Prolonged Breastfeeding and the Risk of *Plasmodium vivax* Infection and Clinical Malaria in Early Childhood

A Birth Cohort Study

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Background: Relatively few Amazonian infants have clinical malaria diagnosed, treated and notified before their first birthday, either because they are little exposed to an infection or remain asymptomatic once infected. Here we measure the proportion of children who have experienced *Plasmodium vivax* infection and malaria by 2 years of age in the main transmission hotspot of Amazonian Brazil.

Methods: We measured IgG antibodies to 3 blood-stage *P. vivax* antigens at the 1- and 2-year follow-up assessment of 435 participants in a population-based birth cohort. Children's malaria case notifications were retrieved from the electronic database of the Ministry of Health. We used multiple Poisson regression models to identify predictors of serologically proven *P. vivax* infection and clinical vivax malaria during the first 2 years of life.

Results: Overall, 23 [5.3%; 95% confidence interval (CI): 3.5–7.8%) children had antibodies to ≥2 antigens detected during at least one follow-up assessment, consistent with past *P. vivax* infection(s). Fifteen (3.4%; 95% CI: 2.1–5.6%) children had clinical vivax episodes notified during the first 2 years of life; 7 of them were seronegative. We estimate that half of the infections remained unnotified. Children born to women who experienced *P. vivax* infection during pregnancy were more likely to be infected and develop clinical vivax malaria, while those breast-fed for ≥12 months had their risk of being *P. vivax*-seropositive (which we take as evidence of blood-stage *P. vivax* infection during the first 2 years of life) decreased by 79.8% (95% CI: 69.3–86.7%).

Conclusion: *P. vivax* infections in early childhood are underreported in the Amazon, are associated with anemia at 2 years of age, and appear to be partially prevented by prolonged breastfeeding.

Key Words: malaria; *Plasmodium vivax*; breast-feeding; infants; Amazon

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Plasmodium falciparum causes significant morbidity and mortality in children across sub-Saharan Africa, but neonates and young infants rarely develop clinical disease.[1] Since the mid-1950s, exclusive breastfeeding is known to reduce malaria risk in early childhood.[2] Additional protective factors are specific maternal antibodies transferred to the fetus, the high content of fetal hemoglobin in host cells and the lower infants' exposure to mosquito vector bites, compared to adolescents and adults.[3] Children >6 months of age are often infected and remain at increased risk of severe disease and death over the next years,[3] until immunity gradually develops and protects most adolescents and adults from clinical disease.[4] *Plasmodium vivax*, the second most prevalent human malaria parasite worldwide, remains rare in Central and West Africa, but is increasingly common elsewhere in the tropics and can cause substantial childhood morbidity.[5] Anemia is particularly common in young children with vivax malaria.[6]

The epidemiology of childhood malaria is understudied in Latin America, where *P. vivax* accounts for >80% of the infections.[7] Findings from a population-based birth cohort suggest that Amazonian infants rarely develop clinical malaria before the age of 12 months, but older children are as vulnerable to malaria as their mothers.[8] It remains unclear whether younger infants are less exposed to infection or less susceptible to disease, once infected with malaria parasites.[3] To address this knowledge gap, here we use serology to estimate the proportion of cohort participants who experienced blood-stage *P. vivax* infection during their first 2 years of life. Multiple Poisson regression models were built to identify independent correlates of protection from malarial infection and disease.

METHODS

Study Design

The Maternal and Child Health and Nutrition in Acre, Brazil (MINA-Brazil) study is a population-based birth cohort set-up to measure the impact of a range of early exposures on child health and development in the Amazon.[9] Mother-baby pairs (n = 1551) were enrolled in the city of Cruzeiro do Sul (CZS; population, 64,000), Acre State, during pregnancy or at birth. Between July 2015 and June 2016, delivering mothers were interviewed at the Women and Children's Hospital of Juruá Valley—the only maternity hospital

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of CZS—to obtain sociodemographic, lifestyle, and morbidity information. Birth weight was measured to the nearest 0.5 g, using a Toledo Junior portable scale (Toledo, São Bernardo do Campo, Brazil) with 15 kg capacity. The infant mortality rate in CZS, estimated at 13.8 deaths per 1000 live births in 2017, is higher than the country´s average of 12.8 (https://www.ibge.gov.br/cidades-e-estados/ac/cruzeiro-do-sul.html). CZS experiences year-round malaria transmission, with an annual malaria index (API; defined as the number of laboratory-confirmed cases per 1000 people per year) of 231.9 in 2016, one of the highest in Brazil.[10] *P. vivax* accounts for approximately 85% of the infections in the study site.

Study Population

Figure 1 shows the study flow chart. Mothers of liveborn children, except for those from hard-to-reach rural communities (n = 305), were invited to bring their children to follow-up assessments at 6–8 months, 1 year, 2 years and 5 years of age.[9] Here we focus on 435 children who had anti-*P. vivax* antibodies were measured and sociodemographic, morbidity and nutritional information (including infant feeding practices) were updated during assessments at the age of 1 year (mean, 12.7 months; range, 11.1–16.2 months) and 2 years (mean, 23.7 months; range, 16.0–32.1 months). Hemoglobin concentrations were measured during the follow-up visit at the age of 2 years using an ABX Micro 60 cell counter (Horiba, Montpellier, France). Study participants included in the present analysis (65.4% of the 665 children attending both follow-up assessments) had similar perinatal health profiles compared with children lost to follow-up (n = 581) or lacking blood samples (n = 230). However, the proportions of children from poorest families (lowest quartile; 20.0% vs. 12.5%, $P < 0.001$) and those born to younger (<19 years; 20.5% vs. 14.9%, $P = 0.025$) and less educated (≤9 years of schooling; 39.6% vs. 28.5%, $P = 0.001$) mothers and those with inadequate gestational weight gain (34.0% vs. 26.7%, $P = 0.004$) were significantly higher (by χ^2 tests) among nonparticipants, compared to participants. These differences are mostly due to the exclusion from follow-up assessments of children from poorer families living in remote rural sites.

Antibody Measurements

Exposure to *P. vivax* blood stages is known to induce specific IgG antibody responses that can be long-lived even following a low-density asymptomatic infection.[11–14] We used enzyme immunoassay (ELISA) to detect plasma IgG antibodies to 3 recombinant blood-stage antigens of *P. vivax*: (1) the apical membrane protein (PvAMA1) ectodomain, Brazilian strain, expressed in *Pichia pastoris*[15]; (2) the C-terminal, 19-kDa region of merozoite surface protein (MSP) 1 ($PvMSP1_{19}$), Belém strain, expressed in *Escherichia coli*[16] and (3) the Duffy-binding protein (PvDBP) erythrocyte-binding domain, Salvador-I strain, expressed in *E. coli*.[17] Assays were carried as described with the following solid-phase antigen concentrations: PvAMA1, 1 μg/mL; $PvMSP1_{19}$, 0.025 μg/mL and PvDBP, 0.025 μg/mL (50 μL/well).[8] Reactivity indices (RIs) were calculated as the ratio between the absorbance values of each test sample and a cutoff value for each antigen, corresponding to the

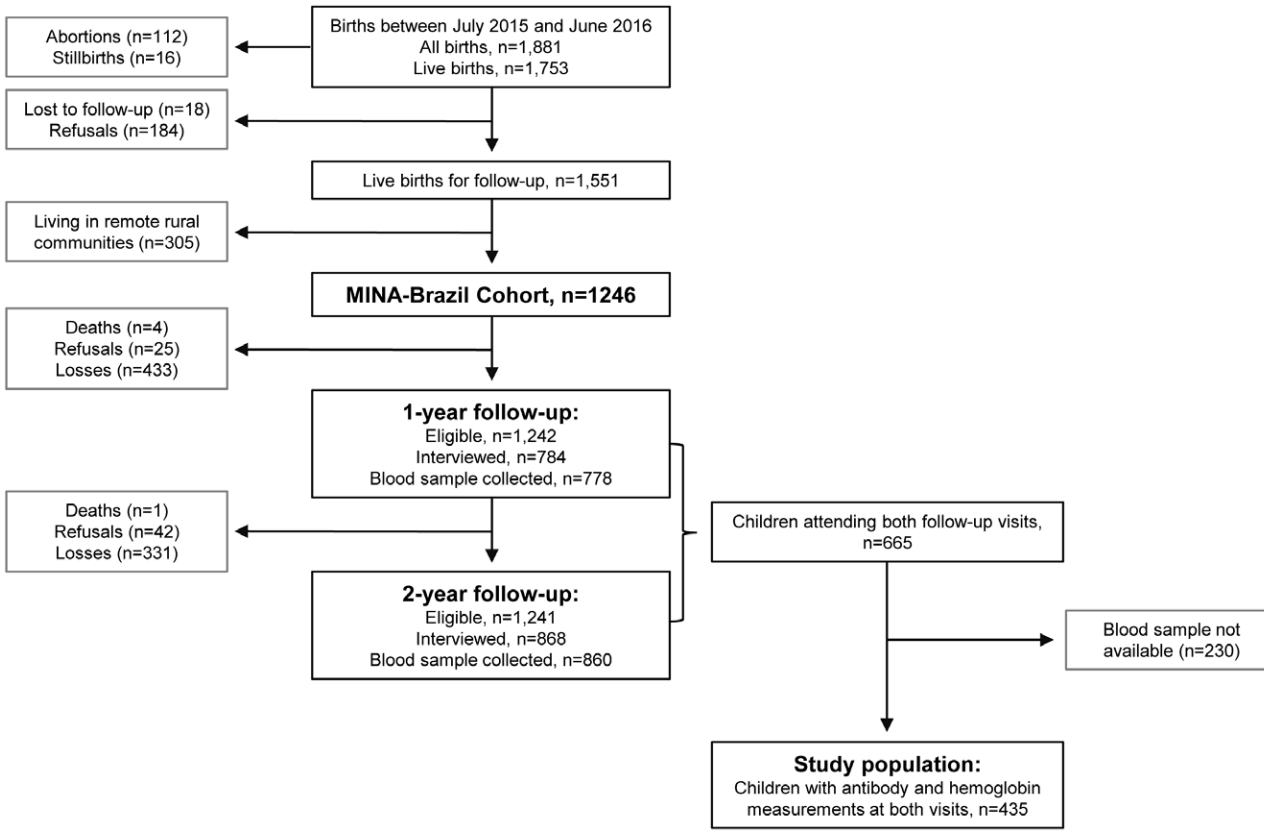

FIGURE 1. Study flowchart. Between July 2015 and June 2016, pregnant women attending antenatal clinics or admitted for delivery to the maternity ward of the Women and Children's Hospital of Jurua´ Valley in Cruzeiro do Sul, Brazil, were invited to participate. We analyze antibody data obtained during the follow-up visits at 1 and 2 years of age and hemoglobin measurement data obtained during the 2-years visit. Reasons for exclusion and the final number of subjects analyzed for each study outcome are indicated.

average absorbance for plasmas from 42 malaria-naïve donors plus 3 standard deviations. Positive samples had RIs greater than 1. IgG positivity rates among *P. vivax*-infected subjects have been previously estimated at 73% for PvAMA-1,[14] 95% for PvMSP1$_{19}$[14] and 72% for PvDBP.[18]

Outcomes

We considered IgG positivity to ≥2 antigens at one or both follow-up assessments as evidence of blood-stage *P. vivax* infection since birth, regardless of any clinical symptoms. Given that maternal IgG antibodies are expected to wane over a period of 6–12 months,[19] we assume that anti-*P. vivax* IgG of maternal origin was undetectable at the time of measurement when children were aged between 11 and 32 months. We additionally used a more relaxed definition of serologically proven *P. vivax* infection (IgG positivity to at least one of the 3 antigens tested) in exploratory analysis. We used vivax malaria case notifications as evidence of clinical malaria episodes experienced by study children. We searched the electronic database of the Ministry of Health of Brazil (SIVEP Malaria)[20] for malaria case notifications between July 2015, when the first participants were born, and July 2018, when all study participants had already completed 2 years,[8] aiming to identify study participants with one or more *P. vivax* malaria episodes diagnosed, treated and notified from birth until the age of 2 years. We retrieved case records that matched study children's name, sex and age, in addition to their mothers' name.[8] Because malaria is a notifiable disease in Brazil and diagnostic testing and treatment are not available outside government-run health care facilities, as many as 99.6% of laboratory-confirmed malaria episodes across the Amazon are estimated to be routinely entered in the SIVEP Malaria database.[21] As the vast majority of case notifications refer to infections diagnosed during sick visits, we define them as clinical vivax malaria cases. The secondary outcome, anemia at the 2-year follow-up assessment, was defined by a hemoglobin concentration <110 g/L (https://apps.who.int/iris/bitstream/handle/10665/85839/WHO_NMH_NHD_MNM_11.1_eng.pdf).

Statistical Analysis

Data were analyzed with STATA 15.1 (StataCorp, College Station, TX). Separate multiple Poisson regression models[22] were built to identify factors associated with binary (yes/no) outcomes: (1) serologically proven *P. vivax* infection within the first 2 years of age, (2) one of more vivax malaria episodes notified from birth to 2 years of age and (3) anemia diagnosed at 2 years of age. To account for children's clustering into localities with different levels of malaria transmission, as estimated by their API, we used mixed-effects models with API quintile as a contextual covariate and robust variance to analyze malaria-related outcomes. Standard regression models were used to analyze anemia as an outcome. Two critical explanatory variables were prolonged breastfeeding, defined as exclusive or partial breastfeeding lasting for ≥12 months,[23] and malaria in pregnancy, defined as one or more infections diagnosed by microscopy during sick visits to health facilities or by real-time PCR at delivery.[24] All variables considered in the unadjusted analysis are listed in Figure 1, Supplemental Digital Content 1, http://links.lww.com/INF/E760 and Figures 2, Supplemental Digital Content 1, http://links.lww.com/INF/E761. Only variables associated with the outcome at a significance level <20% were entered in multiple Poisson regression models. We used a hierarchical approach with distal, intermediate and proximal levels of disease determination[25] to select covariates that, in addition to age and wealth index, were retained in downstream analyses because (1) they were associated with the outcome at a significance level of <10% or (2) their inclusion in the model changed the risk measures by ≥10%. Participants with missing values for API (n = 4) were excluded from malaria models; those with missing values in categorical covariates were maintained in the malaria and anemia models by creating a new missing-value category. Adjusted prevalence ratio (PR) estimates are provided along with 95% confidence intervals (CIs) to quantify the influence of each predictor on the outcome while controlling for all other covariates.[22] Statistical significance was defined at the 5% level.

Ethics Statement

The study protocol was approved by the institutional review board of the School of Public Health, University of São Paulo (# 872.613, 2014). All mothers or their parents or guardians (if mothers were <18 years old) provided written informed consent.

RESULTS

Antibody Positivity and Vivax Malaria in Early Childhood

Figure 2 shows the proportions of the 435 children with IgG antibodies to each individual antigen and those who recognized ≥2 antigens. Few children—15.6% (95% CI: 12.5–19.4) at 1 year and 13.6% (95% CI: 10.6–17.1) at 2 years—had antibodies detected to at least one antigen. Only 4.4% (95% CI: 2.8–6.8) of the children at 1 year and 2.5% (95% CI: 1.4–4.5) of those at 2 years recognized ≥2 antigens. PvAMA1 was recognized by only 0.9% at 1 year and 1.1% at 2 –years. Overall, 23 (5.3%; 95% CI: 3.5–7.8) children had antibodies to ≥2 antigens detected during at least one follow-up assessment (See Figure, Supplemental Digital Content 3A, http://links.lww.com/INF/E762), which we take as evidence of blood-stage *P. vivax* infection during the first 2 years of life. In contrast, only 15 (3.4%; 95% CI: 2.1–5.6%) children had vivax malaria episode(s) retrieved from the case notification database during the same period. Seven children with one or more vivax malaria episodes notified were seronegative at the time of both follow-up assessments. Although we have used a very stringent definition of serologically proven *P. vivax* infection, this outcome was 1.5 times more frequent than clinical vivax malaria. Overall, 6.9% (95% CI: 4.9–9.7%) of children had evidence of *P. vivax* infection—seropositivity to ≥2 antigens and/or one or more vivax malaria case notifications—during their first 2 years of life. There were relatively few changes in antibody status between the 1-year and 2-year follow-up assessments (See Figure, Supplemental Digital Content 3B, http://links.lww.com/INF/E762). Most seropositive children at the 2-year assessment (7 of 11, 63.6%) had already serological evidence of *P. vivax* infection at the age of 1 year (See Figure, Supplemental Digital Content 3A, http://links.lww.com/INF/E762), although very few clinical malaria episodes had been diagnosed in this cohort and notified to the Ministry of Health during the first year of life.[8]

Correlates of *Plasmodium Vivax* Infection and Clinical Malaria

We next sought to identify correlates of serologically proven *P. vivax* infection and clinical vivax malaria for 431 participants included in multivariable analysis, which comprised 23 (5.3%) children with antibodies to ≥2 antigens detected during at least one follow-up assessment (Table 1) and 15 (3.4%) children with one or more vivax malaria episodes notified during the first 2 years of life (Table 2).

Children born to mothers with one or more *P. vivax* infection diagnosed during pregnancy or at delivery[23] had a significantly increased risk of serologically proven *P. vivax* infection (Table 1) and clinical vivax malaria during their early life (Table 2; see also reference 8), after adjustment for transmission intensity in the children's area of residence (API quintiles). Maternal anemia at

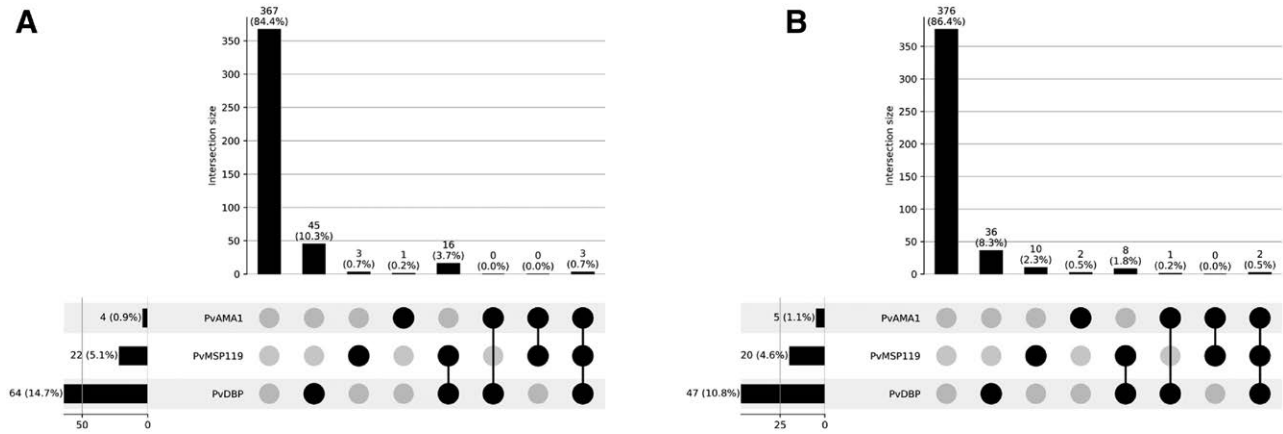

FIGURE 2. Proportions of study participants (n = 435) with IgG antibodies to individual *Plasmodium vivax* blood-stage antigens (PvAMA1, PvMSP$_{19}$, and PvDBP) (black dots) and proportions of those who recognized more than one antigen. A: follow-up visit at 1 year of age; B: follow-up visit at 2 years of age. Gray dots indicate no response.

TABLE 1. Factors Associated with the Presence of IgG Antibodies to ≥2 Blood-Stage *P. Vivax Antigens* in Amazonian Children at the Age of 1 or 2 Years (n = 431)

		Unadjusted			Adjusted		
	n positive/total	PR	95% CI	*P*	PR	95% CI	*P*
Child's age (months)		1.124	(0.884–1.428)	0.341	1.139	(0.864–1.501)	0.357
Wealth index quartile							
1st (poorest)	6/93	Ref.			Ref.		
2nd	6/114	0.816	(0.272–2.449)	0.717	1.039	(0.420–2.570)	0.934
3rd	8/113	1.097	(0.394–3.054)	0.859	1.468	(0.496–4.341)	0.488
4th (wealthiest)	3/111	0.419	(0.108–1.632)	0.210	0.452	(0.114–1.786)	0.257
Malaria in pregnancy							
No	19/398	Ref.			Ref.		
Yes	4/33	2.539	(0.916–7.036)	0.073	2.606	(1.268–5.357)	0.009
Breast-feeding duration							
<12 months	15/136	Ref.			Ref.		
≥12 months	8/292	0.248	(0.108–0.572)	0.001	0.202	(0.133–0.307)	<0.001
Annual parasite index (API) quintile							
1st (lowest)	5/101	Ref.					
2nd	3/104	0.583	(0.143–2.378)	0.452			
3rd	6/91	1.332	(0.420–4.223)	0.626			
4th	1/83	0.243	(0.029–2.048)	0.193			
5th (highest)	8/52	3.108	(1.069–9.036)	0.037			

CI indicates confidence interval; PR, prevalence ratio.

delivery was a predictor of the risk of clinical malaria in these children (Table 2).

Over two-thirds of the 428 children with complete information (292 or 68.2%) were breastfed for ≥12 months (Tables 1 and 2), but the median duration of exclusive breastfeeding was as short as 16 days in this population.[26] Interestingly, prolonged breastfeeding appeared to partially protect children from serologically proven early-life *P. vivax* infection (Table 1), although not from clinical vivax malaria using notified episodes as a proxy (Table 2). Similar results were obtained when we repeated the multiple regression analysis using a more relaxed definition of serologically proven *P. vivax* infection (IgG positivity to at least one antigen), as shown in Table, Supplemental Digital Content 4, http://links.lww.com/INF/E763.

Plasmodium Vivax Infection, Clinical Malaria, and Anemia at 2 Years of Age

All children with antibody and hemoglobin measurements (n = 435) were included in multivariable analyses of risk factors for anemia at 2 years of age. Serologically proven *P. vivax* infections (regardless of symptoms) and clinical vivax malaria episodes (regardless of the antibody status) were both significantly associated with increased anemia risk, after controlling for sex and age (in months), gestational age at birth (in weeks), wealth index (stratified into quintiles), maternal anemia at delivery (yes vs. no), and prolonged (≥12 months) breastfeeding (yes vs. no; Fig. 3). We next tested whether clinically silent, unnotified *P. vivax* infections—those retrospectively diagnosed by serology but missing in the case notification database—also contributed to increased anemia risk. No significant association was found (Figure 3).

DISCUSSION

We show that young Amazonian children are twice more frequently exposed to *P. vivax* than previously estimated from case notification records from the Ministry of Health of Brazil. Infections retrospectively diagnosed by serology have often failed to elicit a sick visit to a health post, with timely laboratory diagnosis

TABLE 2. Factors Associated with One or More *Plasmodium vivax* Malaria Episodes Notified in Amazonian Children During Their First 2 Years of Life (n = 431)

		Unadjusted			Adjusted		
	n positive/total	PR	95% CI	*P*	PR	95% CI	*P*
Child's age (months)		1.120	(0.792–1.583)	0.522	1.220	(0.945–1.573)	0.127
Wealth index quartile							
1st (poorest)	4/93	Ref.			Ref.		
2nd	6/114	1.224	(0.355–4.214)	0.749	1.449	(0.904–2.322)	0.123
3rd	4/113	0.823	(0.211–3.207)	0.779	1.407	(0.253–7.834)	0.697
4th (wealthiest)	1/111	0.209	(0.024–1.846)	0.159	0.356	(0.086–1.474)	0.154
Malaria in pregnancy							
No	9/398	Ref.			Ref.		
Yes	6/33	8.040	(3.044–21.235)	<0.001	5.926	(2.159–16.264)	0.001
Maternal anemia at delivery							
No	4/256	Ref.			Ref.		
Yes	11/156	4.513	(1.461–13.943)	0.009	3.451	(1.432–8.318)	0.006
Breast-feeding duration							
< 12 months	3/136	Ref.			Ref.		
≥12 months	12/292	1.864	(0.534–6.508)	0.329	1.325	(0.171–10.266)	0.788
Annual parasite index (API) quintile							
1st (lowest)	1/101	Ref.					
2nd	1/104	0.971	(0.061–15.366)	0.983			
3rd	3/91	3.330	(0.352–31.527)	0.294			
4th	2/83	2.434	(0.224–26.444)	0.465			
5th (highest)	8/52	15.538	(1.992–121.203)	0.009			

CI indicates confidence interval; PR, prevalence ratio.

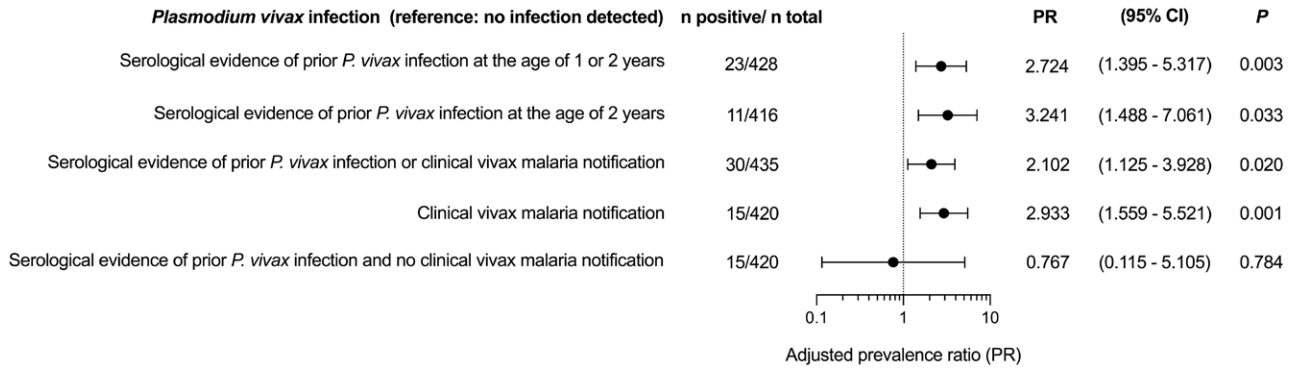

FIGURE 3. Association of anemia at 2 years of age with serologically proven *Plasmodium vivax* infection and clinical vivax malaria notified during the first 2 years of life. Prevalence ratios (PR) indicate the change in anemia risk associated with *P. vivax* infection or disease, compared with children with negative serology and no clinical vivax malaria notification, while controlling for sex and age, wealth index, maternal anemia at delivery, mother's gestational age at birth, and prolonged (≥12 months) breastfeeding. PR estimates for each exposure and their respective 95% confidence intervals (95% CIs) and *P* values were derived from separate multiple Poisson regression models. The total number of study participants in each analysis vary according to the criteria used to define "no infection detected". Accordingly, from the comparison of *P. vivax* infection detected by serology at 1 or 2 years vs. no infection, we excluded 7 children with malaria case notifications who were negative by serology, giving a total of 428. From the comparison of *P. vivax* infection detected by serology at 2 years vs. no infection, we excluded 7 children with malaria case notifications who were negative by serology and 12 children who were seropositive at 1 year but not at 2 years (total analyzed, 416). From the comparison of vivax malaria vs. no infection, we excluded 15 children with positive serology but no malaria case notification (total analyzed, 420). Finally, from the comparison of *P. vivax* infection detected by serology only vs. no infection, we excluded 15 children (8 of them seropositive) with malaria case notifications (total analyzed, 420).

followed by treatment and case notification. These findings imply that active case detection—for example, systematic screening for malaria parasites of infants and young children attending routine care, regardless of any clinical sign and symptom—is required to properly estimate the incidence of childhood infection in this and likely in other similar malaria-endemic settings. The clinical impact of early-life *P. vivax* infections that remain undiagnosed and untreated is unclear in our population. Reassuringly, we found no association between unnotified infections and the risk of anemia (Fig. 3), a common adverse consequence of vivax malaria in young children.[6]

Breastfeeding protects infants from a broad range of infections—for example, diarrhea, respiratory tract infections, necrotizing enterocolitis, meningitis and otitis media—through the passive transfer of immunoglobulins and other bioactive substances such as cytokines, oligosaccharides, lactoferrin and

lysozyme.[27] Whether specific antibodies acquired via breastfeeding play a major protective role against childhood malaria remains undetermined, but breastmilk IgA from malaria-exposed mothers has been shown to significantly inhibit the growth of *P. falciparum* in vitro.[28] Moreover, breastmilk components actively regulate the infant's immune system[29] and favor healthy microbial colonization of the gut.[30] Soluble malaria antigens are shed in breastmilk and may potentially elicit protective immune responses in offspring who are breastfed.[31] Importantly, protection from infections differs according to dose, duration, and exclusivity of breastfeeding.[29]

Exclusive (but not necessarily partial) breastfeeding has been suggested to reduce the risk of *P. falciparum* infection in neonates and young infants from the Gambia,[2] most likely because maternal milk is deficient in para-aminobenzoic acid, which is required for de novo folate synthesis by malaria parasites.[32,33] More recent evidence that exclusive breastfeeding protects infants from clinical malaria comes from a birth cohort study in Malawi[34] and cross-sectional surveys in the Democratic Republic of Congo[35] and Cameroon,[36] all in Sub-Saharan African settings where *P. falciparum* is the dominant human malaria parasite.

There is much less evidence that partial breastfeeding may protect from malaria well beyond early infancy. Here we show that children who regularly received human breastmilk for ≥12 months, regardless of any complementary foods, are less likely to develop antibodies to blood-stage *P. vivax* antigens (Table 1), a proxy of infection. Similarly, current total (partial or exclusive) breastfeeding was associated with a significantly lower risk of *P. falciparum* infection in 6–15 months old HIV-exposed children in Uganda, but not necessarily at the age of 16–24 months.[37] Total breastfeeding for ≥24 months was also associated with protection from severe falciparum malaria in a case-control study in Mali.[38] We have no clear-cut explanation for the fact that, in our study, children breastfed for ≥12 months did not differ from other children according to the risk of having a vivax malaria episode during their first 2 years of life (Table 2). Given the infrequency of childhood malaria in our study population, larger studies are needed to examine the association between breastfeeding and the risk of *P. vivax* infection and clinically apparent disease.

The present study has some limitations. First, only 34.9% of the original MINA cohort (n = 1246 participants) were eligible for the current analysis (Fig. 1) and children lost to follow-up differed from study participants in some of the key explanatory variables, such as socioeconomic status and place of residence. Second, to minimize the risk of false-positive diagnosis, we applied a strict definition of serologically proven *P. vivax* infection that is admittedly poorly sensitive, as it requires the simultaneous antibody recognition of at least two unrelated blood-stage antigens. Accordingly, only 8 (53.3%) of 15 children with clinical vivax malaria notified during the study period met our criteria of serologically proven infection. Antibody responses to PvAMA1, for example, were particularly infrequent in our study children, consistent with the notion that strong antibody responses to this recombinant antigen may require repeated exposure to *P. vivax* blood stages.[13,14] We, therefore, have underestimated the burden of *P. vivax* infection. However, the association between prolonged breastfeeding and reduced risk of *P. vivax* infection remained highly significant when we repeated the multiple regression analysis using a less strict definition of past infection (Table 1, Supplemental Digital Content, http://links.lww.com/INF/E760). Third, vivax malaria episodes occurring in study children were retrospectively identified, with no blood samples available for further confirmatory diagnostic tests. Although we assume that nearly all clinical malaria episodes confirmed by microscopy and treated in our cohort participants were retrieved from the notification database,[17] passive surveillance overlooks children with transient or chronic submicroscopic (often asymptomatic) infections who do not seek malaria diagnosis. Despite these potential limitations, the present study provides new insights into the epidemiology of *P. vivax* infection and vivax malaria in young Amazonian children and its relationship with breast-feeding—a topic that merits further exploration in the regional context.

CONCLUSION

Early-life *P. vivax* infections are substantially underreported in the main malaria transmission hotspot of Amazonian Brazil, but are significantly associated with increased risk of anemia at the age of 2 years. Importantly, these infections appear to be partially prevented by prolonged breastfeeding.

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