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TEMPORAL AND SPATIAL PATTERNS OF MALARIA REINFECTION IN NORTHEASTERN VENEZUELA

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Abstract. We stratified the risk of malaria transmission (*Plasmodium vivax*) in 35 villages along a coastal range in northeastern Venezuela (51 km^2) where the main vector is the mosquito *Anopheles aquasalis.* After 20 years without local malaria transmission, reinfection of the entire area occurred from May to December 1985 by local (continuous) and jump (discontinuous) dispersal. Epidemiologic, environmental, and vector variables were investigated with the aid of a Geographic Information System. Risk factors for malaria transmission were human population density, proximity to pre-adult mosquito habitats ($<$ 500 m), and the number of pre-adult habitats nearby. Most inhabitants, immature mosquito habitats, and malaria cases were located at low elevations and on gentle slopes. High prevalence of malaria during the dry seasons was associated with the presence of permanent bodies of water containing *An. aquasalis.* Occurrence of a La Niña event in 1988 (wet and cool phase of the El Niño Southern Oscillation) triggered malaria transmission to unusually high levels, consolidating infection in the area, and rendering traditional control efforts useless. We recommend tracking malaria persistence per village and associated risk factors as methods to reduce the cost of malaria control programs.

A malaria focus is a circumscribed locality situated in a current or former malarious area that includes the epidemiologic factors necessary for malaria transmission. These include a human community, at least 1 source of infection, a vector population, and the appropriate environmental conditions.[1] However, health departments in countries where malaria is a problem may not have the historical information to delineate the potential foci of malaria. The locations where malaria cases occur are commonly known and the addresses of the reported cases usually correspond to the dwellings of the infected individuals. Accordingly, it may not be necessary to apply control measures to the entire community but only to provide medical care to individual cases of malaria.

It would also be useful to know the importance of local malaria transmission since various foci may produce cases at different rates that can be connected by dispersal of infected vectors or humans. For example, a focus in an area where humans congregate (e.g., market place, school) may lead to more dissemination than isolated or less connected foci. Thus, a malarious area can be made of localities with cases where there is no local transmission (cold spots), foci of high or persistent transmission (hot spots), and foci of moderate to low local transmission (cool spots) where the disease may disappear by itself if the locality were isolated.[2] The underlying hypothesis is that a simultaneous and drastic reduction of malaria transmission in the hot spots will prompt a subsequent fade out[3] of malaria in the cool spots, and will eliminate cases in cold spots.

The present study examined several aspects of malaria transmission in an area of northeastern Venezuela that was free of malaria for more than 20 years (1965–1985).[4] We attempted to stratify 35 localities in the reinfected area in terms of malaria persistence (the maximum number of consecutive months with cases) as a means of identifying areas that may be considered hot spots. We also investigated with the aid of a Geographic Information System (GIS), the relationship between several environmental variables (altitude, terrain slope, number of inhabitants, number of immature mosquito habitats within 500 m of the human settings, distance to the nearest immature mosquito habitat) and the incidence and persistence of malaria.

MATERIALS AND METHODS

Study area. The study area (51 km^2) is located in Sucre State in northeastern Venezuela (Figure 1; 10°14′–10°18′N, 64°21′–64°30′W), and corresponds to the coastal ecoregion.[5] It is a mountain range (altitude = 0–700 m) along the Caribbean Sea (Santa Fe Bay). Mean annual rain in the nearest meteorologic station (located 17 km west) was 654 mm (1985–1994), with a rainy season from May to December (97.7% of the total rainfall). Vegetation along the coast is mainly halophytes (plants from saline habitats), mangroves, and coconut groves. Inland, vegetation varies from deciduous to semi-evergreen forests, with patches of land cleared for small-scale agriculture, and short grasses and shrubs derived from disturbances such as logging and fire. There are riparian forests and tall grasses in the open areas along the main streams into the sea. Economic activities are fishing, subsistence agriculture, and tourism. An estimated population of 8,836 inhabitants existed in the area in 1991. The villages are interconnected by primary (paved) and secondary (dirt) roads. The main human concentration is the town of Santa Fe where administrative, educational, and health care facilities are located. The decision to work on this area was made because it had traditionally been considered problematic in terms of control,[6] more than 95% of the malaria cases in the entire region were reported from this area, and maps were available.

Epidemiologic data. This was a retrospective study of malaria incidence in which no personal identifiers were used. The study was reviewed and approved by the Venezuelan Council for Scientific Research. Malaria cases were reported by local personnel in the Department of Health who prepared blood smears. Symptomatic cases were detected by surveillance at the local hospital and by visiting households where individuals were suspected to be ill. Most (99%) positive

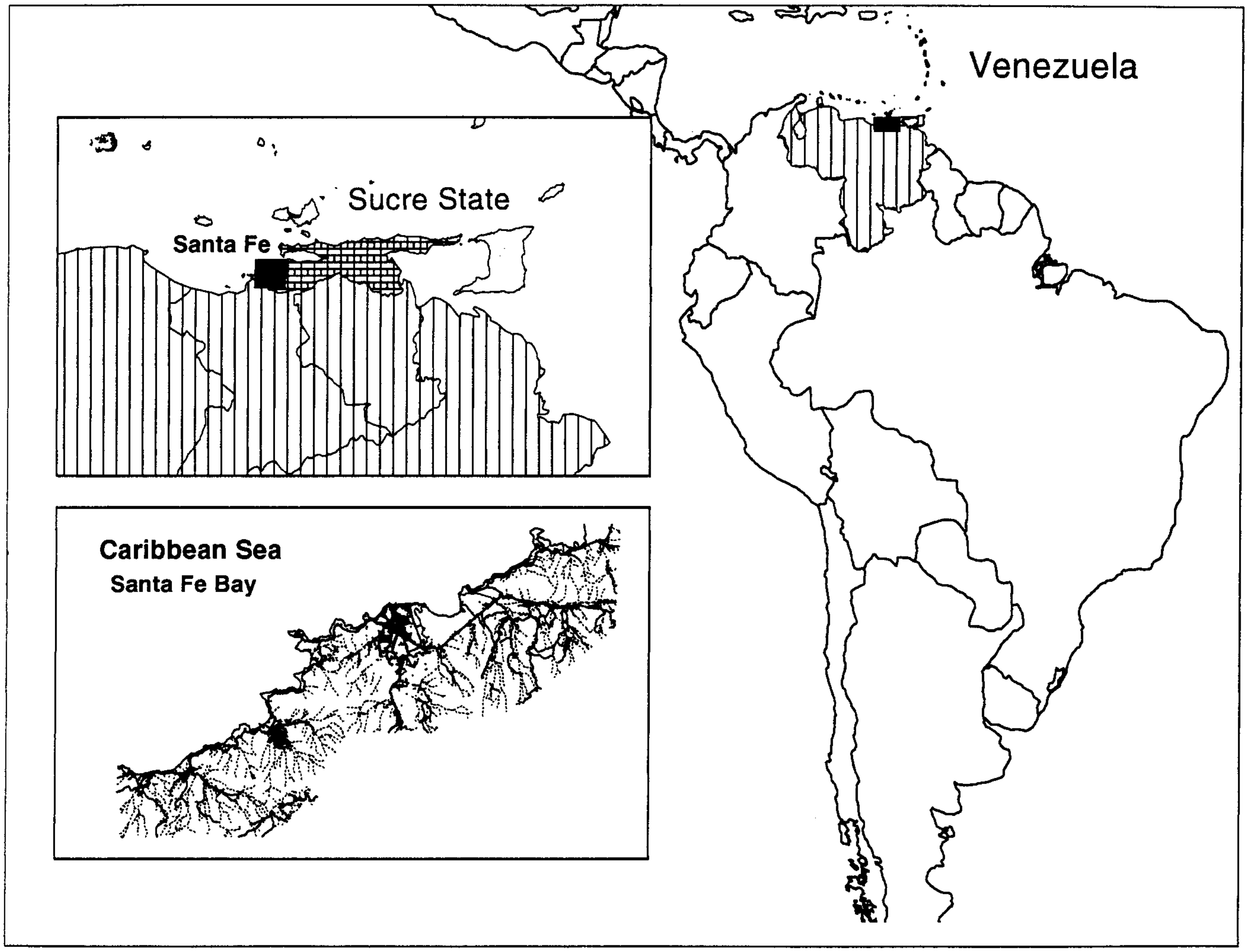

FIGURE 1. Study area in northeastern Venezuela, Sucre State, Santa Fe Bay along the Caribbean Sea. The lower **inset** shows the coastline, major roads, and streams.

blood smears showed *Plasmodium vivax* with very few cases of *P. falciparum.* We analyzed data in the period from 1985 to 1997. A study of malaria prevalence by age group and sex was conducted using data from 1989 (a year of high incidence) to determine whether the structure of the population with malaria was different from that of the entire local population (census data of 1990). A longitudinal study of malaria incidence was performed to describe its relationship with rainfall. The maximum number of consecutive months a locality had malaria cases (malaria persistence) was calculated from monthly malaria incidence data per human setting.

Environmental variables. The following variables were studied: elevation, terrain, slope, number of inhabitants per village, number of immature *Anopheles aquasalis* habitats within 500 m of the center of the villages, and the distance from the nearest immature *An. aquasalis* habitat. These variables were derived from a GIS developed for the study area.[7] The aquatic habitats of the immature vectors were surveyed during the dry seasons in 1996 and 1997 because previous surveys did not cover the entire study area (Berti and others[8]). Mosquito species composition, abundance, and geographic locations of current and past pre-adult surveys were incorporated into the GIS. We attempted to find most anopheline aquatic habitats with the aid of the inhabitants who indicated to us where bodies of water were distributed in the study area. Geographic features (rivers, roads, buildings, contour lines) were digitized in ARC/INFO (Environmental Systems Research Institute, Inc., New York, NY) from 1:5,000 topographic maps (Department of Transportation, 1992). Elevation and slope were calculated with the GIS from the contour lines (every 5 m). The geographic layers were imported into Atlas GIS (Environmental Systems Research Institute, Inc.).

Statistical analyses. A 2 × 2 contingency table (infected males or females versus all males or females in the population) was used to test the null hypothesis that malaria prevalence was independent of gender. A 16 × 2 contingency table (infected population divided into 16 ages classes versus the entire population in the same age classes) was used to test the null hypothesis that malaria prevalence was independent of age. A Pearson correlation analysis was used to

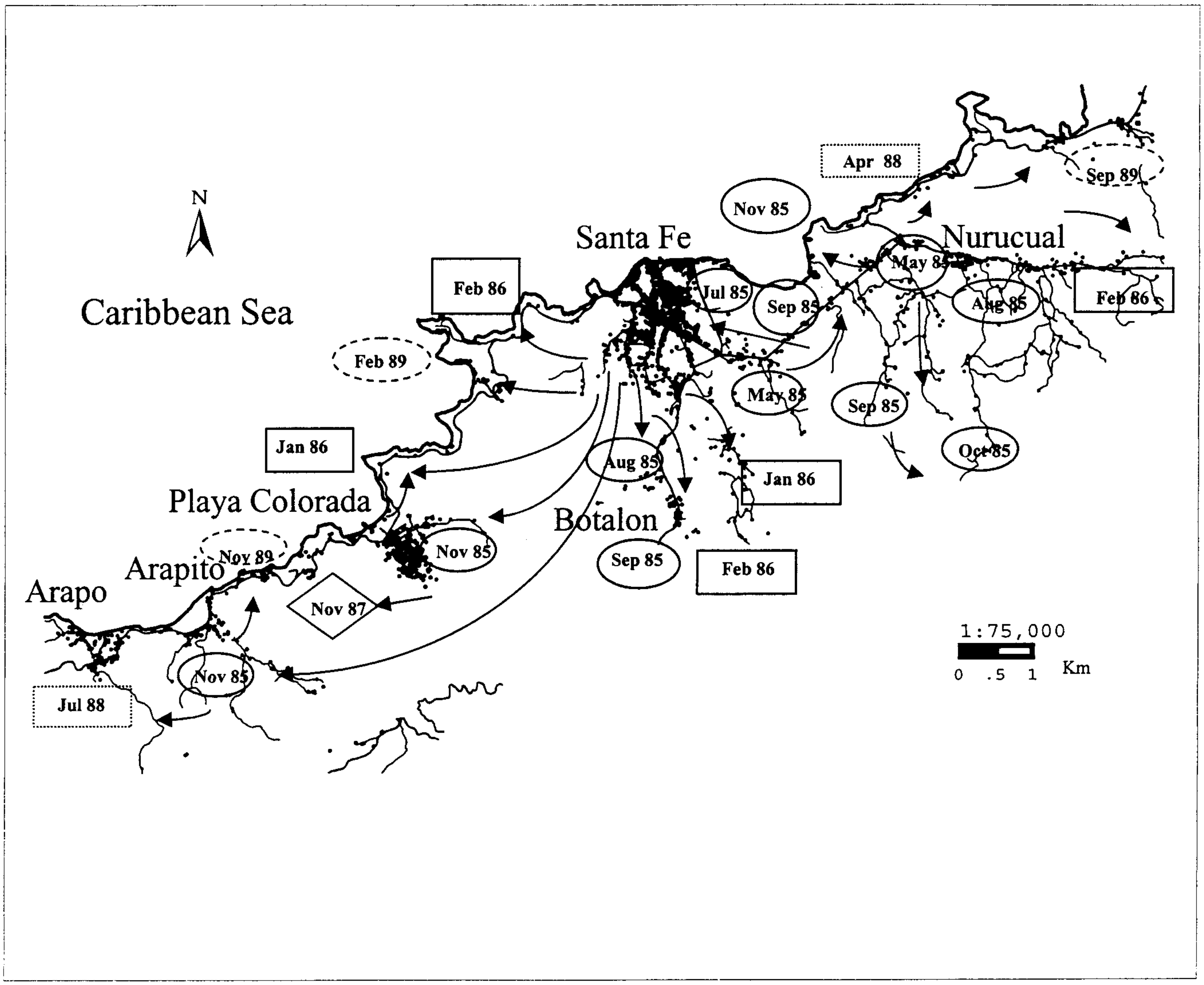

FIGURE 2. Historical account of malaria reinfection in the Santa Fe area. The **arrows** show likely direction of spread. **Solid ovals** indicate the dates and locations of new cases reported in 1985, the year of reinfection. **Solid rectangles** are for 1986, **closed triangles** for 1987, **dotted rectangles** for 1988, and **dashed ovals** for 1989. The map contains roads, enlarged houses, and the names of important locations.

test the null hypothesis that malaria incidence and precipitation per month were independent. The correlation between the maximum consecutive months with cases and the mean monthly cases of malaria was evaluated with a Spearman correlation coefficient. A multiple stepwise regression was used to test the possible effects of environmental variables on malaria persistence and prevalence. Statistical analyses were performed with the software SPSS.

RESULTS

Temporal patterns. The first officially reported cases of malaria occurred in May 1985 near the main town of Santa Fe (Figure 2). Malaria spread rapidly in this town (July 1985; 2 km), Nurucual (August 1985; 3 km), Botalon (August 1985; 4 km), and the other major human settings in the area (November 1985; Playa Colorada, 10 km and Arapito, 13 km). It is interesting to note that Arapo (distance of 14.5 km) did not show malaria cases until July 1988 in spite of its proximity (1.7 km) to Arapito (Figure 2). The smaller human settings were slower in acquiring cases than the larger ones, with the exception of Arapo (Figure 2).

Reinfection proceeded rapidly since the first cases were reported at the end of the dry season, and the epidemics increased throughout the rainy season in 1985 (Figure 3). Control efforts during 1986 and 1987 (primarily indoor spraying) may have had an impact on malaria incidence but transmission persisted through those years (Figure 3). In 1988, precipitation was unusually high and malaria was out of control until 1991 when new control measures were applied (outdoor spraying of mosquito resting sites and breeding site treatment with pathogenic bacteria).[9] Malaria was successfully controlled in the following years (Figure 3), and only 3 cases of imported malaria were reported from 1994

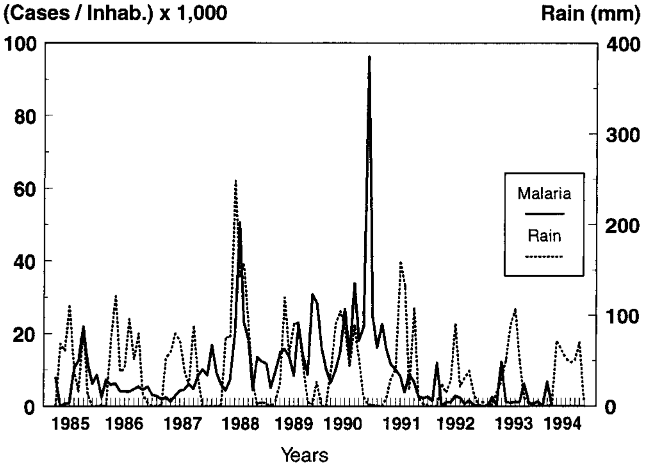

FIGURE 3. Temporal changes in the annual parasite index and precipitation per month for the study area. Inhab. = inhabitants.

to 1997. A distinct pattern of the epidemic was the high level of malaria transmission that occurred during the dry seasons. Indeed, only 6.7% of the total rainfall occurred from January to May 1985–1994, a period when 45% of all malaria cases were reported. There was no linear correlation between precipitation and malaria incidence per month (r = 0.09, $P > 0.05$).

Spatial variation in malaria persistence. Some villages showed a long period of sustained malaria incidence, such as Santa Fe, which had cases for as much as 81 consecutive months. Only 9 of 35 human settings showed a maximum consecutive incidence of malaria greater than 10 months. Five had consecutive cases between 5 and 9 months, whereas 21 had consecutive cases for 4 months or less. Human settings with few consecutive months with cases showed a characteristic pattern of interrupted malaria incidence (e.g., Estebita and Chorro Frio; Figure 4). There was a positive correlation between the length of the maximum consecutive months with cases and the mean monthly cases of malaria (Spearman correlation, rs = 0.97, $P < 0.01$; Figure 5). Therefore, most localities from where malaria cases were reported had low malaria incidence and persistence. Also, human settings with the highest malaria prevalence and persistence had the largest populations (Figure 6). It should be noted that a positive relationship between these 2 variables exists only for human settings with the highest persistence of malaria (Figure 6).

Environmental variables associated with malaria prevalence and persistence. The human settings with high malaria persistence and prevalence had a larger population, were closer to immature mosquito habitats, and were located at low elevations on gentle slopes (Table 1). A stepwise multiple regression on the $\log_{10}$ number of consecutive months with malaria cases per village was significant (F = 22.3, $P < 0.01$, by analysis of variance [ANOVA]), with significant regression coefficients for the independent variables of number of inhabitants ($\log_{10}$; 0.37 ± 0.14 [± SE]; $t = 2.68$, $P < 0.01$) and the number of nearby immature mosquito habitats ($\log_{10}$; 0.10 ± 0.04; $t = 2.78$, $P < 0.01$). The regression on the total number of malaria cases plus one was also significant (F = 25.9, $P < 0.01$, by analysis of variance), with significant regression coefficients for the independent variables of number of inhabitants ($\log_{10}$; 0.81 ± 0.17; $t = 4.85$, $P < 0.01$) and distance to mosquito habitats ($\log_{10}$; -1.00 ± 0.24; $t = -4.09$, $P < 0.01$). Therefore, malaria prevalence and persistence in the study area seem to have been influenced by a combination of the size of the human settlement and the existence of nearby immature mosquito habitats.

Vertical study. The number of males with malaria (285)

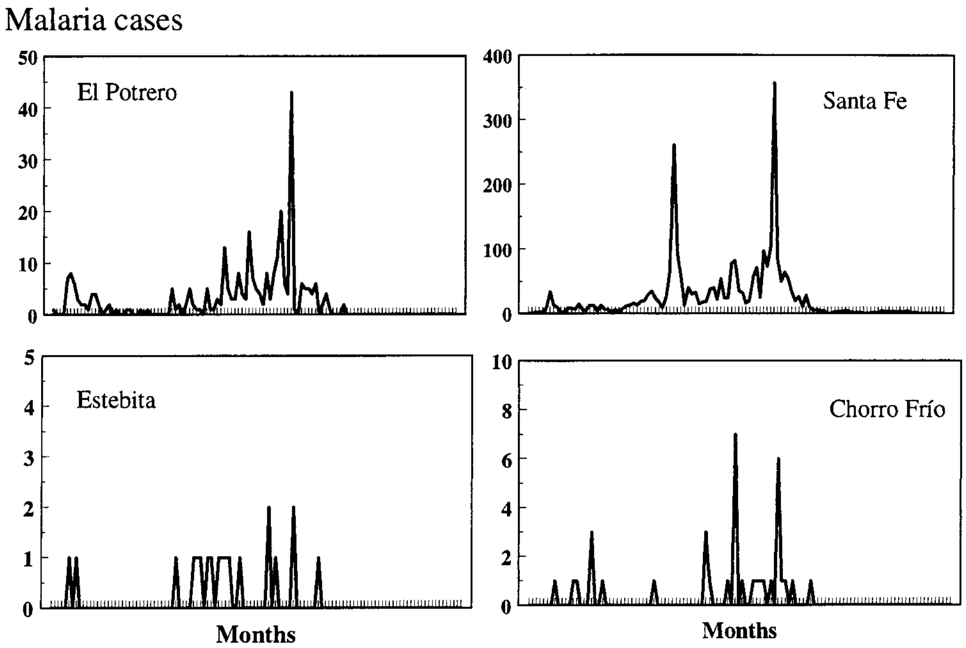

FIGURE 4. Sample representation of 4 towns with different levels of monthly malaria incidence to show the continuous or intermittent nature of malaria.

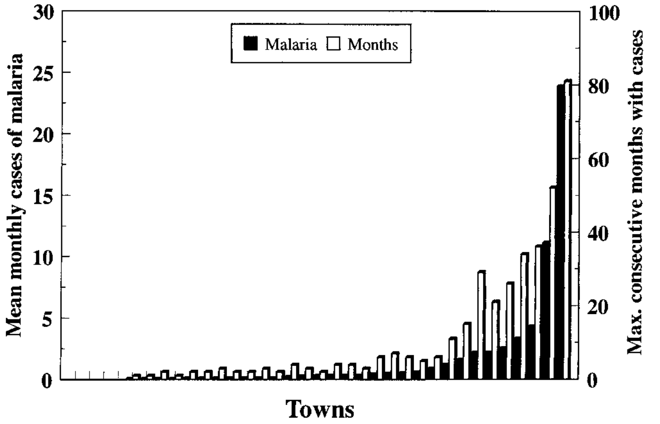

FIGURE 5. Relationship between mean monthly cases of malaria (1985–1994) and malaria persistence for each village in the study area. The values were ordered from villages with the lowest mean number of cases.

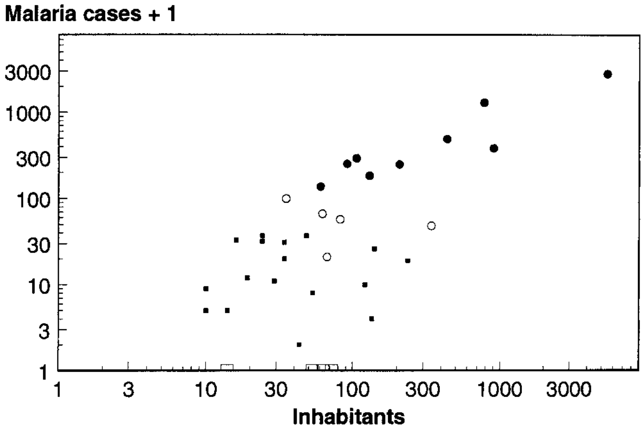

FIGURE 6. Relationship between ($\log_{10}$) malaria prevalence plus 1 (1985–1994) and the density of ($\log_{10}$) inhabitants per village. Symbols represent villages with different levels of malaria persistence: **solid circles,** more than 10 months; **empty circles,** between 5 and 9 months; **solid squares,** between 1 and 4 months; **empty squares** are villages with no cases reported.

in 1989 was higher than the number of females (235). Contingency analysis showed that the frequency of infected males and females did not significantly differ ($\chi^2 = 0.3$, $P > 0.05$) from that of the entire population in the region. However, the age structure of the population with malaria did significantly differ from that of the entire population ($\chi^2 = 57.5$, $P < 0.01$). The observed frequencies of very young (0–9 years old; 111 cases) and very old (> 70 years; 5 cases) individuals infected with malaria were lower than expected (160 and 14 cases, respectively), whereas the observed frequency of teenagers (10–14 years old, 107 cases) with malaria was much higher than expected (75 cases). Other age groups showing more cases (159 cases) than expected (129 cases) were the young adults (20–39 years old).

DISCUSSION

The pattern of malaria reinfection of the Santa Fe area showed that it proceeded rapidly, since in a few months there were cases in the entire zone (Figure 2). Reinfection seemed to have occurred by local (continuous) spread and jump (discontinuous) dispersal into neighboring towns. Exchange among human settings and the main town of Santa Fe was probably high because this was where most services exist. Santa Fe showed the highest persistence and prevalence of malaria throughout the epidemic and it is likely that most of the cases were initially exported from there. The high incidence of malaria during the dry seasons (Figure 3) was another factor contributing to both the spread and high prevalence of malaria in the area, since the parasites could survive locally. A dry season survey of habitats of immature *An. aquasalis* in the study area showed the presence of permanent pre-adult habitats close to human settlements.[7] Dry season transmission of malaria has been reported in areas with permanent bodies of water where anophelines develop.[10]

Two El Niño Southern Oscillation events (1987 and 1992) occurred during this study, which for Venezuela meant less rain than expected. There was also 1 La Niña event in 1988 in which more rain fell than expected (Figure 2).[11] It appeared that the occurrence of La Niña triggered malaria transmission to unusually high levels in 1988, consolidating the reinfection of the area, and rendering traditional control efforts (indoor spraying) useless (Figure 2). On the other hand, newer control measures in 1991 may have been facilitated by the occurrence of El Niño in 1992. These new control measures were based on entomologic observations that showed that *An. aquasalis* was resting outdoors in the vegetation around houses and also biting outdoors. Therefore, control efforts included applications of insecticides around houses and distribution of *Bacillus thuringiensis israelensis* to sites in which pre-adults live. It has been recently determined that malaria in Venezuela significantly increased 1 year after an El Niño event.[11] It is worth noting,

TABLE 1

Comparison of 35 human settings grouped in four classes with varying levels of malaria persistence and the corresponding means ± standard errors of malaria prevalence, number of inhabitants, and related environmental variables

Maximum consecutive months with cases	n	Malaria cases	Annual parasite index*	Inhabitants	Distance to mosquito habitats (m)	Larval habitats within 500 m	Terrain slope (%)	Terrain elevation (m)
>10	9	672 ± 289	160 ± 29	908 ± 577	410 ± 146	4.0 ± 1.0	6 ± 2	15 ± 5
5–9	5	57 ± 11	99 ± 43	118 ± 51	1,086 ± 172	0.4 ± 0.4	20 ± 3	88 ± 43
1–4	17	17 ± 3	58 ± 14	58 ± 15	1,125 ± 187	0.2 ± 0.1	21 ± 4	41 ± 19
0	4	0	0	50 ± 13	1,343 ± 204	0	26 ± 5	70 ± 38

* (cases/inhabitants) × 1,000.

however, that the highest peak of malaria prevalence observed between 1990 and 1991 was not directly related to any apparent meteorologic effects. Problems associated with the administration of the malaria programs (e.g., strikes and budget cuts) were likely involved.

The analysis of malaria persistence suggests that only 9–14 of 35 human settlements may have been real foci or hot spots, with continuous or consistent seasonal malaria transmission. On the other hand, 17 human settings may have been cold spots, or places where malaria was transient. Malaria infections in those places may have been acquired elsewhere in the study area. The stratification of the human settings based on the persistence of malaria is consistent with the identified risk factors. The closer proximity of villages with high persistence of malaria to pre-adult habitats of *An. aquasalis* or the abundance of these habitats within 500 m of the center of those villages (Table 1) indicates a higher risk of vector-human contacts. Thus, if historical data on malaria incidence are available for a given region, the analysis presented here may be used to perform a preliminary stratification of the malarious area. From a practical point of view, this implies a reduced need for vector control operations, although medical attention should be given to all human settings showing malaria cases regardless of where the infection was acquired.

This study exemplifies the importance of locating and mapping the aquatic habitats of immature anopheline vectors. It has been shown that the risk of malaria can be 6.2 times higher for individuals living less than 200 m from pre-adult habitats than for individuals living at a distance of 500 m or more from pre-adult habitats in an urban area in Mozambique.[12] Other studies have found that the highest parasite rates were in close proximity to major pre-adult habitats, and most *An. gambiae s.l.* adults were collected within 300 m of pre-adult habitats in an urban area in Burkina Faso.[13,14] Other investigators observed a reduced density of adult *An. gambiae s.l.* within 350 m of the bottom of a valley in Cameroon in which most pre-adult habitats were located.[15] This study also showed the importance of dense urban habitats and slope as factors limiting anopheline dispersion. In our study area, high malaria persistence and prevalence existed in settlements located at low altitudes and on gentle slopes (Table 1). However, multiple stepwise regression showed that human density and closeness to the aquatic habitats of the vectors independently explained malaria persistence and prevalence. We suggest performing spatial analysis to determine the overlap between human settlements and anopheline breeding places as a tool to identify areas at risk of malaria, or to decide where to allocate limited resources for control operations. The use of GISs can greatly facilitate this process.[7]

Our study showed that age but not gender was a risk factor for malaria infection. The lower than expected frequencies among very young and very old people and the higher than expected frequencies among teenagers and young adults suggest that *An. aquasalis* is more exophilic than endophilic, and that it is abundant near houses. This is consistent with previous studies that showed that *An. aquasalis* bites outdoors between 7:00 PM and 11:00 PM.[16]

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