

THE EPIDEMIOLOGY OF MALARIA IN AN EPIDEMIC AREA OF THE PERUVIAN AMAZON

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Abstract. A longitudinal study of malarionometric indicators and their association with potential risk factors was conducted during August 1997–July 1998 at Padre Cocha, a village of 1,400 residents in the Peruvian Amazon. The incidence of *Plasmodium falciparum* infections during the study year was 166/1,000 persons; that of *P. vivax* was 826/1,000 persons. The mean duration of symptoms prior to diagnosis was 2 days; presenting geometric mean parasite densities were 3,976 parasites/ μ l for *P. falciparum* infections and 2,282 parasites/ μ l for *P. vivax*. There were no malaria-associated deaths. Consistent with the epidemic nature of malaria in the area, the incidence of both parasite species increased with age and there were no age-specific differences in mean parasite densities. No specific occupational risks for malaria were identified. Activities significantly associated with malaria risk reflected local vector behavior and included strolling outdoors after 6:00 PM and arising before 6:00 AM for adults, and attending evening church services for children.

The incidence of malaria in Peru has increased dramatically since the early 1990s.^{1,2} Data collected by the Peruvian Ministry of Health (MOH) demonstrate a 7-fold increase in malaria incidence from 13 per 10,000 inhabitants in 1990 to a peak of 88 per 10,000 in 1996 (Figure 1). Malaria caused by *Plasmodium falciparum*, which previously occurred only sporadically at the Ecuadorian, Colombian, and Brazilian borders, became established in 1992, and now accounts for 30% of all cases in Peru.¹

The Amazonian Department of Loreto (Figure 2) has been a major focus of the malaria epidemic. In 1990, 641 cases of malaria were reported to the Loreto Health Department, of which 123 were caused by *P. falciparum*. By 1997, the number of registered cases in Loreto reached 121,268, producing an annual incidence rate of 148 per 1,000 inhabitants and accounting for 67% of all malaria cases in Peru (Figure 1). Parasitologically confirmed *P. falciparum* cases exceeded 54,000, yielding an incidence of 66 per 1,000 inhabitants, with 85 associated deaths.^{1,2} The rapid spread of both malaria species in Loreto is thought to be related to the appearance of *Anopheles darlingi* as the dominant vector in the area, combined with changing patterns of river use and jungle exploitation occurring in the region and reductions in residual house spraying programs that were conducted regularly prior to the 1990s.^{1,2,4,5}

While considerable resources have been expended on malaria treatment and control in the Amazon basin during the last two decades, few epidemiologic studies of Amazonian malaria transmission and risk factors have been performed. In the state of Rondônia, Brazil, where most available research has been conducted, transmission appears to be predominately occupational and extra-domiciliary.^{6–9} In a recent overview of the malaria epidemic in Loreto, the reported age-specific attack rates were highest in adults, particularly males, suggesting that occupation is an important risk factor in Peru as well.⁴ Peruvian studies of malaria-associated behavioral risks have been limited to the northern coastal region of Piura and the southern Amazonian region of Madre de Dios, where infections were caused exclusively by *P. vivax*, and the research focused on sleeping patterns and bed net usage.^{10,11} To date, there have been no published studies

exploring specific risk factors associated with malaria acquisition in the northern Peruvian Amazon. To better understand malaria epidemiology and transmission in the Department of Loreto, we conducted a longitudinal study of malarionometric indicators and their association with potential risk factors in a village located in a high transmission zone near Iquitos, the largest city in the Peruvian Amazon.

MATERIALS AND METHODS

Study site and population. Padre Cocha is a village of 1,400 inhabitants located on the Rio Nanay 5 km northwest of Iquitos, the departmental capital of Loreto, Peru (latitude 3°41'55"S, longitude 73°16'39"W). The central portion of Padre Cocha lies in a cleared area of approximately 1 km². The surrounding area consists of scrub and secondary growth forest, some of which is inundated during the rainy season. Between the village and the Rio Nanay lies a *cocha*, or river-fed lake, that expands and contracts with the river level. The elevation above sea level is 122 meters, the mean mid-day temperature is 30°C (range = 23–35°C), the mean humidity is 90% (range = 60–100%), the average annual rainfall is 4.3 meters, and the river level fluctuates an average of 10 meters during the year, peaking in April–May. Travel to and from Iquitos is by boat.

Malaria transmission in Loreto and Padre Cocha occurs year round. In 1997 and 1998, a pronounced seasonal pattern emerged, with highest monthly malaria incidence rates during the wetter months of January–June (Figure 3). Both *P. vivax* and *P. falciparum* were first detected in Padre Cocha in 1994, and the incidence of both species has subsequently undergone explosive growth. A cross-sectional parasitemia survey of 71% of Padre Cocha inhabitants performed by the MOH during the first week of February 1997 revealed a community point prevalence of 6.5% *P. vivax* malaria and 7.4% *P. falciparum* malaria (Magill A, unpublished data). Crude tallies of Padre Cocha malaria treatment records for the months of January–July 1997 showed similar numbers of *P. falciparum* and *P. vivax* cases (973 and 1,121, respectively) and peak monthly incidence rates (Figure 3). The combined annual cumulative incidence was approximately 2

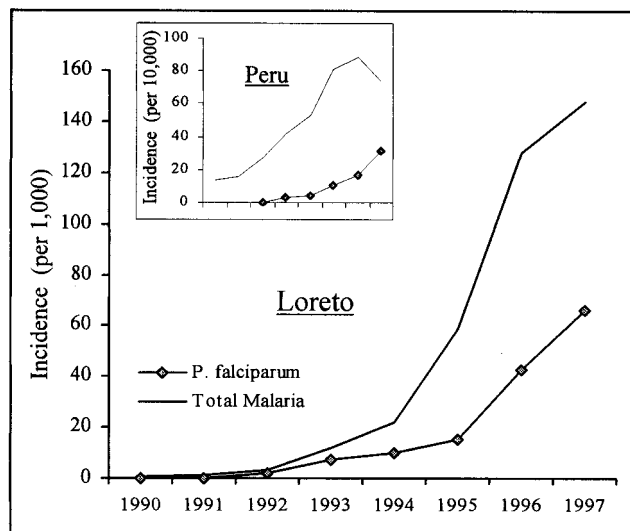


FIGURE 1. Annual incidence of malaria in the Department of Loreto and Peru (inset), 1990–1997.

malaria episodes per person for the 1996–1997 transmission year (Roper M, unpublished data).

The presence of *An. darlingi* has been documented in Padre Cocha since 1995.⁵ Pilot entomologic investigations performed in Padre Cocha during March–July 1998 found *An. darlingi* to be the predominant species collected both inside and outside of capture stations. Anopheline biting occurred throughout the night, with highest hourly collection totals in human-biting studies between the hours of 6:00 PM and midnight. *Anopheles darlingi* larvae were detected in every type of potential breeding site that was sampled in the village and surrounding area, including the *cocha*, small streams, natural springs, artificial fishponds, and water-filled pits (Chan A, unpublished data). Vector control measures have been limited to occasional applications of the larvicide temephos (O,O'-(thiodi-4,1-phenylene)bis(O,O-dimethyl phosphorothioate)) in some of the larger ponds and the *cocha*, and indoor residual insecticide spraying with pyrethroid compounds performed on an irregular basis. A lack of personal and MOH resources have precluded impregnation of bednets with insecticide.

Malaria treatment program. The Padre Cocha Health Post is open 5½ days per week and is staffed with nurses and community health workers trained in obtaining blood smears and dispensing antimalarial medication. Malaria infections are microscopically confirmed prior to treatment administration, except in the face of severe illness without possibility of prompt smear assessment. Treatment is dispensed in accordance with Peruvian MOH guidelines directed at both asexual parasites and gametocytes. Patients are required to report to the Health Post to receive their daily doses of medication except on Sundays when they take medication given to them the day before. Due to widespread chloroquine (CQ) resistance in the Iquitos area, falciparum malaria is treated with sulfadoxine/pyrimethamine (SP), 25 mg/kg and primaquine (PQ), 0.75 mg/kg administered as single doses. Cases resistant to SP are treated with 7-day courses of quinine and tetracycline or clindamycin. Follow-up smears to assess therapeutic response are obtained on days 3, 7, and

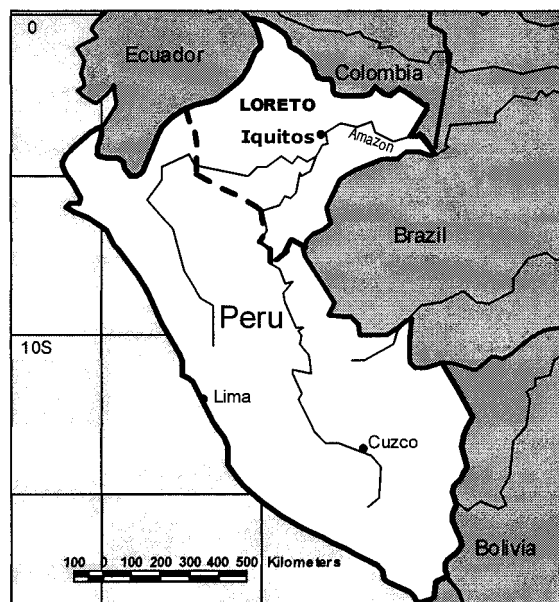


FIGURE 2. Map of Peru. The Department of Loreto occupies 25% of the landmass of Peru and has a population of approximately 820,000, or 3.3% of the total population of Peru. The study site, Padre Cocha, lies 5 km northwest of Iquitos, the capital of the Department of Loreto.

14 following treatment for *P. falciparum* infections. Treatment failure is defined as parasitemia on day 3 that is as high or higher than at the time of diagnosis, any parasitemia on days 7 or 14, or clinical deterioration in the presence of persistent parasitemia. Vivax malaria is treated with CQ, 25 mg/kg over a 3-day period and concurrent therapy with PQ, 0.5 mg/kg/day for 7 days. Follow-up smears for *P. vivax* infections are taken on days 7 and 14. There have been no reports of CQ-resistant *P. vivax* in Peru to date (Ministerio de Salud del Perú, unpublished data).

All malaria diagnostic and therapeutic services are provided free of charge. There are no alternative medical facilities or providers in Padre Cocha, and few residents seek treatment for acute febrile illnesses outside the village. The MOH policy prohibits private pharmacies from dispensing antimalarial drugs to symptomatic individuals; however, there is no attempt to monitor adherence to this policy, and all anti-malarial agents used by MOH facilities are available in Iquitos pharmacies. Chloroquine is also sold in several shops in Padre Cocha.

Census. A community-wide census was performed at the beginning of the study year to enumerate all inhabitants, assign unique personal identification numbers, and collect basic demographic information. Census information was continuously updated during the course of the study year, and in May 1998, a repeat community-wide census was performed to identify newcomers missed by the updating procedure and to confirm the departure of residents who had moved away. The population of Padre Cocha at the beginning of the study was 1,396. In May 1998, 1,405 inhabitants were identified. During the course of the year, 61 residents moved away from Padre Cocha while 70 individuals moved in. There were no significant differences in age or sex between those members of the population who remained

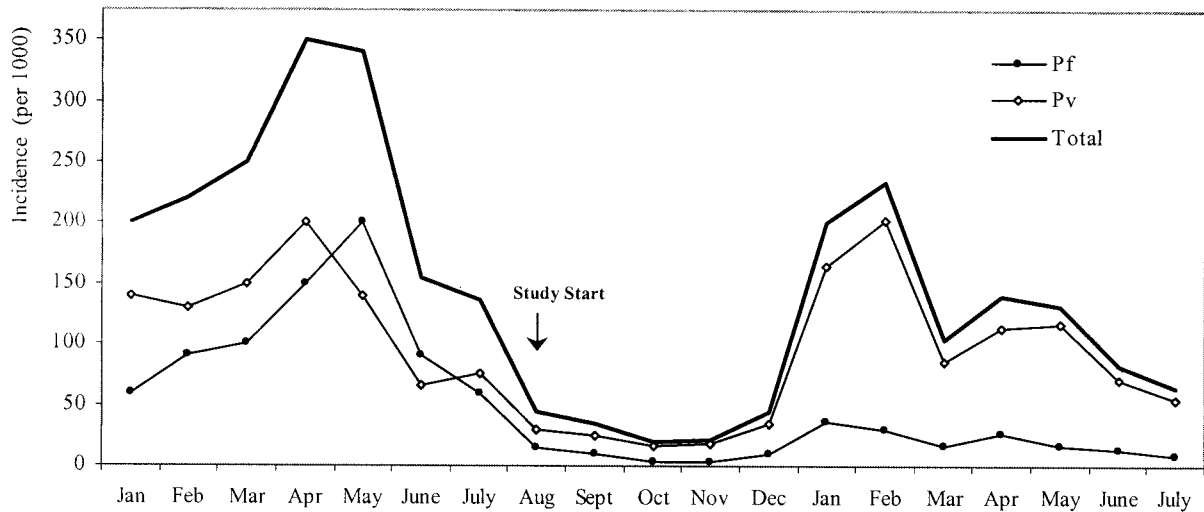


FIGURE 3. Monthly malaria incidence in Padre Cocha, 1997–1998. Pf = *Plasmodium falciparum*; Pv = *P. vivax*.

throughout the study and those who moved in or out during that time. The number of residents present throughout the year was calculated as the average of the 2 census totals, or 1,400. Average numbers of residents in each age group and of each sex were calculated in the same manner to provide estimates of persons at risk for stratified analyses. Children were defined as residents 16 years of age or younger, adults as those 17 years of age or older.

Risk factor survey. A risk factor survey was administered to all available inhabitants or their guardians by trained community health workers at the time of the May 1998 census using a structured questionnaire. The survey addressed activities and occupations that could confer an increased risk of malaria, especially those performed between dusk and dawn. Adults were questioned about knowledge of malaria transmission and avoidance, and self-administration of antimalarial medications. All completed questionnaires were checked by at least one supervising member of the research team prior to data entry, and validation of accuracy and consistency of responses was performed in 8%. To assure uniformity of risk, survey respondents who were not present in Padre Cocha during the entire study year were excluded from the analysis.

Case detection. Malaria infections were detected in two ways. Individuals presenting to the Padre Cocha Health Post with symptoms suggestive of malaria were tested for microscopic evidence of parasitemia using fingerprick blood samples. Those whose blood smears showed malaria parasites were registered as cases at the Health Post, and treatment was initiated. Registry information was abstracted and entered into a computerized database. To avoid missing cases of individuals who sought initial diagnosis in Iquitos clinics or hospitals, the registries of all relevant facilities in the Iquitos area were reviewed. Less than 0.2% of patients sought initial assessment at other facilities, and all follow-up was performed at the Padre Cocha Health Post.

The second means of identifying cases was two week-long community blood smear surveys performed by the MOH. In the first cross-section in February 1998, any available and willing resident had a blood smear taken. Information about symptoms and history were not recorded; therefore, distinc-

tions between symptomatic and asymptomatic infections could not be made. During the second cross-sectional survey performed in April 1998, only individuals who were not symptomatic and not receiving malaria treatment were sampled, permitting an estimate of asymptomatic parasitemia. To minimize biases that could be introduced by including episodes of recurrent malaria due to treatment failure in the total counts of *P. falciparum* and *P. vivax* infections, we adopted the following definitions to distinguish recrudescence or relapse from new infections. In the case of falciparum malaria, all therapeutic failures identified at the health post in accordance with MOH guidelines were counted only at the time of initial diagnosis and therapy. In addition, any episode of recurrence of *P. falciparum* parasitemia identified after negative day-7 and day-14 smears but within 30 days of initial treatment was considered a possible late treatment failure (late RI) and was excluded from further analysis.¹² For vivax malaria, any episode of *P. vivax* parasitemia recurring within the interval of 60 days following initial therapy was also excluded. This 60-day interval was chosen to encompass recrudescence due to CQ resistance, which would be expected to recur within 30 days of therapy,¹³ and early relapses due to non-compliance with the 7-day PQ regimen or to insensitivity to PQ.¹⁴

Parasitology. All diagnostic Giemsa-stained smear examinations were performed by experienced MOH microscopists who used a standard semi-quantitative scoring system based on the number of parasites/oil-immersion field (1,000×) found on thick smears.¹⁵ A minimum of 100 fields on each slide was scrutinized before assigning a negative result. A second reading was performed by a research-team microscopist during the peak transmission months of January–April 1998. Parasite counts from the second reading were estimated per 200 white blood cells (WBCs) and parasite densities were calculated based on the assumption of 8,000 WBCs/ μ l of blood. A minimum of 200 oil-immersion fields was examined during the second reading prior to considering a smear negative.

Statistical analysis. The incidence of each malaria species was calculated as the number of new infections divided by the average number of persons at risk in the community

TABLE 1
Parasitologic results from the 1997–1998 transmission year at Padre Cocha*

	No. of smears performed	<i>P. falciparum</i>		<i>P. vivax</i>		Total malaria	
		n	(%)	n	(%)	n	(%)
Passive surveillance	4,046	243	(6)	1,213	(30)	1,436	(36)
Active detection	2,117	17	(1.3)	87		103	
February	1,022	13	(0.4)	65	(6.4)	77	(7.5)
April	1,095	4		22	(2.0)	26	(2.4)
Total episodes of parasitemia		260†		1,300		1,540†	
Possible recrudescence/relapse‡		28	(11)	143	(11)	171	(11)
Total new infections		232	(17)	1,157	(83)	1,369	
Population affected§		196	(14)	858	(61)	930	(66)
1997–1998 annual incidence (per 1,000 persons)		166		826		978	
Geometric mean parasite density [95% CI]		3,976 [2,951–5,378]		2,282 [1,998–2,592]			
Children ≤ 16 years [95% CI]		3,551 [1,556–8,103]		2,757 [2,298–3,294]			
Adults > 16 years [95% CI]		4,277 [2,392–7,631]		1,957 [1,636–2,345]			

* 95% CI = 95% confidence interval.

† Excludes treatment failures identified at the Health Post (n = 47).

‡ See Materials and Methods for the definition of possible recrudescence and relapse.

§ Residents with ≥ 1 infection during the study year. Total population N = 1,400 individuals.

during the study year (n = 1,400). Incidence rate ratios (RRs) were used to measure the association between potential risk factors and malaria incidence in the sample of risk survey respondents who were present in Padre Cocha for the entire study year (n = 1,250). Statistical significance was defined as a *P* value <0.05 or 95% confidence intervals (95% CIs) that did not include 1.

Data entry and population analyses were performed using Epi-Info, version 6.04 (Public Domain Software, Centers for Disease Control and Prevention, Atlanta, GA). Proportions and categorical data were compared by chi-square testing; means were compared using one-way analysis of variance. Poisson regression modeling of numbers of malaria episodes per person during the one-year study period, with correction for intra-household correlation, was used for univariate and multivariate analyses of the risk factor survey data using Statistical Analysis Software (SAS) software, version 6.12 (SAS Institute, Cary, NC). Final regression models of risk factors significantly associated with malaria incidence were chosen using a backward selection technique of factors identified in preliminary univariate and bivariate analyses. Separate models were used for children and adults due to interaction noted in preliminary age-stratified analyses.

Ethical review. The malaria risk survey was conducted in accordance with a research protocol that was approved by the scientific and human use committees of the Walter Reed Army Institute of Research (WRAIR Protocol No. 727) and the corresponding ethical review committee of the Direccion Regional de Salud de Loreto. Review and use of Padre Cocha malaria registry data was conducted under Technical and Scientific Letters of Intention between the U.S. Naval Medical Research Center Detachment, Lima, Peru, and the Direccion Regional de Salud de Loreto and the Vice-Minister of Health (RM No. 237-97-SA/DM, May 5, 1997) for the Government of Peru. Verbal informed consent was obtained from all participants in the study protocol.

RESULTS

Demographics. Padre Cocha residents are of mestizo (Amerindian-Spanish) background and all are native to the Peruvian Amazon. The age distribution of the population is

typical of developing countries: 48% are 16 years of age or less (665) and 52% are 17 years of age or older (735). The population is evenly divided between males (704) and females (696). More than half of all adults are literate and 41% have attended at least some secondary school. Ninety-seven percent of children between the ages of 6 and 17 years attend school. The village economy is based on small-scale agriculture with 52% of adults spending some time working in family plots. Fishing, hunting, and gathering forest produce also contribute to family sustenance and income. Pottery and clay figure production supplement income in 25% of households. Fifteen percent of adults work outside of Padre Cocha as laborers, service industry workers, vendors, riverboat drivers, and guides. There were 235 inhabited houses in Padre Cocha during the study year with a mean ± SD household size of 6 ± 3 persons (range = 1–20). Ninety-nine houses (42%) were constructed with traditional wood walls and thatched roofs, 83 (35%) were made of brick and concrete with aluminum roofs, and 54 (23%) were a combination of new and traditional structures. Eaves in most houses were open, and windows and doorways were un-screened. An increasing number of homes situated in the village center have electricity supplied by gasoline-powered generators, but the majority of houses lack power. There is no community sewage disposal system and drinking water is obtained from the river, *cocha*, and natural springs.

Parasitology. The parasitologic results for the August 1997–July 1998 study year are shown in Table 1. There were 4,046 visits to the Health Post by residents with symptoms suggestive of malaria. The mean ± SD duration of symptoms was 2 ± 2 days and ranged from 2 hr to 30 days. Of the 4,046 smears obtained, 36% were positive for malaria parasites (17% *P. falciparum* and 83% *P. vivax*). Mixed infections were diagnosed in a total of 20 cases (1.3%), and were counted in both the *P. falciparum* and *P. vivax* totals, but only once in the category of total malaria infections. Treatment was initiated on the same day as diagnostic smear preparation in 75% of the cases. There were no malaria-associated deaths during the study year, and only one patient required hospitalization for severe disease.

During the February community cross-section, 7.5% of the obtained smears were positive for malaria (Table 1). This

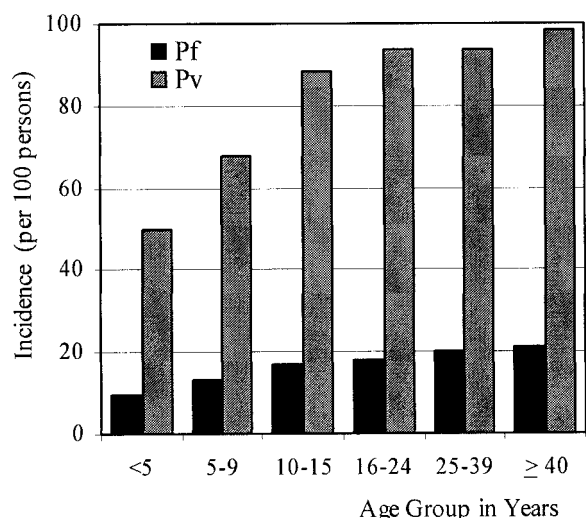


FIGURE 4. Age-specific annual malaria incidence by species. Pf = *Plasmodium falciparum*; Pv = *P. vivax*.

survey included symptomatic individuals and those in treatment for malaria, thus representing the prevalence of all malaria infections in the community during the week of sampling. In April, 2.4% of the smears taken exclusively from asymptomatic community members were positive. This estimate of asymptomatic parasitemia agreed with the community point prevalence survey performed in February 1997, in which 2% of those sampled had parasitemia without symptoms (Magill A, unpublished data). Since asymptomatic parasitemia is uncommon, passively and actively detected cases were pooled and considered together in the following analyses. Monthly incidence rates are shown in Figure 3; annual incidence rates are displayed in Table 1.

Age and gender. The distribution of *P. falciparum* and *P. vivax* infections by age group are shown in Figure 4. The

cumulative incidence of malaria increased significantly with age (χ^2 for trend = 53.4, $P < 0.0001$). Adults were 49% more likely to develop falciparum malaria than children, and had a 38% greater chance of developing vivax malaria during the course of the year (Table 2). Furthermore, adults were three times more likely to have multiple episodes of falciparum malaria than children (RR = 3.37, 95% CI = 1.37–8.29, $P < 0.01$) and 67% more likely to have multiple episodes of vivax malaria (RR = 1.67, 95% CI = 1.32–2.12, $P = 0.00004$). Neither malaria species produced infections with significantly different geometric mean parasite densities in children compared to adults (Table 1). There were no differences in malaria incidence when analyzed by gender (Table 2), including when adults alone were considered (RR = 1.02, 95% CI = 0.91–1.14, $P = 0.73$).

Risk factor survey. During the May 1998 census, the risk factor questionnaire was administered to 1,301 (93%) of Padre Cocha residents, 1,250 of whom had been present in Padre Cocha for the entire year and were included in the risk factor analysis (89% of the total population). There was no significant difference in the demographic composition between the included respondents and the community as a whole.

The results of univariate analysis of the association between hypothesized risk factors and malaria incidence during the study year are shown in Table 2. Since the patterns of association between risks and the incidence of each malaria species were similar, and the relatively low number of *P. falciparum* cases prohibited detection of differences in risk between the two species, the combined number of *P. falciparum* and *P. vivax* episodes per person were used for age-controlled analysis (Table 2) and multivariate modeling (Table 3). Additional analyses of the relationship between risks and the incidence of multiple infections (more than one infection during the study year), or the incidence of first infection of either species, led to the same overall pattern of associations.

TABLE 2
Univariate analysis controlled for household correlation; age-adjusted relative risks for malaria of either species*

Risk factor	n	(%)	<i>P. falciparum</i> Univariate		<i>P. vivax</i> Univariate		Total malaria			
			RR	95% CI	RR	95% CI	Univariate		Age adjusted	
							RR	95% CI	RR	95% CI
Age ≥ 16 years old	637	(51)	1.49	1.09–2.04	1.38	1.24–1.54	1.40	1.26–1.56		
Gender–male	632	(51)	1.21	0.92–1.59	1.00	0.91–1.10	1.03	0.94–1.12	1.01	0.92–1.10
Traditional bedroom type	479	(38)	0.90	0.64–1.26	0.92	0.80–1.05	0.92	0.80–1.06	0.91	0.79–1.05
Bedtime at 8:00 PM or later	779	(63)	1.42	1.03–1.97	1.32	1.18–1.49	1.34	1.20–1.51	1.20	1.06–1.36†
Time of arising at 6:00 AM or earlier	237	(19)	1.31	0.92–1.84	1.09	0.95–1.25	1.13	0.99–1.28	1.03	0.90–1.17†
Bath time after 6:00 PM	146	(12)	1.34	0.89–2.02	1.20	1.02–1.40	1.24	1.07–1.43	1.13	0.97–1.32
Farming	425	(34)	1.39	1.04–1.86	1.31	1.17–1.47	1.32	1.18–1.48	1.14	1.00–1.29†
Fishing	124	(10)	1.83	1.18–2.85	1.20	1.02–1.42	1.29	1.11–1.50	1.11	0.94–1.30
Forest work	77	(6)	1.08	0.56–2.06	1.15	0.94–1.40	1.14	0.94–1.38	1.02	0.83–1.23
Farming before 6:00 AM, after 6:00 PM	88	(21)‡	0.90	0.46–1.79	1.07	0.86–1.33	1.04	0.82–1.31	0.89	0.75–1.40
Fishing before 6:00 AM, after 6:00 PM	89	(72)‡	1.69	1.03–2.76	1.17	0.96–1.42	1.24	1.02–1.49	1.06	0.86–1.29
Forest before 6:00 AM, after 6:00 PM	50	(65)‡	0.64	0.23–1.73	1.09	0.80–1.48	1.02	0.75–1.38	0.90	0.66–1.22
Strolling after 6:00 PM	242	(19)	1.17	0.79–1.75	1.30	1.14–1.49	1.27	1.10–1.45	1.22	1.07–1.40†
Attending church after 6:00 PM	128	(10)	1.40	0.90–2.19	1.16	0.98–1.36	1.19	1.03–1.38	1.08	0.92–1.26†
Dancing (at disco) after 6:00 PM	104	(8)	1.29	0.78–2.16	1.09	0.91–1.31	1.13	0.96–1.34	1.10	0.93–1.31
Drinking (at bar) after 6:00 PM	115	(9)	1.61	1.03–2.51	1.00	0.82–1.22	1.11	0.93–1.33	1.00	0.83–1.20
Watching TV (out of home; after 6:00 PM)	412	(33)	0.98	0.70–1.36	1.00	0.90–1.12	1.00	0.90–1.11	1.02	0.92–1.24

* RR = incidence rate ratio, 95% CI = 95% confidence interval.

† Age-specific interaction, RR not meaningful.

‡ % of those engaged in the activity.

TABLE 3
Risk of malaria in adults and children: results of final Poisson regression model*

Risk factor	Total Malaria			
	Adults (>16 years)		Children (≤16 years)	
	RR	95% CI	RR	95% CI
Age (in years)	1.00	0.99–1.00	1.05	1.04–1.07†
Arising before 6:00 AM	1.22	1.04–1.20†	0.85	0.67–1.09
Strolling after 6:00 PM	1.23	1.06–1.43†	1.12	0.91–1.40
Church after 6:00 PM	0.93	0.77–1.02	1.29	1.00–1.67†
TV after 6:00 PM	0.84	0.73–0.96†	0.91	0.75–1.09
Work in Padre Cocha	1.50	1.19–1.87†		

* RR = incidence rate ratio, 95% CI = 95% confidence interval.

† $P < 0.05$.

Residents of Padre Cocha spent the majority of their time between 6:00 PM and 6:00 AM in bed. The mean \pm SD reported bedtime for respondents was 8:00 PM \pm 1 hr. The mean \pm SD bedtime was 7:00 PM \pm 1 hr for children and 9:00 PM \pm 1 hr for adults. Eighty percent of the population arose at 6:00 AM or later. Bednets were used by 99.7% of the population. Typically, there were several bednets per household, and the use of individual bednets by 2 or more household members was common. The risk of having one or more episodes of malaria increased with later bedtimes; however, in the multivariate models, this association was no longer present. There was no association between malaria risk and arising before 6:00 AM upon univariate and age-adjusted analysis. When adults were considered separately in the multivariate model, those who arose before 6:00 AM had a 22% higher risk of malaria. The risk for children arising before 6:00 AM was 15% lower; however, this negative association was not significant, and was present only for infants and small children <3 years old; for children \geq 3 years old, there was no association between the hour of arising and malaria incidence (RR = 0.97, 95% CI = 0.75–1.26, P = 0.82). The construction type of sleeping quarters (traditional wood and thatched roof versus brick and concrete with metal roof) was not associated with malaria risk.

Three types of bathing sites were used by the population: the main river and *cocha*, small streams, and natural water holes. Bathing site preferences changed depending on water levels and day-to-day availability, precluding meaningful testing of this potential risk factor. The majority (88%) of respondents bathed before 6:00 PM, and once controlled for age, bathing after 6:00 PM was not associated with malaria incidence.

Since malaria occurs more frequently in adults in Padre Cocha, it seemed possible that some occupational activities would be associated with increased malaria incidence. Although working in the Padre Cocha vicinity, as opposed to outside the area, was associated with 50% more malaria episodes, there were no associations between malaria incidence and specific occupations. The RRs for occupational activities were as follows: farming = 1.10 (95% CI = 0.94–1.28), fishing = 1.06 (95% CI = 0.89–1.25), pottery production = 1.15 (95% CI = 0.97–1.38), homemaking = 1.01 (95% CI = 0.89–1.15), and teaching = 0.67 (95% CI = 0.38–1.16). When the time of travel to and from agricultural plots was considered, there was no higher incidence of malaria in individuals who left home for their fields before 6:00 AM and/or returned after 6:00 PM (Table 2). Similarly, the hours dur-

ing which workers traveled to and from the forest, or were out fishing, were not associated with malaria risk. There was also no relationship between malaria incidence and level of education attained by adults when analyzed by number of years of school completed (RR per year of school = 0.99, 95% CI = 0.84–1.17), or by categories of highest level of educational institution attended.

Evening recreational activities were postulated as sources of increased malaria risk. Since only parts of the central village are electrified, residents who leave their homes in the evenings tend to congregate at locations with power and associated music, television, or church services. Television viewing at the community recreational center or at windows of homes with televisions was the most commonly reported evening activity, involving 412 (33%) of the population. For adults, there was a significantly decreased risk of malaria among those who reported watching television in the evening (Table 3). This negative association was also present to a lesser degree for children, but was not statistically significant. Strolling around the village was another common evening activity involving 19% of the population and 24% of all adults. Adults who went out strolling had a 23% greater malaria incidence than those who did not. Children who reported strolling or playing outdoors had an increased malaria incidence, but this was not statistically significant. However, children who attended evening church services did have a 29% increase in malaria risk. Other evening activities such as going dancing or drinking at one of the local bars showed no association with malaria incidence.

Malaria knowledge and risk avoidance. Despite MOH efforts to educate the population about malaria transmission and protective measures, half of all adults denied knowledge of how malaria is acquired or spread (Table 4). Of those offering explanations of malaria transmission, most correctly identified mosquitoes as a cause of malaria. When residents were asked what means (other than bednets) they used to avoid being bitten by mosquitoes, 84% of adults denied using any specific protective measures (Table 4). The use of protective clothing was cited by 13%; the use of repellent, pyrethroid coils, and other household fumigants was rare. While the MOH prohibition of anti-malarial self-administration is well known to the residents of Padre Cocha, 58 adults from 42 households admitted to buying and using CQ without Health Post assessment. Reasons cited for CQ self-administration included to treat fever (52%), to prevent malaria (45%), and to alleviate pain (3%). Self-administration of CQ

TABLE 4

Knowledge of malaria transmission and prevention among Padre Cocha adults (n = 707); more than one response could be selected

Causes of malaria	n	%
Mosquitoes	308	44
Water	33	5
Poor housekeeping/hygiene	25	4
Sun	10	1
Forest	8	1
Do not know	351	50
Protection against Malaria	n	%
Bednets	700	99
Protective clothing	91	13
Burn coils/fumigants	16	2
Repellant	4	6
Nothing (other than bednets)	593	84

was not associated with reduced malaria incidence (RR = 1.09, 95% CI = 0.81–1.47).

DISCUSSION

The epidemiology of malaria is the product of complex interactions between host, vector, and parasitic factors that are specific to each location in which malaria occurs.¹⁶ In settings such as sub-Saharan Africa and New Guinea, where transmission is intense and malaria exists in hyperendemic proportions in the population, children bear the major burden of malaria morbidity and mortality.^{17–21} Immunity develops with repeated exposures to malaria parasites, and the incidence of clinical episodes of malaria and the prevalence of malaria parasitemia decreases with age. Where malaria transmission intensity is low or unstable, as has been described in Southeast Asia, northern Africa, and the Americas, natural immunity is slow to develop; all age groups are affected and incidence often increases with age.^{6–9,22,23} The epidemiologic characteristics of malaria in Padre Cocha, Peru are typical of newly-established transmission of low-moderate intensity: the incidence of both *P. falciparum* and *P. vivax* infections increases with age, symptoms occur with low parasitic densities, and asymptomatic parasitemia is uncommon. Unlike similar settings where malaria is long-standing, we found no evidence of naturally acquired immunity in older age groups. The highest incidence of both malaria species was found in residents 40 years of age or older, and there were no significant differences in parasite density of either species when comparing adults to children.

The distribution of malaria in the Department of Loreto is uneven. There are several zones of high transmission and mesoendemic malariometric indices, and larger areas where malaria transmission and disease occur at lower levels.^{1,3,4} Socioeconomic status, access to health care, and cultural characteristics also differ between locations and populations, and contribute to the diversity of malaria characteristics in the region. Padre Cocha is a relatively stable, prosperous, and well-educated community, and its Health Post provides prompt diagnostic and therapeutic services. The rapidity with which residents report for assessment following the onset of symptoms reflects the community understanding that malaria is best treated early, and the confidence with which

the Health Post is regarded. Consequently, parasite densities are relatively low at presentation, malaria-related complications and hospitalizations are rare, and there have been no deaths associated with malaria. In contrast, patients enrolled in malaria studies in Iquitos clinics, for whom health care services are less accessible, had symptoms prior to diagnosis for twice as long, mean parasite densities up to twice as high, and higher rates of complications than those of Padre Cocha patients (Stennies G, Magill A, unpublished data).

Unlike the communities studied in the Brazilian Amazon,^{6–9} Padre Cocha is populated by individuals who have lived their entire lives in the village or surrounding area. Most adults work within walking or easy paddling distance from the village throughout the year, and spend their nights at home in the village. This contrasts not only with occupational patterns in the Brazilian Amazon, but with some of the other high malaria transmission areas in the Peruvian Amazon, where there are encampments of adult males working in the petroleum industry, jungle clearance and road construction, and frontier military service,⁴ or where adult family members leave home to farm at distant sites on a seasonal basis. Despite the strong association between working in Padre Cocha and malaria incidence in Padre Cocha adults, we were unable to identify specific occupations that conferred increased malaria risk. The lack of association between gender and malaria risk in adults is also consistent with the interpretation that occupational activities are not important risk factors in this community. Since more than 80% of those who work outside of Padre Cocha are day laborers who return to the village by nightfall, it is not clear what the association between place of work and malaria incidence signifies, but it seems likely that it is a surrogate for other factors that we were unable to identify.

Since the incidence of malaria is related to the magnitude of exposure to infectious mosquitoes,^{24–27} we attempted to measure variables related to the probability of human-vector contact. In Padre Cocha, the predominant anopheline mosquito found in preliminary adult mosquito collections is *An. darlingi*, a species that has been shown to be an efficient malaria vector in other Amazonian locations. *Anopheles darlingi* is highly anthropophilic, susceptible to both *P. falciparum* and *P. vivax* infection, both endophagic and exophagic, and, depending on the location, either endophilic or exophilic.^{28–34} Flight ranges for adult *An. darlingi* mosquitoes as far as 7 km have been described.³⁵ Entomologic investigations at Puerto Almendras, another village lying beside the Nanay river outside of Iquitos, have shown peak indoor and outdoor biting activity between 8:00 PM and midnight, with a smaller peak of activity just before dawn (Jones J, unpublished data). Extrapolating these findings to Padre Cocha, one might expect to find higher incidence rates of malaria associated with activities placing individuals out of doors and outside the protection of bednets during evening and pre-dawn hours.

The results of our risk factor assessment show that for adults, strolling about the village after 6:00 PM and arising before 6:00 AM were significantly associated with malaria incidence. For children, age itself was the most important malaria risk factor, followed by evening church attendance. Other factors such as bathing in the evening, traveling from forest and farming plots during evening and early morning

hours, and night fishing did not show any association with malaria risk. Television viewing in the evenings was negatively associated with malaria incidence, and while this may reflect the fact that television viewing often takes place in crowded settings where the probability of receiving mosquito bites is diminished, there is also a potential geographic explanation that will be discussed further below. All of the associations we identified were modest. Taken together, they are consistent with the hypothesis that evening and early morning activities, especially outside the home, are related to increased malaria risk; however, the lack of strong associations suggest that we either failed to measure important individual determinants, or that understanding malaria risk in Padre Cocha requires a group-level or geographic analytic approach.

Analysis of the household and spatial distribution of malaria in Padre Cocha using Geographic Information System (GIS) techniques has provided additional insight into the pattern of malaria incidence in the village.³⁶ Virtually all households in the village had at least one malaria episode during the 1997–1998 study year, and there was no correlation between malaria household incidence and family size, neighborhood population density, or house construction type. However, the GIS analysis did show that the spatial pattern of malaria distribution was distinctly heterogeneous within the village. Several areas had consistently high incidences of malaria of both species, and the central portion of the village had the lowest concentration of malaria infections throughout the year. Furthermore, preliminary spatial analysis of entomologic findings showed higher adult *An. darlingi* abundance in areas with higher malaria infection densities. The finding that evening television viewing was associated with diminished malaria risk is interesting in the context of malaria infection and vector distribution because most television watching occurs in the central portion of the village where there were fewer *An. darlingi* collected and malaria incidence was lowest. Most evening dancing and drinking also took place in the central area of low malaria incidence and mosquito abundance, offering a possible explanation of why these activities were unrelated to malaria risk.

There are several factors that may have affected the validity of our assessment of risks associated with malaria incidence. We have attempted to distinguish reinfection from recrudescence or relapse in a context in which the accuracy of our definitions cannot be tested. *Plasmodium falciparum* resistance to SP in the Iquitos area has been well documented,^{4,37} and it is possible that some recrudescence infections did not become apparent until after the 30 day cut-off we used, inflating the total number of *P. falciparum* infections included in our analysis. The incidence of falciparum malaria was so low during the study year that the converse, misclassifying a new infection as recrudescence during the 30-day period following initial therapy, is unlikely. Distinguishing *P. vivax* relapse from reinfection is more complicated because the relapse profile for *P. vivax* parasite strains in the area has never been determined. Since the annual incidence of *P. vivax* infection in the population is less than one infection per person, we assumed that a *P. vivax* reinfection within 2 months of prior therapy would be unlikely, and that a “tropical”,¹⁴ or early relapse pattern would be a

more probable explanation for those cases of *P. vivax* recurring within 60 days of therapy with CQ and PQ. By excluding those recurrent infections, we may have underestimated the true incidence of vivax malaria in the population. On the other hand, it is equally possible that recurrent infections identified more than 60 days following treatment for an episode of vivax malaria were due to relapse, in which case our estimate of total new *P. vivax* infections would be inflated. We compared associations between risk factors and several outcome measures, including the incidence of new infections of either or both species, the incidence of multiple infections (more than one during the study year), and the incidence of first infections. Because the patterns of association and their strength showed little variation among these different analyses, we believe that the effect of any misclassification introduced by our choice of exclusion criteria did not seriously compromise our results.

A second source of misclassification may have been introduced by respondent bias in data collected during the risk factor survey. Some of the activities included in the questionnaire may have been perceived by the participants undesirable (such as self-administration of CQ, drinking, or dancing), leading to under-reporting of these activities. Furthermore, our dichotomous categories of activity participation were crude. We were unable to quantify the frequency with which participants engaged in specific activities, or even the proportion of time individuals spent inside or outside their homes during the evenings before retiring to bed. While these biases and measurement limitations may have affected the magnitude of association between risks and malaria incidence, the likely consequence would be an underestimation of the strength of association that we detected. We therefore believe that the relationships identified as significant are likely to be valid, if underestimated.

The results of our study have several implications for malaria control in Padre Cocha. Prompt assessment and treatment can reduce malaria morbidity and mortality, and the use of PQ to abolish gametocytes should aid in reducing transmission. Despite adherence to these principles, malaria in Padre Cocha has continued to flourish, and it is unlikely that there is room for marked improvement at the Health Post where malaria diagnosis and treatment are already rapid and appropriate. If a significant proportion of malaria transmission occurs out of doors and during hours when the population is not in bed, vector control measures such as indoor residual insecticide spraying and insecticide-impregnated bednets may not be sufficient to bring about dramatic reductions in malaria incidence. There is, however, historical evidence,^{2,38} and a recent trial comparing lambda-cyhalothrin and DDT residual indoor house sprays in Rondônia, Brazil³⁹ that support the efficacy of this approach. In addition, pyrethroid-impregnated curtains have been shown to be effective in reducing malaria prevalence and the numbers of anophelines found in houses with open windows and eaves in Suriname, where *An. darlingi* is also the principle malaria vector.⁴⁰ Thus, insecticide-impregnated bednets or curtains may be worth testing, particularly if local *An. darlingi* are found not to rest indoors. The use of mosquito repellents, coils, and electric insecticide vaporizers are beyond the means of most Padre Cocha residents, rendering these measures impractical. Furthermore, the ubiquitous distribution of

larval breeding sites during the wetter months of high malaria transmission makes effective larviciding a daunting proposition. Rational approaches to outdoor mosquito abundance reduction will require a better understanding of vector behavior and requirements in the village and its surroundings than is currently available. It seems likely that a combination of indoor and outdoor vector control measures, in conjunction with continued prompt and effective treatment of clinical malaria, will be required to achieve a marked reduction in malaria transmission in the village.

A final phenomenon observed during the study year deserves comment. The total number of malaria cases diagnosed in Padre Cocha decreased by approximately 40% from 1997 to 1998, and the entire reduction was in *P. falciparum* infections. This decrease in falciparum malaria was also true for Loreto as a whole, where the total number of microscopically confirmed cases decreased by 31%, and 72.5% of the reduction was in *P. falciparum* infections.⁴ Several possible explanations for this disproportionate reduction in falciparum malaria present themselves. A change to more effective therapeutic agents for *P. falciparum* infections could produce this result; however, SP has been used continuously in Padre Cocha since 1995, and there had not been any major drug policy changes elsewhere in Loreto. Earlier diagnosis and treatment, particularly PQ administration, could also produce this effect,^{29,41} but there is no evidence that a systematic reduction in the duration of malaria infections prior to treatment has been achieved in either Padre Cocha or Loreto as a whole. If recent MOH residual insecticide spraying and larviciding campaigns achieved this result, one must infer that the two malaria species are transmitted by different vectors that were not equally affected by these measures. Given the marked predominance of *An. darlingi* in the Iquitos area, this too seems unlikely. Finally, the occurrence of a prolonged and unusually hot dry season during the months of August–November 1997, related to an *El Niño* year, has been offered as a potential cause of the decrease in malaria incidence.⁴ This climatic explanation is an intriguing possibility, which fits with historical associations of reduced malaria during *El Niño* years in Venezuela.⁴² However, the exact mechanism of the *El Niño* effect that would lead to reductions in *P. falciparum* transmission, while *P. vivax* transmission remained intact, is obscure. If high ambient temperatures produce a failure in *P. falciparum* sporogony more readily than in *P. vivax* reproduction, the dry season *P. falciparum* reservoir could be disproportionately depleted. Alternatively, it is possible that anopheline vectors other than *An. darlingi* are responsible for maintenance of transmission during dry season conditions, and that this secondary vector population reproduces and transmits *P. falciparum* less efficiently than *P. vivax*. The effects of such a shift in vectors might only become apparent if the dry season were unusually prolonged, permitting a marked reduction in the human reservoir of falciparum parasites through diagnosis and treatment of *P. falciparum* infections during a time when vector reproduction has also slowed.

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