Differential perpetuation of malaria species among Amazonian Yanomami Amerindians

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Abstract. To determine whether malaria perpetuates within isolated Amerindian villages in the Venezuelan Amazon, we surveyed malaria infection and disease among 1,311 Yanomami in three communities during a 16-month period. Plasmodium vivax was generally present in each of these small, isolated villages; asymptomatic infection was frequent, and clinical disease was most evident among children less than five years of age (odds ratio [OR] = 6.3, 95% confidence interval [CI] = 1.4–29.2) and among persons experiencing parasitemias ≥ 1,000 parasites/mm³ of blood (OR= 45.0, 95% CI = 5.5–370.7). Plasmodium falciparum, in contrast, was less prevalent, except during an abrupt outbreak in which 72 infections resulted in symptoms in all age groups and at all levels of parasitemia, and occasionally were life-threatening. The observed endemic pattern of P. vivax infection may derive from the capacity of this pathogen to relapse, while the epidemic pattern of P. falciparum infection may reflect occasional introductions of strains carried by immigrants or residents of distant villages and the subsequent disappearance of this non-relapsing pathogen.

Malaria stands out as one of the more devastating health burdens borne by the Yanomami Amerindians of the Venezuelan Amazon. Although malaria has been endemic in the region for more than a century, certain Yanomami communities became exposed only after the discovery of gold in 1989. In 1990, 60% of all Yanomami deaths were attributed to malaria.

This severe loss of life stimulated a series of clinic-based surveys in the region during the past decade. Observations on ill residents of various Yanomami villages suggested that both Plasmodium falciparum and P. vivax had become endemic and that both infections were highly prevalent throughout the year. Superimposed on this perennial pattern of transmission, sporadic malaria outbreaks produced intense morbidity and mortality in widely separated villages. The apparently continuous high-level transmission, however, seemed inconsistent with the observed severity of the resulting disease episodes.

It may be that each malaria species perpetuates differently in small and isolated communities. A study design based on a longitudinal survey of malaria infection and disease in diverse villages would detect differences in the perpetuation of each species. To compare the epidemiology of diverse malaria species in such isolated human populations, we therefore used both passive and active case detection during sequential surveys of three separated Yanomami villages in the Venezuelan Amazon between August 1993 and November 1994.

Materials and methods

Based on a review of historical documents and previous epidemiologic studies of malaria in the region, we designed a 16 month prospective study of three villages: Coyoweteri, Parima B, and Coshiloweteri. The study was carried out under the auspices of Centro Amazonico para la Investigacion y Control de Enfermedades Tropicais ‘Simon Bolivar’ (CAICET), and informed consent was obtained orally from all participants. The human subjects committees of CAICET, the Venezuelan Ministry of Health (MSAS), and the Harvard School of Public Health approved the study.

Study population. The population of the Venezuelan Amazon consists of 37% indigenous persons, of whom about 9,000 persons or one-third are Yanomami. These Amerindians live near the border of Venezuela and Brazil in semi-permanent villages that are separated by a half to five-day walk from each other. The 50–200 inhabitants of each village occupy a single shabono, a ring-like, thatched roofed communal shelter with no walls or internal partitions. Residents usually spend 7–8 months per year working in gardens and hunting in forests in the vicinity of their shabono and the rest of the year on prolonged forest treks visiting other villages and hunting and gathering food. Although villagers generally return to their shabono after such treks, every several years they abandon their dwellings and establish new homes elsewhere. In some instances, shabones have been replaced by enclosed single family houses located in missionary (Catholic and Protestant) villages. Yanomami mainly rely on traditional medicine and occasionally seek assistance for diseases such as malaria at health posts operated by the MSAS or by missionaries.

Antimalarial medications are available only at these health posts. The Yanomami interpret illness as an evil brought on by enemy villages or by spirits, and during epidemics, they attempt to flee from the source.

Study sites. The study was performed in the State of Amazonas, in southern Venezuela, a region of approximately 178,000 km², with a population of 79,000, or roughly 0.4 inhabitants per km² (Figure 1). This roadless region is heavily forested and has temperatures ranging between 22°C and 27.5°C and relative humidities between 60% and 100%. During the wet season (May through October), rainfall measures roughly 200 mm per month. Rain is intermittent during the dry season. The three study sites are separated by a 10-day walk, or by 0.5–2 hr of travel in a small aircraft. Coyoweteri and Parima B are located in the Sierra Parima, in the upper Orinoco Basin, Department of Rio Negro. Coyoweteri is located at sea level in dense forest, and Parima B is located at an altitude of 850 m in a region of mixed rain forest and savanna. Coshiloweteri is located at sea level along the bank of the Padamo River, in the Alto Orinoco, in the Department of Atapabo. The original forest that surrounded Coshilow-
eteri disappeared following settlements of foreigners and increasing river traffic in the last 15 years. Study sites were chosen based on ecologic differences between sites and the presence of missionary or governmental infrastructure that could support the study activities.

**Study design.** From August 1993 to November 1994, successive cross-sectional surveys of 3–8 weeks duration were repeated in the three study sites. On each occasion, the survey was initiated with a house-to-house census of the main population of each of the three sites, which included all Yanomami living in the principal village and surrounding villages within a 0.5-day walk (about 4–5 km). The census was followed by visits to each household to obtain demographic information and information on symptoms suggestive of malaria and use of antimalarial drugs, and to obtain fingerprick blood samples for microscopy and polymerase chain reaction–based analysis. The same protocol was followed for the remote populations, consisting of persons living beyond the 4–5-km perimeter visiting the principal villages for medical or social/cultural reasons, typically in large groups (10–100 individuals), during the survey. The study design allowed complete population sampling of the main populations and convenience sampling of the remote populations.

Coyoweteri, Parima B, and Coshiloweteri were surveyed four, three, and two times, respectively. We attempted to re-study all participants at subsequent cross-sectional surveys, including individuals from the remote populations. The number of participants, however, varied across surveys largely as a result of absences from the site.

**Laboratory studies.** Smears of peripheral blood were stained with Giemsa and examined by light microscopy in the field. Parasites were identified to species and quantified...
by counting the number of asexual parasites per number of white blood cells in 100 oil-immersion fields. The parasite density per mm$^3$ was calculated, assuming that a microliter of blood contained $8 \times 10^3$ white blood cells and $5 \times 10^6$ red blood cells. Subjects with symptomatic infection were treated and followed for clinical improvement, and with malaria smears for up to 28 days or until the subject or study team left the area. Pretreatment urine samples from 477 persons were examined for the presence of chloroquine using the Dill-Glazko test.16,17

**Clinical studies.** Symptomatic malaria was defined as documented fever or a history of fever with or without headache, chills, or myalgia in the setting of a positive blood smear. Asymptomatic malaria was defined as the presence of parasites in the absence of symptoms. Of infected persons, all asymptomatic infections and less than 10% of symptomatic infections were identified during the house-to-house survey. The majority of symptomatic persons presented to the health post for diagnosis. Some of these subjects had not yet been visited by the study team, while others had not been infected when examined earlier during the house-to-house survey. According to the MSAS and CAICET protocols, infected persons received standard doses of chloroquine phosphate or, in the presence of high parasitemia or signs of severe malaria, other schizonticidal drugs. Primaquine phosphate was given for three days if there were gametocytes of *P. falciparum* on the smear and for 14 days if the infecting species was *P. vivax*.

**Entomologic studies.** Larval anopheline mosquitoes were collected from accumulations of clear water in and around each main village and preserved in ethanol. Every night for two weeks at each site, adult mosquitoes were captured in Center for Disease Control (CDC) (Atlanta, GA) light traps set inside two shabones or by human landing catches performed by two residents who volunteered to assist us. Mosquitoes were identified to species at the Harvard School of Public Health and at the Florida Medical Entomology Laboratory (Vero Beach, FL).18-20

**Analysis of data.** Data were recorded using Epi-Info (Version 5.01a, CDC, 1991). *Plasmodium falciparum*—and *P. vivax*-infected subjects were tabulated separately. Mixed infections were counted both as *P. falciparum* and *P. vivax*, but were not included in the calculation of rates of disease caused by each species or in the measurement of parasitemia. Wilcoxon rank sum tests were used to compare parasitemias among age groups and between persons with symptomatic and asymptomatic infections.

Logistic regression was used to examine the relationships of age group, sex, type of housing, and season on both infection and symptoms. Models examining symptoms also evaluated the effects of levels of parasitemia. Because only one type of dwelling was present in Coyoweteri and Coshiloweteri, models examined the effect of house type only in Parima B. Due to ecologic differences between the three study sites and different methods of sampling residents of the main and remote populations, analyses were adjusted for study site and residence in the remote population.

Because the level of endemicity influences the age-specific distribution of both infection and disease,21-24 we examined possible interactions between age and study site, residence in the main or remote populations, or parasitemia levels in our multivariate models. To account for the correlation among repeated observations on subjects across different cross-sectional surveys, generalized estimating equations24,25 were used to calculate robust variance estimates, confidence intervals, and Wald $\chi^2$ tests.

### RESULTS

**Demography.** A total of 1311 persons participated during the 16-month study, or approximately 14% of the Yanomami population in the Venezuelan Amazon (Table 1). Forty-four percent of the population was younger than 15 years of age except in Coyoweteri, where fewer young people were present, perhaps because of decreased fertility reported by women during the previous five years. The population structure observed in the study villages, similar to that of developing countries, likely reflects the overall Yanomami population structure. All residents of Coshiloweteri and 27% of residents of Parima B lived in single-family houses. The pre-treatment Dill-Glazko examination indicated that about 6% of the study population (20 of 207 persons in Coyoweteri, 1 of 169 persons in Parima B, and 8 of 101 persons in Coshiloweteri) had used chloroquine recently.

**Epidemiology of *P. vivax.*** *Plasmodium vivax* infections were detected in each study site in all but one survey (in Parima B, Figure 2). During the 16-month period of observation, 1,309 subjects experienced 231 infections due to *P. vivax* (two study subjects had missing information). Rates in males did not differ from those in females. Multivariate analysis demonstrated a different relationship between age and infection in each village (Wald $\chi^2$ test for age by village interaction = 29.3, degrees of freedom = 6, $P < 0.001$; Table 2A); the association was strongest in Coyoweteri, where rates were particularly high in children. In Parima B, where both types of housing were present, the odds of *P.
vivax infection were higher for subjects living in single family houses than for those who lived in shabonos (Table 2B). Episodes of infection occurred mainly at the beginning and at the end of the rainy season.

Young children, especially those living in the main populations, experienced more P. vivax-related illness than adults (Table 3). The 106 symptomatic subjects had higher levels of parasitemia (geometric mean = 489 parasites/mm³, median = 354, range = 11–22,624) than did the 66 asymptomatic residents (geometric mean = 124 parasites/mm³, median = 113, range = 8–5,169) (<0.001, by Wilcoxon rank sum test). Multivariate analysis of all episodes of vivax malaria confirmed that symptoms were most evident among children less than five years of age, members of remote populations, and persons experiencing parasitemias ≥ 1,000 parasites/mm³ of blood (Table 4). Delay in seeking medical attention did not appear to account for the high rate of symptomatic infection in the remote populations, since duration of symptoms prior to diagnosis was not different between the main and remote populations, regardless of levels of parasitemia.

Epidemiology of P. falciparum. There were 116 infections due to P. falciparum during the 16-month survey; 72 of these occurred during an outbreak in the last two months of the survey (Figure 2). In all surveys prior to the outbreak, less than 10% of each population had P. falciparum infections, and this pathogen was recorded only 44 times (including 15 mixed infections). Infections that occurred before the outbreak were most frequent at the beginning and the end of the rainy season (Table 5). There was no association of infection with age, sex, site of residence, or type of housing.

Before the outbreak, 45% (13 of 29) of infections due to P. falciparum alone were symptomatic. There was only one person who had greater than 1,000 parasites/mm³, a one-year-old child who died with 89,062 parasites/mm³ in the peripheral blood (geometric mean of 24 parasitemias measured = 122, median = 96, range = 12–89,062 parasites/mm³). Parasitemia was only slightly greater in symptomatic persons than in asymptomatic persons (geometric means = 150 parasites/mm³ versus 103 parasites/mm³, respectively; P = 0.62, by Wilcoxon rank sum test).

An outbreak of P. falciparum infection occurred in Coyoweteri during the final survey in October–November, 1994, at the end of the rainy season (Figure 2). More than 40% of the population became infected, and 93% (51 of 55) of persons infected by P. falciparum alone and 89% (8 of 9) of those with mixed infections for whom we have clinical information were symptomatic (Table 3). Many subjects who were asymptptomatically infected with P. falciparum during previous surveys became symptomatic during this epidemic. Symptomatic infection was observed in nearly all infected persons in both populations and in all age groups. Symptoms occurred at all levels of parasitemia, which ranged from 17 to 620,000 parasites/mm³ in those for whom we have information on parasitemia (n = 60, geometric mean = 483, median = 396). More than 60% of the subjects in both populations for whom data were available had been symptomatic for one day or less at the time that they presented at the health post.

Anopheine vectors. A list of anopheines present in the sampling sites was developed based on mosquitoes captured in light traps and when attempting to feed on volunteers. Collections taken in Coshiloweteri in September 1994 and in Coyoweteri in October and November 1994 during the outbreak of P. falciparum malaria included Anopheles darlingi, A. intermediately, and A. oswaldi. Anopheles intermedius was particularly abundant during the outbreak, and A. neivai was discovered solely during the outbreak near a single shabono inhabited by members of a remote population in Coyoweteri where nu-
merous bromiliads were present. No anopheline mosquitoes were captured during collections in Parima B.

**DISCUSSION**

Patterns of malarial infection and disease in these isolated Yanomami communities differed according to species and over space and time. *Plasmodium vivax* was present at almost all surveys, and nearly 30% of the residents of two of these communities were infected at the end of the first rainy season. Immunity provoked by successive infections with *P. vivax* may account for the numerous asymptomatic episodes that we observed, particularly among older residents. In areas where transmission was most intense (as in Coyoweteri), children experienced the highest rates of both infection and disease, while adults infrequently had *P. vivax* infection. Self-administration of antimalarial drugs does not appear to explain the preponderance of asymptomatic *P. vivax* infec-

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**Figure 2.** Prevalence of infection with *Plasmodium vivax* and *P. falciparum* in the three study sites, by survey.

**Table 3**

Symptomatic malaria infection among persons infected with *Plasmodium vivax* or *Plasmodium falciparum* by age and population type

<table>
<thead>
<tr>
<th>Age (years)</th>
<th>P. vivax No. of symptomatic infections/no. of total infections (% symptomatic)</th>
<th>P. falciparum (prior to the epidemic)</th>
<th>P. falciparum (during the epidemic)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. of symptomatic infections/no. of total infections (% symptomatic)</td>
<td>Main</td>
<td>Remote</td>
</tr>
<tr>
<td>0–4</td>
<td>13/17 (76)</td>
<td>7/8 (88)</td>
<td>1/3 (33)</td>
</tr>
<tr>
<td>5–14</td>
<td>15/49 (31)</td>
<td>11/15 (73)</td>
<td>2/6 (33)</td>
</tr>
<tr>
<td>15–39</td>
<td>26/56 (46)</td>
<td>24/28 (86)</td>
<td>4/9 (44)</td>
</tr>
<tr>
<td>≥40</td>
<td>7/21 (33)</td>
<td>6/8 (75)</td>
<td>2/3 (67)</td>
</tr>
</tbody>
</table>

* Two individuals not included due to missing data on symptoms.
† Odds ratio = 5.3 (95% Confidence interval = 1.6–17.2; *P* = 0.006) for 0–4 year old age group vs. all others.
‡ One individual not included due to missing data on symptoms.
§ Six individuals not included due to missing data on symptoms.
tions because only a few infected persons had evidence of recent chloroquine use by Dill-Glazko testing. In comparison, infections with *P. falciparum* were infrequent before the epidemic in Coyoweteri in October and November 1994. Lower levels of immunity to *P. falciparum* may have placed persons at risk for the severe and life-threatening illness that was observed in all age groups during the outbreak. The asymptomatic *P. falciparum* infections observed prior to the epidemic may have resulted from exposure to strains antigenically different from the single variant which caused the epidemic (Laserson KF, unpublished data).

Clinical records maintained by both local medical missionaries and CAICET in prior years confirm our finding that infection with *P. vivax* but not *P. falciparum* was relatively constant in these villages. *Plasmodium vivax* may be able to persist in the community because of its tendency to relapse after prolonged periods of dormancy and because attempts at radical cure with primaquine often fail. Non-relapsing *P. falciparum* infections would persist in the community for shorter periods of time and thus require periodic re-introductions to perpetuate. The migratory lifestyle of the Yanomami appears to facilitate periodic exchanges or introductions of parasites only occasionally during social and/or medical treks or short journeys to neighboring villages or from intrusions by immigrants to the region. Anti-parasite or anti-disease immunity to *P. falciparum* would be slow to develop under these conditions, if it develops at all.26

Because each Yanomami village, including as many as 200 residents, resides under only a single, non-partitioned *shabono*, an introduced infection can rapidly affect the entire village. Each introduction of *P. falciparum* may involve a unique strain, as was observed during the outbreak in this study (Laserson KF, unpublished data), given the prolonged time between outbreaks and the rapidity with which the parasite undergoes recombination in the *Anopheles* vector.30–32 Furthermore, each strain may lead to differing pathogenesis, and the introduction of a virulent strain that the population had not previously encountered would have devastating consequences, as were observed in the outbreak in Coyoweteri.30–33

Malaria infection and disease were not associated with gender, suggesting that transmission occurs in or around dwellings, or during treks when families migrate together. As in the Brazilian Amazon,34 the highest rates of transmission occurred at the beginning and the end of the rainy season, when partially sunlit and clean pools of water tend to form near human habitations. *Anopheles darlingi*, the principal vector in the region, would breed in such water, particularly where drainage has been disturbed. The relative absence of transmission during periods of heavy precipitation may be due to a flushing action that inhibits the development of larval anopheline mosquitoes. The significant association between *P. vivax* infection and living in a single family home observed in Parima B may reflect greater proximity to breeding sites among these Yanomami, and warrants further investigation.

Owing to the migratory lifestyle of the Yanomami, the prospective nature of this study was limited by the irregular follow-up of individuals across surveys, especially those living in the remote villages. Furthermore, we were unable to sample Yanomami from all known villages in the region due to great distances between villages and logistic difficulties in reaching all areas. Nevertheless, the main and remote populations did not demonstrate major differences in the relationships between covariates and outcomes, and seasonal patterns of malaria were similar across the three study sites, suggesting that our sampling was representative of the Yanomami population in general. While *in vitro* tests of acquired immunity are necessary to confirm the hypothesis that the Yanomami enjoy greater acquired immunity to *P. vivax* than to *P. falciparum*,35–36 malaria appears to be two distinct disease complexes in the Venezuelan Amazon. Our results demonstrate that the uniquely isolated *shabono* lifestyle of the Yanomami, combined with the current conditions of pathogen introduction by other Yanomami or through invasions by outside groups, influences the perpetuation of malaria in this region. These findings should be taken into account in the targeting of appropriate control strategies to areas and at time points where there is the greatest need.

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