population.

Association between malaria and risk factors

Frequencies for risk factors among cases and controls and associated bivariate and multivariate odds ratios are presented in table III. Eighteen variables out of 99 were significantly associated ($p < 0.05$) with increased risk of malaria. Correlation coefficient values were less than 0.8 in all cases, indicating no colinearity. The percentages of cases and controls that referred to a place of birth other than the locality where they were living were 23.5 and 13.45%, respectively, and this condition was associated with a three-fold (OR 3.16, 95% CI 1.16-6.13) greater risk of malaria infection.

The risk of malaria infection increased in subjects who spoke only an autochthonous language (13.4%, OR 2.48, 95% CI 1.19-3.77) compared to subjects who spoke Spanish and both Spanish and an autochthonous language (4.2%).

The proportions of cases in the medium and low MKI levels were 48.7% and 31.0%, respectively, compared to 48.3 and 24.4 % of controls, respectively. These knowledge levels were associated with an increased risk of disease (OR 1.75, 95% IC 1.93-3.30, P < 0.030 and OR 2.26 95% IC 1.10-4.66 P < 0.02, respectively).

The coverage of vegetation surrounding 60 to 100% and 30 to 59% of the house occurred in 34.8% and 63.0% of cases, respectively, and in 18.4% and 50.0% of controls, respectively. These coverages were strongly associated with risk of malaria infection (OR= 20.43, 95% IC 5.98-70.87, $P < 0.000$; OR= 3.78, 95% IC 1.21-11.80, respectively).

A larger number of the cases (75.6%) had houses constructed with perishable materials, incomplete walls and no bedrooms, compared with those of controls (51.3%). The risk due to housing was OR= 2.85 (95% IC 1.62-5.01), after controlling for occupation and place of

Table I

Total malaria cases recorded in the villages during the study period (from January 2002 to July 2003) and number of cases included in the study in the residual transmission focus of Oaxaca, Mexico

Municipalities	*Villages*	*Cases in 2002*	*Cases in 2003*	*Cases (%) included in the study*
Candelaria Loxicha	Cuajinicuil	2		2 (100)
	Los Horcones	3	14	15 (88.2)
	Azulillo		18	16 (88.2)
San Agustín Loxicha	Río Santa Cruz	1		1 (100)
	Quelove	2		2 (100)
	Sirena Miramar	3	2	5 (100)
	Piedra Virgen	1		1 (100)
San Miguel del Puerto	Arroyo Xuchil	1	1	2 (100)
San Pedro Pochutla	San Isidro Apango		1	1 (100)
	Lagunilla	1		1 (100)
	Arroyo Rico	1	5	6 (100)
	Tomatal	1	1	2 (100)
	Arroyo del Inquente	2		2 (100)
	San Rafael Totoltepec	10	9	11 (57.8)
	Las Cuevas	2		2 (100)
	San Roque	2	3	2 (40)
	San José Chacalapa	6		3 (50)
	Comala	8	9	15 (88.2)
Santa María Colotepec	El Camalote	1		1 (100)
	San José el Palmar	1		1 (100)
Santa María Huatulco	Bajos del Arenal		2	2 (100)
Santa María Tonameca	Escobilla	1		1 (100)
	San Francisco Cozoaltepec		2	2 (100)
	Valdeflores	5	4	4 (44.4)
Sto Domingo de Morelos	Santo Domingo de Morelos	1	2	3 (100)
	Cerro Campana	1	4	5 (100)
	Piedras Negras	3	5	4 (50)
	Caña Brava		5	2 (40)
	Yerba Santa		2	1 (50)
	Bajos de Santo Domingo		2	1 (50)
	Paso San Antonio	3	2	3 (60)
Total		62	93	119 (76.7)

residence. The adjusted fraction and risk of infection attributable to poorly constructed houses was 48.6%.

The proportion of cases living in houses located far from the centre of the villages (56.3%) was higher than in controls (19.4%) and the risk of infection in houses located at the periphery of the villages was six-fold compared to those living downtown (OR 6.43, 95% IC 3.46-11.92). The adjusted fraction for risk of infection was 47.2%.

Sleeping on a dirt floor, a mat or a mattress on the floor was indicated by 40.3% of the cases, corresponding to a two-fold (OR 2.98, 95% CI 1.78-5.01) increased risk of malaria than controls (20.6%). Sixty-one percent of cases and 48.7% of controls indicated sleeping with two

Table II
DISTRIBUTION OF *PLASMODIUM VIVAX*-INFECTED PEOPLE BY AGES INCLUDED IN THE STUDY IN THE RESIDUAL TRANSMISSION FOCUS OF OAXACA, MEXICO, FROM JANUARY 2002 TO JULY 2003

Age stratum	*Proportion*
0-4	17.65
5-10	37.82
11-15	18.49
16-20	5.04
21-30	7.56
31-40	4.20
41-50	1.68
50 and more	7.56
Total (%)	100

Nonsignificant differences F=1.39 *P*= 0.57

or more people in the same bed, resulting in a higher risk of infection (OR 1.85, 95% CI 1.09-3.14).

The proportion of the cases (26.0%) not using bed nets was higher than that of the controls (17.2), resulting in a higher risk of malaria infection (OR= 2.39, 95% IC 1.18-4.83, $p< 0.014$). Bed nets with holes greater than 2 cm were observed in 34.4% of cases and 20.6 % controls, resulting in a five-fold (RM= 5.2, 95% IC 2.27-13.9) increased malaria risk. The use of smoke, mosquito coils and commercial insecticides had no significant effect on risk of malaria in bivariate and multivariate models.

The frequency of traveling outside villages was higher among cases (23.5%) than controls (16.1%). Subjects who reported traveling compared to those individuals who did not travel had a two-fold increase (OR 2.11, 95% IC 1.08-4.12) in the risk of malaria, and a strong increased risk was observed among individuals who traveled before the onset of their malaria episode (OR 9.16, 95% IC 1.98-42.2). The presence of malaria cases in the household in previous years was reported by 20.6% (49 of 238) of controls and by 57.1% (68 of 119) of the cases, corresponding to an almost six-fold increase (OR 7.25, 95% CI 3.84-13.67) in the risk of malaria infection.

Although a dose-response effect was investigated for several variables, only the amount of vegetation coverage around and the conditions of the bed nets were significant in the logistic model.

Discussion

The number of malaria cases included in the study corresponds to 76% of the total incident cases in the area during the period. The geographic distribution of cases confirms that malaria transmission occurs mainly in foothill localities.[2] The distribution of cases peaked in the 10 to 15 years age group, although a continuous increase of anti- *P. vivax* antibody prevalence with age was documented in the sero-epidemiological survey (Danis *et al*. in preparation). This is indicative of low but persistent malaria transmission similar to that of other unstable hypoendemic areas on the continent where malaria occurs at all ages, but young and middle-age adults are at a higher risk.[12,13]

Previous studies on individual risk factors for malaria in Mexico were limited to the Lacandon Forest in Chiapas.[9] In the focus of Oaxaca, the results of the study herein indicate many similarities between the risks of infection in both foci, with some local peculiarities. Similar to that in other malaria endemic areas in the world,[14,15] the main socio-demographic factor that influenced the individual risk of malaria infection in both Mexican foci was to have been born in a different place from the locality of residence. The immigrant condition as a risk of malaria has been long recognized,[12,16] and it has been adduced that encountering new parasite strains not recognized by the immigrants' immune system is a main determinant for the increase in malaria infection in this group.[17,18] But poor knowledge about the disease and its prevention, as well as a higher exposure to mosquito bites conditioned by the occupational activities during the extended process of integration into the new community,[19] could also contribute to an increase in this risk for incomers.

Social status as a risk factor for malaria within the ethnically diverse localities in the Pochutla focus was evidenced by a higher risk in individuals that speak only an autochthonous language. Ethnicity determines and maintains the position of individuals in the social hierarchy and, as a consequence, their attitude and practices determine the quality of their health.[20] This also affects their interaction with MCP personnel and delays seeking treatment which consequently increases the risk of infection.[21] Disregarding ethnicity, delayed diagnosis and treatment seeking attitudes, conditioned by the perception of the disease, could explain the increased risk of malaria in people who have poor knowledge of malaria,[22,23] as it also occurs in other endemic areas of Mexico[9, 21] and the world.[24,25]

The main environmental factor determining malaria transmission in the area was the number of rivers and streams around the localities.[2] It is shown here that occupants of houses in the periphery of the communities had an increased risk of malaria infection, probably because of their proximity to *An. pseudopunctipennis* larval breeding sites, the main malaria vector in the

Table III

FREQUENCIES OF RISK FACTORS AMONG CASES AND CONTROLS, AND ASSOCIATED BIVARIATE AND MULTIVARIATE ODDS RATIOS IN HABITANTS OF THE RESIDUAL MALARIA TRANSMISSION FOCUS LOCATED ON THE OCEAN PACIFIC COAST OF OAXACA. JANUARY 2002 TO JULY 2003

Risk Factors	*Cases (%)*	*Controls (%)*	*Bivariate unadjusted OR (95 % CI)*	*P*	*Conditional logistic regression adjusted (95 % CI)**	*P*
Birthplace						
Birth in the locality	91 (76.4)	206 (86.6)	1.0	1.0		
Birth outside the locality	28 (23.5)	32 (13.4)	2.13 (1.17-3.89)	0.013	3.16 (1.63-6.13)	0.001
Language						
Speak both Spanish and an autochthonous language	103 (86.6)	228 (95.8)	1.0	1.0		
Speak only an autochthonous language	16 (13.4)	10 (4.2)	4.66 (1.79-12.14)	0.002	2.48 (1.19-3.77)	0.000
Knowledge about malaria						
High	24 (20.2)	65 (27.3)	1.0	1.0		
Medium	58 (48.7)	115 (48.3)	1.36 (0.78-2.37)	0.276	1.75 (1.93-3.30)	0.030
Low	37 (31.1)	58 (24.4)	1.77 (0.93-3.35)	0.080	2.26 (1.10-4.66)	0.026
Coverage of vegetation surrounding of the house (%)						
0-29	5 (4.2)	75 (31.5)	1.0	1.0		
30-59	70 (60.0)	119 (50.0)	2.88 (1.06-7.79)	0.037	3.78 (1.21-11.80)	0.022
60-100	40 (34.8)	44 (18.5)	11.33 (4.01-32.00)	0.000	20.43 (5.89-70.87)	0.000
Housing construction						
Houses constructed with better materials	29 (24.4)	116 (48.7)	1.0	1.0		
Houses constructed with perishable materials	90 (75.6)	122 (51.3)	3.13 (1.86-5.25)	0.000	2.85 (1.62-5.01)	0.000
Location of houses in villages						
Houses located close from the centre of the villages	52 (43.7)	191 (80.6)	1.0	1.0		
Houses located on the periphery of the villages	67 (56.3)	46 (19.4)	6.23 (3.50-11.06)	0.000	6.43 (3.46-11.92)	0.000
Sleeping conditions						
Sleeping directly on the bed	71 (59.7)	189 (79.4)	1.0	1.0		
Sleeping directly on the floor, a mat or matrix on the floor	48 (40.3)	49 (20.6)	2.40 (1.50-3.83)	0.000	2.98 (1.78-5.01)	0.000
People in the bed						
Sleeping alone	46 (38.7)	122 (51.3)	1.0	1.0		
Sleeping with two or more people in the same bed	73 (61.3)	116 (48.7)	1.76 (1.09-2.86)	0.020	1.85 (1.09-3.14)	0.021
Use of bed-nets						
Always	54 (45.4)	139 (58.4)	1.0	1.0		
Sometimes	34 (28.6)	58 (24.4)	1.49 (0.89-2.47)	0.122	1.95 (1.09-3.46)	0.022
Never	31 (26.0)	41 (17.2)	2.19 (1.17-4.11)	0.014	2.39 (1.18-4.83)	0.014
Condition of bed-nets						
Intact	49 (41.2)	110 (46.2)	1.0	1.0		
Holes minor or equal 2 cm	29 (24.4)	79 (33.2)	1.24 (0.54-2.85)	0.598	1.41 (0.56-3.52)	0.453
Holes greater than 2 cm	41 (34.4)	49 (20.6)	9.23 (2.63-32.33)	0.001	5.29 (2.27-13.90)	0.003
Travels outside of villages						
Not traveling outside of the village of residence	91 (76.5)	198 (83.9)	1.0	1.0		
Traveling outside of the village of residence	28 (23.5)	38 (16.1)	1.75 (0.96-3.19)	0.066	2.11 (1.08-4.12)	0.028
Traveled before the onset of their malaria episode						
No	109 (91.6)	236 (99.2)	1.0	1.0	‘	
Yes	10 (8.4)	2 (0.8)	10.0 (2.19-45.63)	0.003	9.16 (1.98-42.21)	0.004
History of malaria cases in the house						
Not malaria cases in the house of residence in previous years	51 (42.9)	189 (79.4)	1.0	1.0		
Malaria cases in the house of residence in previous years	68 (57.1)	49 (20.6)	5.84 (3.33-10.22)	0.000	7.25 (3.84-13.67)	0.000

* Adjusted by job and village

area.[26,27] In this sense, vegetation plays an important role as a refuge and resting place for blood-seeking adult mosquito populations,[28,29] which explains why the amount of vegetation around households was a determinant of the risk level for malaria infection; in fact a significant dose-response effect was found in our logistic mode (table III), reinforcing our conclusion that this environmental condition is associated with malaria infection.

Although *An. pseudopunctipennis* is exophilic, it readily enters houses to feed, thus the need for conditions that allow for its free transit in and out of houses.[30] Most people in the study area lived in poorly constructed houses, but a larger number of cases lived in houses made of perishable materials with incomplete walls. This is a common feature in most endemic malaria areas, and a higher risk of malaria has been associated with housing construction that facilitates human-mosquito contact.[6,31-33]

Sleeping on the floor or mats on the floor, as well as with more than one person in the same bed, also reflect poor economic household conditions, but it is also possible that mosquito attraction increased in these households because of an increase in human-released chemoattractants in reduced spaces.[34]

The efficacy of insecticide-impregnated bed nets in conferring high protection against child mortality has been proven in *P. falciparum* malaria areas.[35-38] As impregnated bed nets have little effect on morbidity, they have not been recommended for the control of *P. vivax* malaria. Nevertheless, untreated bed nets have been proven to protect against *P. vivax* infection in the Lacandon Forest[9] and other endemic areas,[39] and in the present study people that do not use or irregularly use untreated bed nets had a higher risk for *P. vivax* malaria. The increased risk when bed nets had holes bigger that 2cm, documented in both Mexican transmission foci,[9] could be explained by the free entrance and the accumulation of mosquito attractant in the secluded microenvironment of the bed net. A significant dose-response effect as bed net conditions deteriorated was found in our logistic model (table III), indicating that as access to the interior of the bed net increases, the risk of infection also increases. This possibility warrants further study as damaged, untreated bed nets are used with the possible false assumption of protection.

Human movements within the economic development nucleus around Pochutla City was identified as a determinant for high malaria transmission intensity among localities in the residual focus.[2] Here, we documented that traveling outside of the village of residence increased the individual risk of malaria infection, and that the detected risk was much higher in those that traveled before the onset of their symptoms, suggesting that many infections occurred outside the village of residence. Short-term population migrations and circular migrations from non- endemic to endemic areas have been associated with malaria epidemics in other areas,[40,41] and malaria outbreaks have been associated with movements in the interior of endemic areas.[42] Malaria infection in these cases could result from a combination of factors, such as the intensity of malaria transmission, precarious housing conditions and poor protection against mosquitoes in the visiting site.

Plasmodium vivax hypnozoites survive in hepatocytes for extended periods of time.[43,44] The reactivation of these parasite forms are responsible for new malaria episodes (relapses) in patients that were cured of a primary attack of the disease,[45] and the participation of these relapses in the occurrence of malaria outbreaks has been documented.[46,47] In Mexico, the grouping of new and secondary cases results in a concentration of 80% of the malaria cases in 11% of the localities in endemic areas. In these localities, malaria is also focalized only in a small portion of the houses.[48] This is in accordance with a six-fold risk of malaria in houses in the Pochutla focus where another family member had presented a clinical episode of the disease, and indicates that transmission is focalized to houses where the parasite is made available by the initial introduction of an infected family member, and it is maintained for several years by symptomatic or asymptomatic relapses.

The precise time when malaria cases acquired their infections cannot be identified because cases were associated with more than one situation that exposed them to mosquito bites during their daily activities. However, it has been useful to identify the most common individual risk factors for malaria infection in the Oaxaca focus. This could be useful for guiding malaria control strategies in the area. New malaria control strategies have been put in place in this and other residual foci in the country, including the abatement of mosquito abundance by participatory community treatment of larval breeding sites, the reduction of *P. vivax* infection relapses by means of repeated treatment with chloroquine and primaquine,[6] and the special attention provided to indigenous communities by bilingual (Zapotecan and Spanish) MCP health workers. The results of the present study indicate that these strategies could be complemented by other interventions which could incorporate the participation of the community, such as improving housing conditions[49] and the periodical clearing of vegetation around houses. Additional activities should include education interventions to improve knowledge about malaria diagnosis and seeking treatment, as well as the need to increase protection measures while travel-

ing. The use of bed nets deserves particular attention; this protection measure is very effective when bed nets are treated with insecticides,[35-38, 50] even when some holes normally develop as a result of wear-and-tear. The results of the present study indicate that although untreated nets provide protection, when holes appear this protective effect reverts and the risk of malaria infection increases. These results indicate that, on the one hand, the use of bed nets as a participative community anti-malaria intervention should be promoted among inhabitants of the residual focus; but on the other hand, the need to maintain bed nets in good condition should be stressed.

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