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MAJOR ARTICLE

Temporal and Microspatial Heterogeneity in Transmission Dynamics of Coendemic *Plasmodium vivax* and *Plasmodium falciparum* in Two Rural Cohort Populations in the Peruvian Amazon

Angel Rosas-Aguirre,[1,2] Mitchel Guzman-Guzman,[2,3] Raul Chuquiyauri,[2,3] Marta Moreno,[4,5] Paulo Manrique,[3] Roberson Ramirez,[3] Gabriel Carrasco-Escobar,[2,3,4] Hugo Rodriguez,[7] Niko Speybroeck,[1] Jan E. Conn,[8,9] Dionicia Gamboa,[2,3,10] Joseph M. Vinetz,[2,3,10,11] and Alejandro Llanos-Cuentas[2,6]

[1]Research Institute of Health and Society, Université Catholique de Louvain, Brussels, Belgium, [2]Instituto de Medicina Tropical Alexander von Humboldt, Universidad Peruana Cayetano Heredia, Lima, Perú, [3]Laboratorio International Centers of Excellence in Malaria Research-Amazonia, Laboratorios de Investigación y Desarrollo, Facultad de Ciencias y Filosofía, Universidad Peruana Cayetano Heredia, Lima, Perú, [4]Division of Infectious Diseases, Department of Medicine, University of California San Diego, La Jolla, California, USA, [5]London School of Hygiene and Tropical Medicine, Department of Immunology and Infection, London, United Kingdom, [6]Facultad de Salud Pública y Administración, Universidad Peruana Cayetano Heredia, Lima, Perú, [7]Dirección Regional de Salud Loreto DIRESA Loreto, Loreto, Perú, [8]Wadsworth Center, New York State Department of Health, Albany, New York, USA, [9]Department of Biomedical Sciences, School of Public Health, University at Albany, State University of New York, Albany, New York, USA, [10]Departamento de Ciencias Celulares y Moleculares, Facultad de Ciencias y Filosofía, Universidad Peruana Cayetano Heredia, Lima, Perú, [11]Section of Infectious Diseases, Department of Internal Medicine, Yale School of Medicine, New Haven, Connecticut, USA

Background. Malaria is highly heterogeneous: its changing malaria microepidemiology needs to be addressed to support malaria elimination efforts at the regional level.

Methods. A 3-year, population-based cohort study in 2 settings in the Peruvian Amazon (Lupuna, Cahuide) followed participants by passive and active case detection from January 2013 to December 2015. Incidence and prevalence rates were estimated using microscopy and polymerase chain reaction (PCR).

Results. Lupuna registered 1828 infections (1708 *Plasmodium vivax*, 120 *Plasmodium falciparum*; incidence was 80.7 infections/100 person-years (95% confidence interval [CI] , 77.1–84.5). Cahuide detected 1046 infections (1024 *P vivax*, 20 *P falciparum*, 2 mixed); incidence was 40.2 infections/100 person-years (95% CI, 37.9–42.7). Recurrent *P vivax* infections predominated onwards from 2013. According to PCR data, submicroscopic predominated over microscopic infections, especially in periods of low transmission. The integration of parasitological, entomological, and environmental observations evidenced an intense and seasonal transmission resilient to standard control measures in Lupuna and a persistent residual transmission after severe outbreaks were intensively handled in Cahuide.

Conclusions. In 2 exemplars of complex local malaria transmission, standard control strategies failed to eliminate submicroscopic and hypnozoite reservoirs, enabling persistent transmission.

Keywords. Amazon; human biting rate; Malaria; Peru; transmission.

Malaria in the Americas declined from 673 723 reported cases in 2010 to 451 242 in 2015 [1]. In Peru, for instance, the Amazonian department of Loreto (~95% of Peruvian cases) [2] quintupled the reported malaria incidence by *Plasmodium vivax* (12 597 to 47 671 cases) and by *Plasmodium falciparum* (2296 to 9208 cases) from 2010 to 2015 [3]. Leading explanations for this resurgence highlighted the lack of a long-term national malaria control plan able (1) to continue and sustain achievements attained in previous years with support of international donors [4–6] and (2) to anticipate and react to dramatic environmental changes such as severe flooding in riverine villages, like the flooding that occurred in 2012.

Designing one-size-fits-all national malaria control strategies applicable to local situations is not straightforward, given highly heterogeneous and changing malaria microepidemiologies driven by the complex local interactions among *Plasmodium* parasites, human behavior, and the vector habitants influenced by the environment [3]. Data obtained over the past decade in Loreto villages suggest that malaria infections missed by traditional surveillance (ie, asymptomatic and submicroscopic infections, and carriers of *P vivax* hypnozoites) [7–9] together with human movement related to work [10] and highly anthropophilic *Nyssorhynchus darlingi* mosquitoes biting frequently outdoors [11] can result in the human reservoirs of *Plasmodium* parasites moving malaria transmission across

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Correspondence: Joseph M. Vinetz, MD, Section of Infectious Diseases, Department of Internal Medicine, Yale School of Medicine, 25 York Street, Winchester 403D, New Haven, Connecticut 06520 (joseph.vinetz@yale.edu).

[a]J. M. V. and A. L.-C. contributed equally to this study.

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space and time. Knowledge gaps remain to be addressed, particularly to quantify how and to what extent silent malaria reservoirs contribute to the resilience to interventions and the sustaining of malaria transmission [12].

Large prospective population cohorts with rigorous follow-up [13, 14] aim to deepen the understanding of local malaria complexity in the Peruvian Amazon, accurately estimate the burden of silent malaria reservoirs, and explore their impact on malaria transmission in different ecological settings. This study estimates population-based incidence rates of malaria between January 2013 and December 2015 in 2 different ecological settings in the Peruvian Amazon: Lupuna (LUP) with riverine environment and Cahuide (CAH) with road-associated deforestation. These data, combined with entomological and genetic parasite diversity data, enable better understanding of temporal and spatial dynamics of malaria transmission.

METHODS

Study Area

The study sites LUP and CAH (Figure 1) have been previously described. December–May and June–November are typically the tropical rainy and dry periods in the area [8]. Between 2011 and 2015, however, unusually heavy rains generated earlier and higher river level peaks (compared with historical averages), exceeding the threshold for imminent flooding for several weeks (especially in 2012 and 2015) [16].

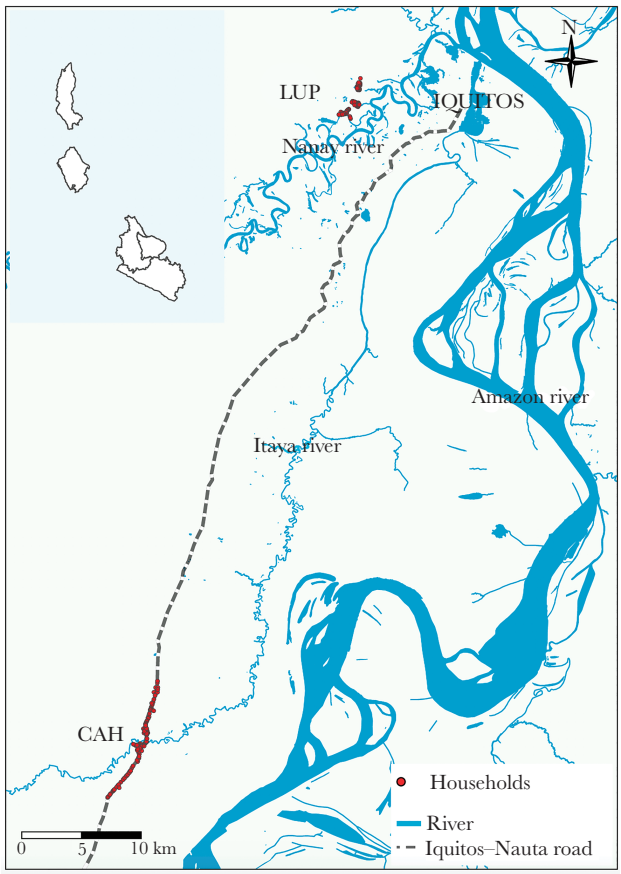

Figure 1. Study area. Adapted from Figure 1 in [15]. Abbreviations: LUP, Lupuna; CAH, Cahuide.

Passive case detection (PCD) data (2009–2012) indicate that malaria is seasonal (March–June) in LUP, predominantly due to *P vivax* [7]. Before the present study, routine malaria interventions in LUP were PCD and long-lasting insecticidal nets (LLINs) delivered in 2008 and 2010 [17]. During the study period, indoor residual spraying (IRS) with 5% deltamethrin was conducted in August 2012, April 2013, October 2013, and December 2014 [11, 18]. Malaria in CAH occurred at low levels from 2009 to 2011 [7]. After May 2012, 2 successive severe malaria outbreaks occurred, triggering 6 rounds of population screening-treatment interventions from May to December 2012 including IRS in May–June 2012 and distribution of 1 LLIN per household in July 2012 [7]. Indoor residual spraying was also conducted in March 2013 and November 2014 [18].

Field Procedures

A 3-year, population-based, longitudinal cohort study was conducted from January 1, 2013 to December 31, 2015, after census July–August 2012, baseline parasitological survey (microscopy, quantitative polymerase chain reaction [qPCR]) September–October 2012 [7], and enrollment November–December 2012. The Ethical Committee of Universidad Peruana Cayetano Heredia (SIDISI code no. 57395) and UCSD Human Subjects Protection Program (Project no. 100765) approved the study protocol.

Residents ≥3 years old providing written informed assent/consent were enrolled. Sample size estimation assumed the following: 20% residents had at least 1 microscopically confirmed malaria infection annually; 2% precision; 25% loss to follow-up; 80% power; and 5% significance level.

Cohort follow-up combined routine PCD at health posts (6 days/week), weekly active case detection of symptomatic individuals (wACDS), and monthly population screenings (mPS). Passive case detection relied on care-seeking behavior of individuals with malaria-compatible symptoms at health posts, where axillary temperature was taken and microscopy-directed treatment was done as appropriate. Household visits enabled weekly registration of axillary temperature and any malaria-compatible symptom. The mPS, conducted in the first visit of each calendar month, involved finger-prick blood sampling for microscopy [19] and dried blood spots (Supplementary Text 1). The wACDS, in the remaining visits (2th–5th) of each month, collected blood samples for microscopy from participants who had malaria-compatible symptoms within the past 7 days. Microscopically confirmed infections in the baseline survey, PCD, wACDS, and mPS were treated by a health worker according to national guidelines [20]. Patients with *P vivax* infections were given chloroquine for 3 days (10 mg/g on days 1 and 2, and 5 mg/kg

on day 3) plus primaquine for 7 days (0.5 mg/kg per day), whereas patients with *P falciparum* infections were given mefloquine (12.5 mg/kg per day for 2 days) plus artesunate (4 mg/kg per day for 3 days). Although only the first day of treatment was directly observed, trained community health workers in sites and weekly visits of our field research teams enhanced treatment adherence. During weekly visits, >95% of participants with a positive microscopy in the previous 2 weeks reported that they had taken the prescribed antimalarial drugs.

DATA ANALYSIS

Cohort and Incidence Data

Every individual with positive microscopy during the cohort follow-up was defined as having a malaria infection, as long as he/she had not had prior infection with the same species in the previous 14 days. This definition prevented double counting of infections. The additional presence or history of fever, headache, chills, or general discomfort in the previous 7 days defined symptomatic malaria.

Incidence rates were calculated as the number of incident infections (×100) divided by the total person-months at risk in a given period from 2013 to 2015. A person-month indicated at least 1 follow-up by any method during the month, and the person-years (PY) were the cumulative person-months divided by 12. It is noteworthy that incidence in years 2012 and 2016 were estimated using weekly reported malaria data from national surveillance [21].

Differences in incidence by age, gender, and residency time were assessed using trend charts, 95% Byar's confidence intervals (95% CIs), and multivariate mixed-effects negative binomial models in R v.3.6.1. Final models yielded an adjusted incident rate ratio (Adj. IRR), indicating a change in the risk from an unexposed to an exposed group. Poisson spatial scan statistics identified clusters of households with high incidence (Supplementary Text 1) [22].

Malaria Prevalence, Entomological, and River Level or Hydrological Data

Malaria prevalence by qPCR [23] and the proportion of submicroscopic infections (qPCR + microscopy−) were estimated quarterly from March 2013 to September 2015 (mPS data) and compared among subgroups with χ^2 tests and post hoc tests (Bonferroni correction). Entomological data from 12-hour mosquito collections by human landing catch before the study in 2012, from January to June in 2013–2015, and in August, October, and December 2013–2014 [11, 18] were used to estimate monthly human biting rates (HBRs). Daily water levels of the Amazon River were averaged monthly [24]. The relationships of monthly incidence rates to HBRs, river levels, and malaria prevalence were assessed with trend charts and correlation analysis.

RESULTS

Cohort Characteristics

A total of 1988 participants (LUP, 891; CAH, 1107) were analyzed in the cohort from 2447 censused people (Figure 2; Supplementary Table 1). Unlike CAH, LUP was primarily inhabited by long-term residents ($P < .001$). More participants reported malaria during their lifetime in LUP (73.5%) than in CAH (64.0%) ($P < .001$) at enrollment, but malaria in the past 12 months was 3 times more common in CAH than in LUP ($P < .001$) (Supplementary Table 2).

Incidence of Microscopically Confirmed Malaria Infections

Participants were followed-up between 4 and 36 months in LUP (median, 34; interquartile range [IQR], 28–36) and CAH (median, 32; IQR, 25–35). In LUP, 1708 *P vivax* infections were detected by microscopy in 627 participants (227 with single infections, 400 with 2–10 recurrent infections), and 120 *P falciparum* infections were detected in 113 participants (106 with single infections, 7 with 2 infections); determining average incidence rates of 80.7 infections/100 PY (95% CI, 77.1–84.5) (Table 1). Approximately 40% of these infections occurred in 2014 (97.1 infections/100 PY, IRR = 1.3, 95% CI = 1.2–1.5, compared with 2013).

In CAH, 1024 *P vivax* infections were detected in 569 participants (314 with single infections, 255 with 2 to 9 recurrent infections), 20 *P falciparum* infections in 19 participants (18 with single infections, 1 with 2 infections), and 2 mixed infections; yielding incidence rates of 40.2 infections/100 PY (95% CI, 37.9–42.7) (Table 1). The vast majority of infections (68.1%) occurred in 2013 (74.9 infections/100 PY, IRR = 4.6, 95% CI = 3.8–5.6, compared with 2014).

The proportion of participants with any *P vivax* infection detected by microscopy was similar between sites during the first year (LUP, 41.5%; CAH, 40.8%; $P > .05$), but it differed at the end of the study (LUP, 70.4%; CAH, 51.4%; $P < .001$). Asymptomatic *P vivax* infections barely exceeded symptomatic ones in LUP, with the lowest (~0.3) and highest (~0.55) proportions (among total *P vivax* infections) in the first months of 2013 and 2015, respectively (Supplementary Figure 1). In CAH, the proportion of asymptomatic *P vivax* infections negatively correlated with *P vivax* incidence (Spearman's rho [r_s] = −0.59, $P < .001$), presenting the highest levels (0.7–1.0) in months with the lowest incidence in 2014 and 2015. The proportion of asymptomatic *P falciparum* infections varied widely, mainly due to the low number of infections (Supplementary Figure 2).

Factors Associated With High Malaria Incidence

Quarterly and yearly incidence rates stratified by demographic groups consistently showed increased *P vivax* incidence in children aged 8–14 years over the study period in LUP (Figure 3A, Supplementary Figures 3A–4A, Supplementary Table 3). Interaction between age and time

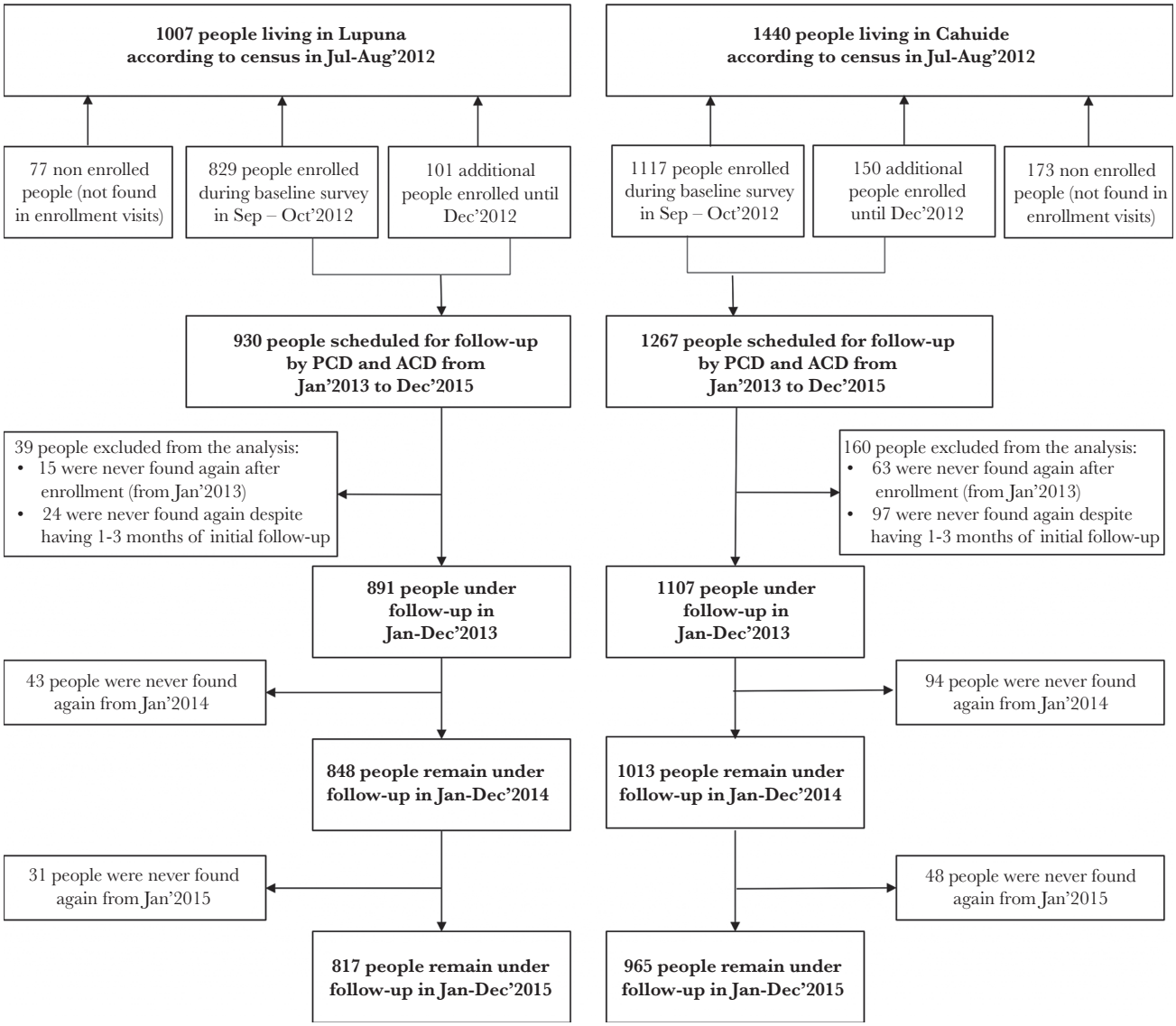

Figure 2. Flow chart of the cohort participants.

of residency in the multivariate model indicated that the association between high *P vivax* incidence and children was significant only among long-term residents (Adj. IRR for $age_{3\text{-}7y}$ = 2.9, 95% CI = 2.1–3.8; Adj. IRR for $age_{8\text{-}14y}$ = 3.2, 95% CI = 2.7–4.1; compared with age >44 years) (Table 2; Supplementary Figures 5A-6A). Increased *P vivax* incidence was associated with short-term residency among individuals aged 15–44 years (Adj. IRR = 2.3, 95% CI = 1.7–3.0) and those >44 years (Adj. IRR = 2.7, 95% CI = 1.7–4.7). It is interesting to note that interyear variations in *P vivax* incidence rates were wider in long-term residents aged 3–7 years ($IRR_{2014\text{-}2013}$ = 0.6, 95% CI = 0.4–0.8; $IRR_{2014\text{-}2015}$ = 0.7, 95% CI = 0.5–1.0) compared with those aged 8–14 years ($IRR_{2014\text{-}2013}$ = 0.7, 95% CI = 0.4–0.8; $IRR_{2014\text{-}2015}$ = 0.8, 95% CI = 0.6–1.0) (Supplementary Tables 4 and 5).

In CAH, stratification of incidence rates by age showed increased *P vivax* incidence rates at >7 years and residence times >5 years, especially during 2013 ($P < .05$) (Supplementary Figures 7A–9A, Supplementary Table 6). Over the cohort period, the multivariate model confirmed this increased *P vivax* incidence in individuals aged 8–44 years (Adj. IRR = 1.4, 95% CI 1.1–1.7, compared with age 3–7 years) and in long-term residents (Adj. IRR = 1.3, 95% CI = 1.1–1.6). Regarding *P falciparum*, males (Adj. IRR = 2.0, 95% CI = 1.4–2.9) and individuals >7 years (Adj. IRR ~2.5) had highest incidence rates in LUP (Supplementary Table 7).

Recurrent Infections Among Total *Plasmodium vivax* Infections

The proportion of recurrent infections detected by microscopy (after a prior infection) steadily increased in both sites during the first year (from 0 to 0.63 in LUP, and

Table 1. Incidence Rates of Microscopically Confirmed Malaria Infections by Study Site

	Lupuna (2264.2 PY)			Cahuide (2598.9 PY)		
Type of malaria infection	Infections	Rate (/100 PY)	95% CI	Infections	Rate (/100 PY)	95% CI
Plasmodium vivax[a]	1708	75.4	71.9–79.1	1024	39.4	37.0–41.9
Plasmodium falciparum[a]	120	5.3	4.4–6.3	20	0.8	0.5–1.2
Mixed infection	0	0.0	0	2	0.1	0.0–0.2
Overall[a]	1828	80.7	77.1–84.5	1046	40.2	37.9–42.7
Asymptomatic *P vivax*[a]	667	29.5	27.3–31.8	601	23.1	21.3–25.0
Symptomatic *P vivax*[a]	1041	46.0	43.2–48.8	423	16.3	14.8–17.9
Asymptomatic *P falciparum*[a]	41	1.8	1.3–2.4	11	0.4	0.2–0.7
Symptomatic *P falciparum*[a]	79	3.5	2.8–4.3	9	0.3	0.2–0.6

Abbreviations: CI, confidence interval; PY, person-years.

[a]$P < .05$.

to 0.75 in CAH). Afterwards, smaller increases were observed in LUP (maximum = 0.92) or fluctuated between 0.56 and 0.83 in CAH (Supplementary Figure 1). Among long-term residents in LUP, individuals aged 8–14 years had the highest proportion of recurrent infections ($P < .001$). No significant differences were found among

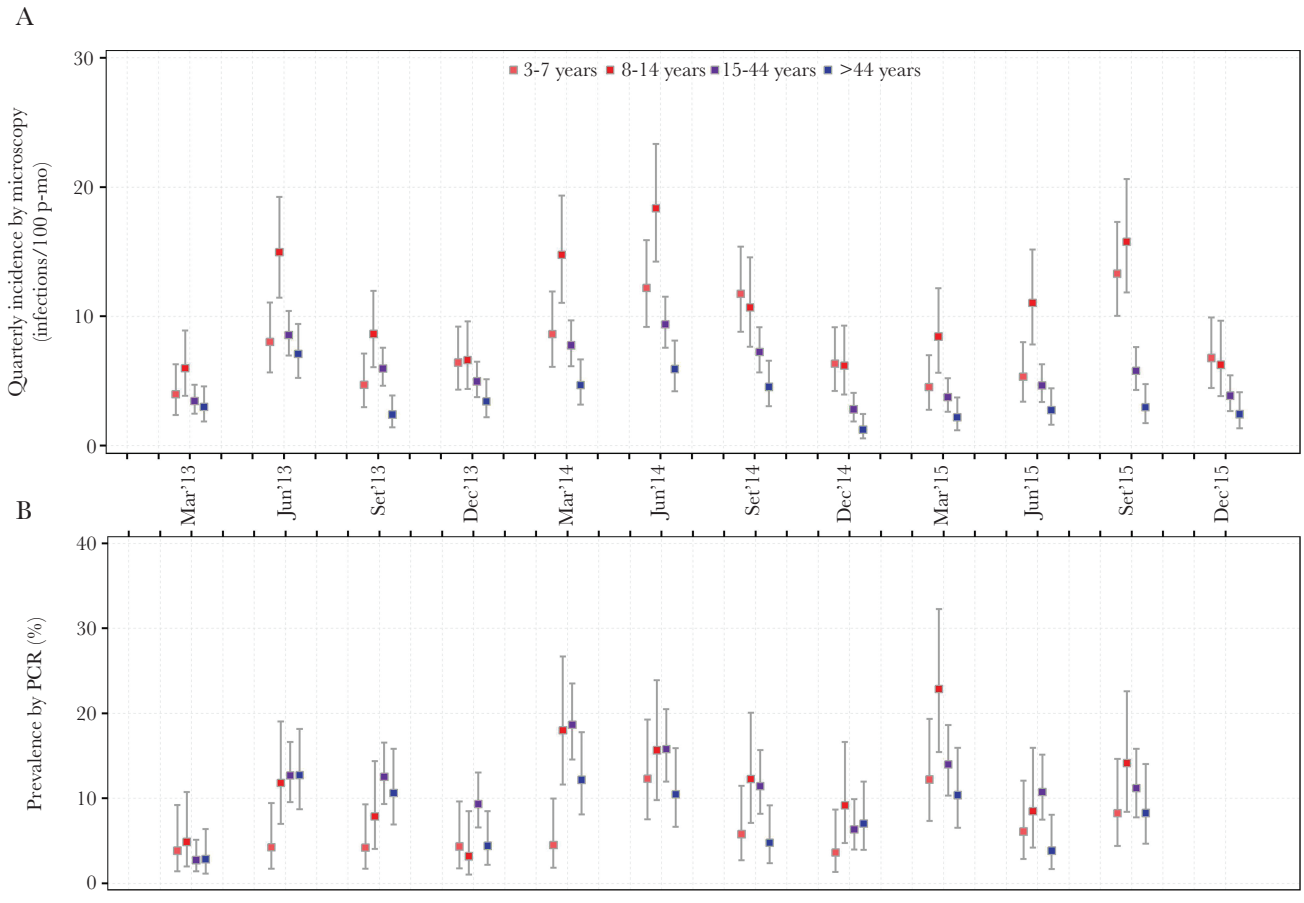

Figure 3. Quarterly incidence of *P. vivax* malaria infections detected by microscopy and prevalence by PCR stratified by age groups in Lupuna from January 2013 to December 2015. Dot plots show incidence/prevalence mean rates, and 95% confidence limits (95% CI) for each age group. The Byar's approximation and the Wilson score method were respectively used to estimate CI for incidence and prevalence rates. Incidence rates were calculated for the periods January-March, April-June, July-September and October-December of each year. Significant differences in incidence rates between age groups were found in almost all trimesters (except January-March 2013 and September-December 2013) ($P < .001$), showing mostly higher incidence rates in individuals aged 8-14 years in comparison with those aged 15-44 years and >45 years (pair-wise post-hoc test, $P < .05$). Significant differences in prevalence between age groups were found in June 2013, March 2014, and March 2015 ($P < .05$). Pair-wise post-hoc tests showed that children aged 3-7 years had lower prevalence than individuals aged 15-44 years ($P = .04$) and >44 years ($P = .04$) in June 2013. Children aged 3-7 years had lower prevalence than those aged 8-14 years ($P = .004$) and 15-44 years in March 2014 ($P = .001$). While in March 2015, individuals aged 8-14 years had higher prevalence than those aged >44 years ($P = .008$).

Table 2. Uni- and Multivariate Risk Factor Analysis for Incidence of Microscopically Confirmed *Plasmodium vivax* Malaria Infections in LUP and CAH

	Infections	PY	Rate (Infections/100 PY)		IRR		Adjusted IRR	
Demographic	n	PY	Rate	95% CI	IRR	95% CI	IRR	95% CI
Lupuna	-	-	-	-	-	-	-	-
Year	-	-	-	-	-	-	-	-
2013	581	830.5	70.0	64.4–75.8	Ref.		Ref.	
2014	679	751.6	90.3	83.7–97.3	1.3[a]	1.2–1.4	1.3[a]	1.2–1.4
2015	448	682.1	65.7	59.8–72.0	0.9	0.8–1.1	0.9	0.8–1.1
Gender	-	-	-	-	-	-	-	-
Female	871	1141.1	76.3	71.4–81.5	Ref.		Ref.	
Male	837	1123.1	74.5	69.6–79.7	1.0	0.8–1.1	1.0	0.8–1.1
Age	-	-	-	-	-			
3–7 y	372	404.0	92.1	83.1–101.8	2.1[a]	1.7–2.6		
8–14 y	436	341.5	127.7	116.1–140.1	3.0[a]	2.5–3.8		
15–44 y	650	941.8	69.0	63.9–74.5	1.6[a]	1.3–1.9		
>44 y	250	576.9	43.3	38.2–49.0	Ref.			
Residency (Time)	-	-	-	-	-	-		
0–5 y	468	458.9	102.2	93.1–111.5	1.5[a]	1.3–1.8		
>5 y	1240	1805.3	68.7	64.9–72.6	Ref.			
Interaction Age[a] Residency	-	-	-	-	-			
For Those With Residency 0–5 y	-	-	-	-	-			
Age 3–7 y	-	-	-	-	-		0.8	0.5–1.2
Age 8–14 y	-	-	-	-	-		1.3	0.7–2.6
Age 15–44 y	-	-	-	-	-		1.2	0.7–2.0
Age >44 y	-	-	-	-	-		Ref.	
For Those With Residency >5 y	-	-	-	-	-			
Age 3–7 y	-	-	-	-	-		2.9[a]	2.1–3.8
Age 8–14 y	-	-	-	-	-		3.2[a]	2.7–4.1
Age 15–44 y	-	-	-	-	-		1.5[a]	1.2–1.8
Age >44 y	-	-	-	-	-		Ref.	
For Those Aged 3–7 y	-	-	-	-	-			
Residency 0–5 y	-	-	-	-	-		0.7[b]	0.6–1.0
Residency >5 y	-	-	-	-	-		Ref.	
For Those Aged 8–14 y	-	-	-	-	-			
Residency 0–5 y	-	-	-	-	-		1.1	0.7–1.7
Residency >5 y	-	-	-	-	-		Ref.	
For Those Aged 15–44 y	-	-	-	-	-			
Residency 0–5 y	-	-	-	-	-		2.3[a]	1.7–3.0
Residency >5 y	-	-	-	-	-		Ref.	
For Those Aged >44 y	-	-	-	-	-			
Residency 0–5 y	-	-	-	-	-		2.7[a]	1.7–4.7
Residency >5 y	-	-	-	-	-		Ref.	
Cahuide								
Year								
2013	695	948.9	73.2	67.9–78.8	Ref.		Ref.	
2014	136	860.0	15.8	13.3–18.6	0.2[a]	0.2–0.3	0.2[a]	0.2–0.3
2015	193	790.0	24.4	21.2–28.1	0.3[a]	0.3–0.4	0.3[a]	0.3–0.4
Gender								
Female	472	1276.1	37.0	33.8–40.4	Ref.		Ref.	
Male	552	1322.8	41.7	38.4–45.3	1.1[b]	1.0–1.3	1.1	1.0–1.3
Age								
3–7 y	148	551.6	26.8	22.8–31.4	Ref.		Ref.	
8–14 y	249	575.8	43.2	38.1–48.9	1.5[a]	1.2–2.0	1.4[a]	1.1–1.7
15–44 y	418	966.8	43.2	39.2–47.5	1.6[a]	1.3–2.0	1.4[a]	1.1–1.7
>44 y	209	504.8	41.4	36.1–47.3	1.5[a]	1.2–2.0	1.3[b]	1.0–1.7
Residency (Time)								
0–5 y	437	1321.7	33.1	30.1–36.3	Ref.			
>5y	587	1277.3	46.0	42.4–49.8	1.4[a]	1.2–1.7	1.3[a]	1.1–1.6

Abbreviations: CAH, Cahuide; CI, confidence interval; IRR, incident rate ratio; LUP, Lupuna; PY, person-years; Ref., reference; y, years.

[a] $P < .05$.

[b] P between .1 and .05.

short-term residents in LUP and total residents in CAH (Supplementary Figure 10).

Spatial Clusters of High Malaria Incidence

Clusters of high *P vivax* incidence in 2013 and 2014 were consistently found in (1) southwest LUP (2013 risk ratio [RR] = 1.7, $P < .001$; 2014 RR=1.5, $P = .02$) and (2) the junction of the Iquitos-Nauta road and Itaya River in CAH (2013's RR = 1.7, $P < .001$; 2014's RR = 2.4, $P < .001$) (Supplementary Figures 11 and 12). In 2015, the *P vivax* cluster in CAH (RR = 4.3, $P < .001$) remained close to the junction, but it was small. There were no significant spatial clusters for *P vivax* in LUP in 2015, nor for *P falciparum* in LUP and CAH in 2013, 2014, and 2015.

Quarterly Malaria Prevalence by Quantitative Polymerase Chain Reaction

Lowest and highest malaria prevalence in LUP were observed in October 2012–March 2013 (≤5%) and March 2014 (20.5%) and December 2014 (1.7%) and June 2013 (14.4%) in CAH (Figure 4; Supplementary Tables 8 and 9). Positive correlation between prevalence by PCR and incidence rates was only found in LUP ($r_{s\ vivax} = 0.64$, $P = .03$; $r_{s\ falciparum} = 0.67$, $P = .02$). Submicroscopic *P vivax* infections predominated over microscopic ones in both sites, with highest proportions in December 2014–March 2015 in LUP (~0.8) and in September 2014–March 2015 in CAH (>0.9). High proportions in CAH were associated with low clinical incidence rates ($r_s = -0.84$, $P = .001$).

Stratified analysis of quarterly prevalence by demographic groups in LUP identified lowest *P vivax* prevalence at ages 3–7 years in June 2013 and March 2014 ($P < .05$) and highest prevalence at ages 8–14 years in March 2015 ($P < .05$) (Figure 3B; Supplementary Figures 3B–9B). In CAH, males had higher prevalence than females in September 2013 and March 2014 ($P = .02$) and in long-term residents in June 2014 ($P = .04$).

Relationship Between Entomological/Environmental Variables and the Incidence of Microscopic Malaria

Amazon River levels from 2012 to 2015 correlated with HBRs in both sites ($r_s = 0.64$, $P < .001$). Significant relationships between overall incidence rates and environmental and entomological variables during the cohort period (when found) occurred at

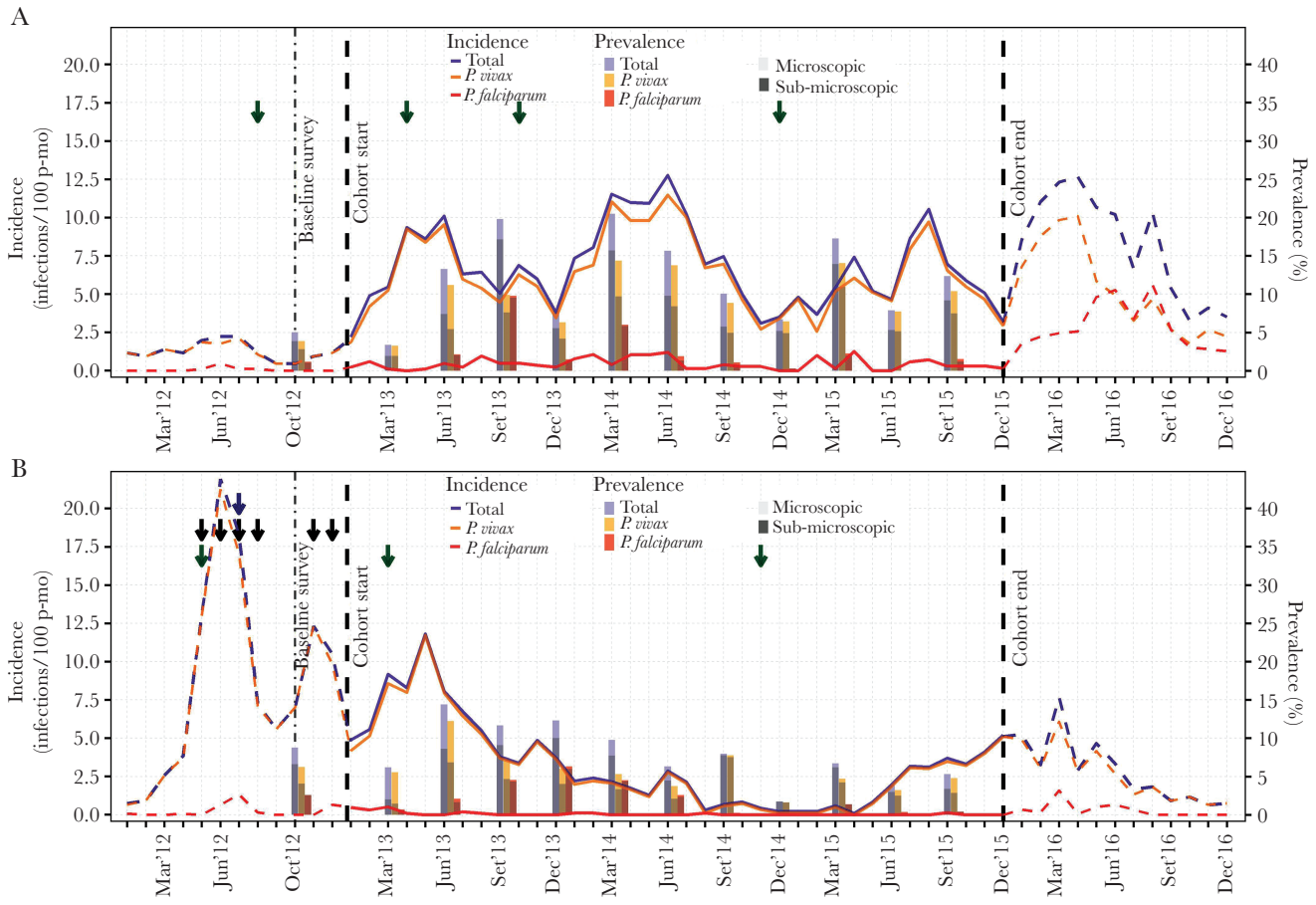

Figure 4. Evolution of monthly incidence of malaria infections by microscopy, and quarterly prevalence by qPCR in Lupuna (*A*) and Cahuide (*B*) from January 2012 to December 2016. Dashed lines represent incidence in 2012 and 2016 estimated with surveillance data, while solid lines in 2013-2015 with cohort data. Green, blue, and black arrows indicate months when respectively indoor residual spraying (IRS) with 5% deltamethrin, distribution of long-lasting insecticidal nets (LLINs), and population screenings using microscopy and treatment of confirmed infections were conducted. The left Y-axis indicates incidence rates (number of infections/100 person-months), while the right Y-axis prevalence in percentage (%). see online version for color figure.

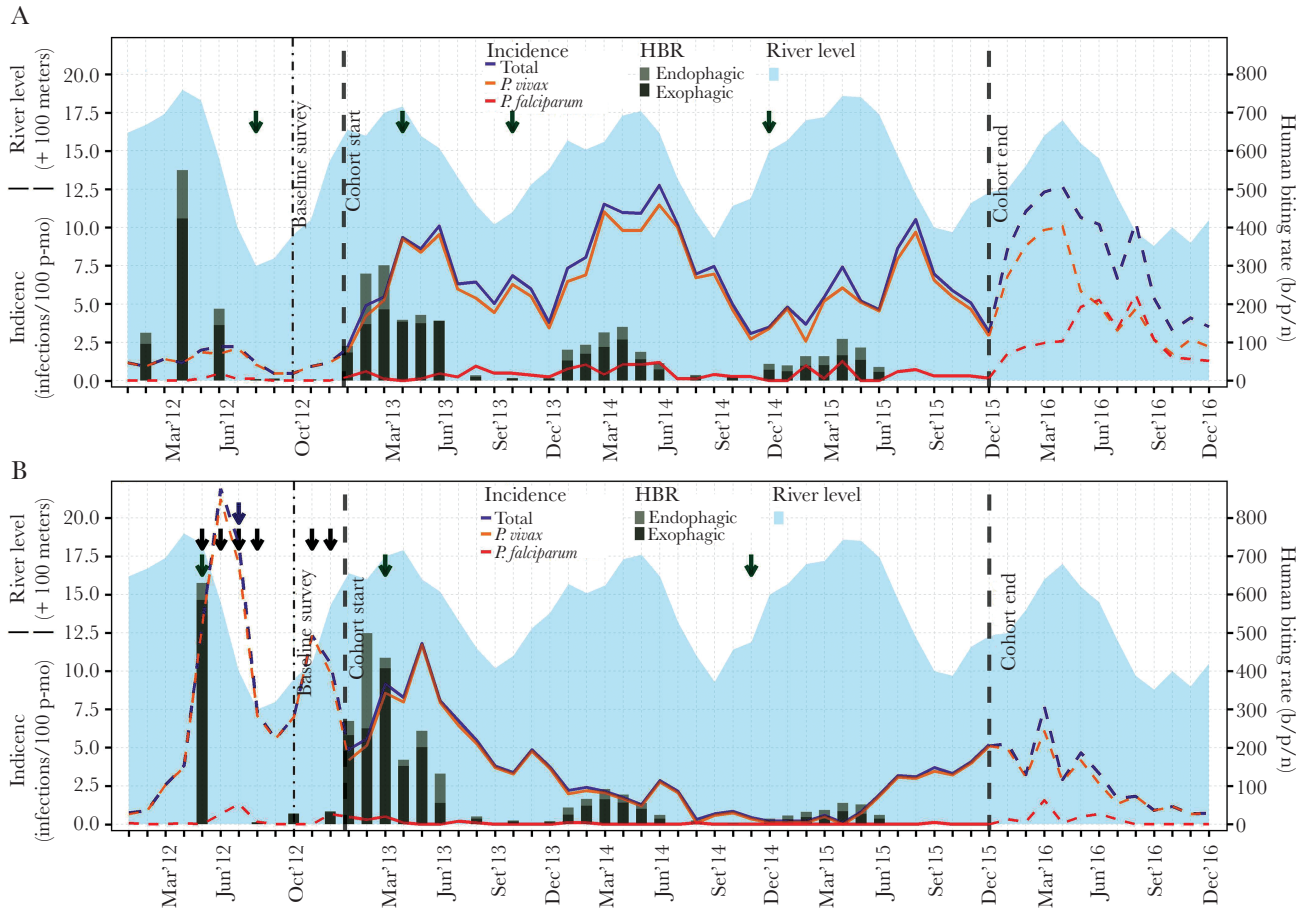

Figure 5. Evolution of monthly incidence of malaria infections by microscopy, Amazon river levels and human biting rates (HBR) in Lupuna (*A*) and Cahuide (*B*) from January 2012 to December 2016. Dashed lines represent incidence in 2012 and 2016 estimated with surveillance data, while solid lines in 2013-2015 with cohort data. HBRs were estimated using published entomological data from 12-h mosquito collections by human landing catch (HLC) before the study in 2012, from January to June in 2013-2015, and in August, October and December 2013-2014. Monthly water levels of the Amazon River were calculated with daily data from the Peruvian Amazon Service of Hydrography and Navigation. Green, blue, and black arrows indicate months when respectively indoor residual spraying (IRS) with 5% deltamethrin, distribution of long-lasting insecticidal nets (LLINs), and population screenings using microscopy and treatment of confirmed infections were conducted. The left Y-axis indicates incidence rates (number of infections/100 person-months) and river levels (meters above 100), while the right Y-axis human biting rates (bites/person/night). see online version for color figure.

2-month lag times, with river levels in LUP ($r_s = 0.54$, $P = .001$) and with HBRs in CAH ($r_s = 0.68$, $P < .001$) (Figure 5).

DISCUSSION

This densely sampled, prospective, longitudinal, population-based cohort study is unique among the few such studies aimed at understanding heterogeneous and changing malaria transmission dynamics in *P vivax* and *P falciparum* coendemic areas [25–27]. The assessment of parasitological rates across time and space, together with published data of genetic parasite diversity [28], entomological observations, river height measurements, and contextual surveillance/control information, provide valuable insights into contrasting epidemiological scenarios in the Peruvian Amazon. Residual transmission persisted after effectively controlling severe outbreaks in a malaria-epidemic prone site (CAH), and under conditions of intense, seasonal malaria transmission resistant to standard control measures (LUP). These sites epitomize the microheterogeneity of malaria transmission in environmentally diverse Amazonian settings, and they are generalizable to other coendemic *P vivax* and *P falciparum* settings.

The location of malaria clusters areas of flood risk [7] and the association of HBRs and malaria incidence support the hypothesis that environmental changes drove increased abundance and wide dispersal of *N darlingi* leading to the 2012–2013 epidemic transmission in CAH. Malaria incidence in CAH in 2012 was similar to the worst epidemic recorded in Loreto (1996–1998) [29]. In 2013, incidence decreased, but malaria transmission remained high despite outbreak responses and intense microscopy-directed treatment.

Standard malaria surveillance is inherently limited in eliminating residual infections, as demonstrated by persistent malaria prevalence in 2014 and early 2015 [30]. Human mobility, not vector movement, was most likely responsible for

dispersing *P vivax* and *P falciparum* infections from outside CAH. Published changes in the genetic diversity of *P vivax* infections during and after malaria outbreaks in CAH are consistent with this proposed mechanism of maintenance *P vivax* transmission [28]. Indeed, CAH's population is not stable, as indicated by the proportion of recent immigrants (>25% individuals with <2 years of residency) and the proportion of participants (one fourth) lost to follow-up due to migration. The accessibility to the 120-km road connecting the major city Iquitos to rural Nauta and to a riverine port make CAH a vulnerable area to imported malaria [31, 32], because of intense human movement related to work and social interactions, within the same community and in proximity to other endemic communities [7, 10]. With favorable conditions for vector development and high human mobility patterns, both endemic and imported strains would have dispersed rapidly across CAH villages and led to uncontrollable malaria outbreaks in 2012–2013 and the resurgence of malaria in all villages in late 2015 (year with floods, but less intense than in 2012). Hence human behavior converts anophelism without malaria to endemic malaria transmission.

Lupuna also withstood floods, more severe in 2012 than in 2013. The spatial cluster of highest incidence close to Nanay River and the rise of seasonal HBRs [11, 18] underlined the contribution of this environmental change to the increase in incidence rates by both *P vivax* and *P falciparum* in 2013. Integrating epidemiological and genotyping data was necessary to understand why malaria driven by floods in LUP did not lead to early increases in the malaria burden since 2012. The low prevalence of parasitemic people (mainly submicroscopic) in October 2012 and the consistent seasonal patterns of low malaria incidence (2009–2012) in the relatively stable LUP population (long residency time and high levels of study retention) suggest that asymptomatic and low-parasite density infections were able to maintain low seasonal malaria transmission before 2013 [7]. These findings also suggest that population exposure to local parasite strains was sufficient for the development of clinical immunity to malaria disease and high-density parasitemia in the precohort period [33, 34]. However, this transmission scenario would have been altered with the introduction and spread of new *P vivax* strains after March 2013 given microsatellite characterization of parasite populations [28]. In 2013, unlike 2012, increased human-vector contacts not only intensified the transmission of local strains, but they also facilitated the spread of new imported ones. As a result, the number of *P vivax* incident infections (mostly symptomatic) reached unusual peaks in high transmission season of 2013, and participants with less-developed immunity such as children and with short-time residency were the most affected groups [27, 35].

The increased contribution of recurrent *P vivax* infections to malaria incidence, the same location of the most likely cluster in 2013–2014, together with the decline in human-vector contacts after less severe flooding and IRS in 2014, also suggest that *P vivax* relapses may have played an important role in keeping high transmission levels in LUP. The rigorous study follow-up facilitated the diagnosis and treatment of microscopic infections (mainly recurrent infections), but it failed to identify the reservoir of low-density blood-stage infections (composed of new and relapsing infections) and the reservoir of hypnozoite carriers (without blood-stage parasites), which together enabled disease across transmission seasons. The decrease in malaria incidence rates in 2015 (greater in young children than in old ones among long-term residents) would likely be due to the gradual acquisition of strain-specific *P vivax* immunity providing some protection to LUP's residents against malaria disease and high-density parasitemia [27, 36, 37] rather than a true reduction in transmission levels. Indeed, the submicroscopic reservoir (mainly by *P vivax*) with persistent high levels (≥0.7) since December 2014 highlighted the potential epidemic risk when conditions for transmission were more favorable. Unfortunately, the decrease of detection efforts after the cohort study ended was associated with an early seasonal peak of *P vivax* incidence and a severe and prolonged outbreak of *P falciparum* in 2016.

The strengths of this study include its large sample size, 3-year prospective design, rigorous parasitological follow-up, and the integration among epidemiological, vector, and environmental data for better understanding malaria transmission dynamics. The main limitations were related to the fixed cohort design [37] and to loss of participants to follow-up (at least in part due to migration) together with the entry of new residents into the study villages after the study onset (who were not additionally enrolled). These findings may have affected incidence and prevalence estimations in unknown ways; for example, by introducing new parasite strains or by the introgression of malaria-naive individuals. However, the higher number of immigrants (12.1% in LUP and 23.8% in CAH) than emigrants (<5% according to population census at end of the cohort) may in part support the validity of our estimations at the population level.

CONCLUSIONS

The data provided here are 2 exemplars of complex local malaria transmission in the Peruvian Amazon. The changing interactions across time between the coendemic and biological distinct *P falciparum* and *P vivax* parasites and human-vector contact rates determined by human mobility and environmental-driven vector behaviors produced contrasting scenarios of malaria transmission with microheterogeneity observed within the same endemic villages. Standard control strategies may have contributed (1) to a reduction of malaria after outbreaks and (2) to possibly reducing increases in morbidity during high transmission season. However, these standard interventions did

not eliminate submicroscopic parasitemics nor hypnozoite reservoirs [30, 39], enabling continued low transmission in areas with variable malaria receptivity such as CAH and areas with high seasonal transmission with relatively permanent malaria receptivity such as LUP. For instance, we observed that prevalence levels of submicroscopic infections as low as 5% can represent a risk for malaria resurgence when conditions become favorable for transmission. Easy-to-use and highly sensitive molecular tests like those based on loop-mediated isothermal deoxyribonucleic acid amplification (still under evaluation) [40] and treatment of confirmed blood-stage infections with shorter courses of effective drugs like tafenoquine (TFQ) [41, 42] will definitively improve test-and-treat interventions, but carriers of liver-stage *P vivax* parasites will remain unidentified [12]. Innovative strategies are needed to support malaria programs aimed to move from low to zero malaria transmission. Evidence from the dynamics of malaria transmission in CAH and LUP suggests the potential of seasonal focal drug administration with artemisinin-based combinations plus TFQ to high-risk individuals—such as individuals with malaria infections within the past 1 to 2 years, multiple recurrences, and/or with high work-related mobility—for targeting and eliminating the parasite reservoir. Modeling [43] and field studies would be required to assess the effectiveness and cost-effectiveness of this strategy before implementation.

Supplementary Data

Supplementary materials are available at *The Journal of Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

Notes

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Authors' contributions. A. L.-C., J. M. V., and D. G. conceived and designed the study. M. G.-G., H. R., M. M., and R. C. supervised the fieldwork. D. G., P. M., and R. R. supervised the laboratory assays. A. R.-A., M. G.-G., and G. C.-E. contributed to the data management and the consolidation of the fieldwork and laboratory data. A. R.-A. and N. S. conducted the analysis. A. R.-A. prepared the figures and tables and wrote the first draft of the paper. J. M. V., A. L.-C., H. R., N. S., J. E. C., P. M., and D. G. contributed to the result interpretation and writing of the paper. All authors read and approved the final manuscript.

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