View metadata, citation and similar papers at core.ac.uk
brought to you by CORE
provided by Carolina Digital Repository

Am. J. Trop. Med. Hyg., 95(4), 2016, pp. 827–830
doi:10.4269/ajtmh.16-0011
Copyright © 2016 by The American Society of Tropical Medicine and Hygiene

Exclusive Breastfeeding and Clinical Malaria Risk in 6-Month-Old Infants: A Cross-Sectional Study from Kinshasa, Democratic Republic of the Congo

Nicholas F. Brazeau,[1]* Martine Tabala,[2] Landry Kiketa,[2] Dyna Kayembe,[2] Jean Lambert Chalachala,[2] Bienvenu Kawende,[2] Bruno Lapika,[3] Steven R. Meshnick,[1] and Marcel Yotebieng[4]

[1]*Gillings School of Global Public Health, University of North Carolina, Chapel Hill, North Carolina;* [2]*School of Public Health, University of Kinshasa, Kinshasa, Democratic Republic of the Congo;* [3]*Department of Anthropology, University of Kinshasa, Kinshasa, Democratic Republic of the Congo;* [4]*College of Public Health, Ohio State University, Columbus, Ohio*

Abstract. The World Health Organization recommends exclusive breastfeeding (EBF) for the first 6 months of life. However, the effect of EBF on malaria risk remains unclear. In the present study, 137 EBF infants and 358 non-EBF infants from the Democratic Republic of the Congo were assessed for fever and malaria infections by polymerase chain reaction, at 6 months of age. EBF was associated with a reduced risk of clinical malaria (odds ratio = 0.13; 95% confidence interval = 0.00–0.80), suggesting a protective effect of EBF against malaria.

INTRODUCTION

In 2013, more than 80% of the world's malaria cases and malaria-related deaths occurred in the World Health Organization (WHO) African region.[1] Most of these deaths occurred in children under the age of 5 years, who lack naturally acquired immunity (NAI) and as a consequence have the highest rates of infection, complications, and mortality.[1,2] However, despite lacking NAI, the first months of life are marked by a low incidence of clinical malaria.[2,3] The low incidence of clinical malaria among infants has been hypothesized to be due to transplacental transfer of protective maternal immunoglobulin G (IgG),[4] although other studies have not supported these findings.[3]

Similarly, the effect of breastfeeding on malaria risk during the first months of life remains uncertain. Recent studies found increased malaria prevalence among exclusively breastfed infants in Uganda and Malawi, whereas a study conducted in Nigeria found a decreased prevalence.[5–7] Despite the different prevalence findings, all three studies suggested that exclusive breastfeeding (EBF) had no significant effect on malaria infection risk.[5–7] However, the study from Nigeria was based on questionnaires and is subject to recall bias, whereas the Malawian study did not use the proper EBF definition as proposed by the WHO.[7,8] These conflicting results need to be resolved, as the WHO recommends that all mothers EBF for the first 6 months in recognition of EBF benefits for infants.[9–11] We sought to determine whether EBF affected the susceptibility to malaria infection and clinical malaria in a cohort of EBF and non-EBF infants residing in Kinshasa, Democratic Republic of the Congo.

METHODS

This cross-sectional study was conducted as an ancillary study to a randomized trial designed to evaluate breastfeeding promotion interventions (Yotebieng and others[12]). The study took place in Kinshasa, the Democratic Republic of the Congo, in six health facilities (Esengo, Kikenda, Kitega, Libikisi, Lukunga, and Luyindu). All mothers who birthed a healthy singleton during the enrollment period (May 24–August 25, 2012) and intended to attend well-baby clinic visits in the same facility were eligible for the original trial. Written consent and dried blood spots (DBS) were collected during the 24-week study visit using Whatman™ filter paper (GE Healthcare Bio-Sciences, Pittsburgh, PA) and shipped to the University of North Carolina at Chapel Hill for laboratory testing. Of 975 infants, 855 enrolled in the parent study were seen at the 24-week visit. However, due to limited supplies, DBS samples were collected for only 495 infants. The human immunodeficiency virus (HIV) status for 480/495 mothers was obtained from the Prevention of Mother-to-Child Transmission registry.

From each patient's DBS card, genomic DNA (gDNA) was extracted as previously described.[13] The samples were then evaluated using real-time polymerase chain reaction (PCR) with each well containing 2 μL of extracted gDNA, 6.25 μL of FastStart Universal Probe Master (Rox; Roche Diagnostics, Indianapolis, IN), 155 nL of 20 μM forward and reverse *Plasmodium falciparum* lactate dehydrogenase (PfLDH) primer, 190 nL of 20 μM PfLDH TaqMan® Probe (Applied Biosystems, Thermo Fisher Scientific, Waltham, MA), and 3.25 μL of molecular-grade water. All plates included a negative control and five positive controls (*P. falciparum* DNA) at sequential log concentrations ranging from 10^{-2} to 10^{-5} ng/μL.

Samples were considered positive if they had a C_t of < 38 or two C_t values of < 39 using the Bio-Rad CFX384™ Real-Time PCR Detection system (Bio-Rad, Clinical Diagnostics, Hercules, CA). Positive samples in the first series or indeterminates were evaluated in duplicate or, if necessary, triplicate using increased concentrations of gDNA (4 μL).

Information on infant feeding was collected through a face-to-face interview during the 24-week visit. Infants were classified as being exclusively breastfed using the definitions proposed by the WHO[14] and recall of the past 24 hours and past 7-day time frames. In addition, mothers were asked during the 24-week interview whether their infant had experienced any fever or had consumed any medications during the month preceding the interview. Mothers were also asked if their infant had slept under a bed net the night preceding the interview. Malaria microscopy was not performed during the 24-week clinical visit and infant's temperatures were not recorded.

The two outcomes of interest for the analysis were malaria infection, which was defined by a positive PCR (since microscopy was not performed), and clinical malaria, which was defined as a positive PCR and reported fever. Other

*Address correspondence to Nicholas F. Brazeau, Department of Epidemiology, University of North Carolina, Chapel Hill, NC 27516. E-mail: nbrazeau@med.unc.edu

TABLE 1

Association between maternal sociodemographic characteristics, use of bed nets, use of antimalarial drugs, and parasitemia or clinical malaria status among the cohort of 495 Congolese infants at 24 weeks of age

	Parasitemia*			Clinical malaria†		
	Yes	No	*P* value‡	Yes	No	*P* value‡
Maternal age in years: median (IQR)	27.0 (21.0–30.0)	27.0 (23.0–33.0)	0.082	24.5 (20.5–28.5)	27.0 (23.0–33.0)	0.025
Maternal education in years: median (IQR)	9.0 (7.0–11.0)	10.0 (8.0–12.0)	0.108	9.0 (8.0–11.0)	10.0 (8.0–12.0)	0.109
Maternal marital status						
Married/live-in boyfriend, *n* (%)	47 (10.8)	289 (89.2)	0.820	17 (3.9)	419 (96.1)	0.293
Never married/separated/divorced, *n* (%)	5 (8.6)	53 (91.4)		4 (6.9)	54 (93.1)	
SES Quintile§						
Fifth, *n* (%)	2 (2.0)	98 (98.0)	0.011	1 (1.0)	99 (99.0)	0.364
Fourth, *n* (%)	11 (11.2)	87 (88.8)		5 (5.1)	93 (94.9)	
Third, *n* (%)	12 (12.5)	84 (87.5)		4 (4.2)	92 (95.8)	
Second, *n* (%)	14 (14.4)	83 (85.6)		6 (6.2)	91 (93.8)	
First, *n* (%)	13 (13.0)	87 (87.0)		5 (5.0)	95 (95.0)	
Previous pregnancies						
Yes, *n* (%)	11 (12.8)	75 (87.2)	0.441	4 (4.7)	82 (16.6)	0.772
No, *n* (%)	41 (10.0)	368 (90.0)		17 (4.2)	392 (95.8)	
Use of bed nets						
Yes, *n* (%)	28 (8.9)	287 (91.1)	0.129	9 (2.9)	306 (97.1)	0.061
No, *n* (%)	24 (13.3)	156 (86.7)		12 (6.7)	168 (93.3)	
Use of antimalarial drugs						
Yes, *n* (%)	9 (16.1)	47 (83.9)	0.164	9 (16.1)	47 (83.9)	< 0.001
No, *n* (%)	43 (9.8)	396 (90.2)		12 (2.7)	427 (97.3)	

IQR = interquartile range; SES = socioeconomic status.
*Malaria parasite detected in the blood using polymerase chain reaction.
†Parasitemia and report of any fever in the last month preceding the sample collection.
‡Using Fisher's exact test or χ^2 test for categorical variables and Wilcoxon rank sum test for continuous variables.
§SES quintiles score calculated using principle component analysis and 11 variables: home ownership, the average number of household members per room, water source, toilet facility type, cooking fuel type, maternal education, and ownership status of durable assets (radio, television, mobile telephone, and refrigerator).

variables considered in the analysis were related to the mother's sociodemographic characteristics (Table 1).

Associations between potential confounders and the outcomes were assessed using Fisher's exact test or χ^2 test, when appropriate, for categorical variables and Wilcoxon rank sum test for continuous variables. Simple and stratified exact Cochran–Mantel–Haenszel odds ratios (ORs) and 95% confidence intervals (95% CIs) were used to assess the strength of the association between EBF and the two outcomes of interest. Analyses were performed using SAS®9.3 (Cary, NC) and assessed at a 0.05 significance level.

RESULTS

At the 24-week interview time point, 137 (27.7%) of the infants were EBF. First-time mothers (primiparous) and younger mothers (median = 27; interquartile range = 22.0–32.0) were less likely to exclusively breastfeed ($P = 0.0078$ and $P = 0.0141$, respectively). In addition, EBF infants were more likely to have used bed nets ($P < 0.0001$) but less likely to have used antimalarials ($P = 0.0439$).

Malaria parasitemia was confirmed by PCR in 52 (10.5%) infants, of whom, 21 were reported to have had a fever in the last month. In bivariate analyses, EBF was associated with a slight, but not significant, reduced risk of malaria infection (prevalence of 9.5% versus 10.9%; OR = 0.82; 95% CI = 0.39–1.62). EBF significantly reduced the risk of clinical malaria (OR = 0.13; 95% CI = 0.00–0.80). Indeed, only one (0.7%) case of clinical malaria was in an EBF infant. Bed net use was associated with reduced risk of malaria infection (OR = 0.63; 95% CI = 0.34–1.19) and clinical malaria (OR = 0.41; 95% CI = 0.15–1.09). The reported use of antimalarial drugs was associated with increased risk of malaria infection (OR = 1.76; 95% CI = 0.71–3.97) and clinical malaria (OR = 6.81; 95% CI = 2.39–18.57). Maternal parity appeared to have little, if any, effect on the rate of malaria infection or clinical malaria (Table 1).

Among the infants without malaria infections, 116 (26.19%) presented with fever. EBF was found to be protective against fever among infants with and without malaria infection (OR = 0.08; 95% CI = 0.01–0.67 and OR = 0.74; 95% CI = 0.432–1.23, respectively).

In stratified analyses, among children who used bed nets, 10 (9.3%) EBF and eight (8.7%) non-EBF infants presented with malaria infection (OR = 1.07; 95% CI = 0.42–2.56; Table 2). Among non-bed net users, malaria infection was found in three EBF (10.3%) and 21 non-EBF (13.9%) infants (OR = 0.71; 95% CI = 0.12–2.67). Overall, controlling for bed net use, there was a tendency toward lower odds of malaria infection among EBF infants (OR = 0.86; 95% CI = 0.41–1.71), although these associations lacked statistical significance. For clinical malaria, among bed net users, one (0.9%) EBF and eight (3.9%) non-EBF infants presented with clinical malaria (OR = 0.23; 95% CI = 0.01–1.78). Among non-bed net users, all 12 cases of clinical malaria recorded were among non-EBF infants. Stratification by bed net use showed decreased odds of clinical malaria among EBF infants (OR = 0.12; 95% CI = 0.00–0.79; Table 2). Similar results were obtained after stratification by use of antimalarial drugs (Table 2). Of 480 mothers, four with known HIV statuses were HIV positive and only one infant with an HIV-positive mother demonstrated malaria infection (zero cases of clinical malaria).

DISCUSSION

The overall prevalence of malaria infection among the 495 6-month-old infants from Kinshasa, Democratic Republic of

TABLE 2
Effect of exclusive breastfeeding on malaria infection and clinical malaria stratified by bed net use and use of antimalarial drugs

		Parasitemia		Odds ratio (95% CI)	
	EBF	Yes	No	Crude	Adjusted*
Bed net use					
Yes	Yes, *n* (%)	10 (9.3)	98 (90.7)	1.07 (0.42–2.56)	0.86 (0.41–1.71)
	No, *n* (%)	18 (8.7)	189 (91.3)		
No	Yes, *n* (%)	3 (10.3)	26 (89.7)	0.71 (0.12–2.67)	
	No, *n* (%)	21 (13.9)	130 (86.1)		
Use of antimalarial drugs					
Yes	Yes, *n* (%)	0	9 (100.0)	NA	0.86 (0.41–1.71)
	No, *n* (%)	9 (19.2)	38 (80.8)		
No	Yes, *n* (%)	13 (10.2)	115 (89.8)	1.06 (0.49–2.18)	
	No, *n* (%)	30 (9.7)	281 (90.3)		
		Clinical malaria			
Bed net use					
Yes	Yes, *n* (%)	1 (0.9)	107 (99.1)	0.23 (0.01–1.78)	0.12 (0.00–0.79)
	No, *n* (%)	8 (3.9)	199 (96.1)		
No	Yes, *n* (%)	0	29 (100.0)	NA	
	No, *n* (%)	12 (7.9)	139 (92.1)		
Use of antimalarial drugs					
Yes	Yes, *n* (%)	0	9 (100.0)	NA	0.12 (0.00–0.79)
	No, *n* (%)	9 (19.2)	38 (80.8)		
No	Yes, *n* (%)	1 (0.78)	127 (99.2)	0.21 (0.01–1.51)	
	No, *n* (%)	11 (3.5)	300 (96.5)		

CI = confidence interval; EBF = exclusive breastfeeding; NA = not available.
*Cochran–Mantel–Haenszel common odds ratio controlling for the stratifying variable. The 95% CIs are exact CIs. Infants are first stratified by bed net use or antimalarial use, followed by stratification by EBF status with respect to the outcomes of interest, parasitemia, and clinical malaria.

the Congo, considered in this study was 10.5%. A concordance of point estimates stratified by bed net use, reported fever, and other factors suggested that EBF at 24 weeks was associated with lower odds of developing clinical malaria, although only the findings of EBF protecting against fever among infants with malaria infection and EBF reducing the odds of clinical malaria were statistically significant.

This reduction in malaria susceptibility among EBF infants may be related to the immunomodulatory effects of breast milk.[15] Previous studies that found a lack of an association between EBF and malaria did not use PCR for malaria detection, measured malaria infection in a broad interval (age 3–12 months), and did not consider clinical malaria as an outcome.

This cross-sectional study has several limitations, including the small number of malaria cases, the unknown status of genetic factors that affect disease susceptibility (i.e., hemoglobinopathies), and the potential of recall bias. The issue of recall bias and misclassification was attenuated in that mothers and interviewers were blinded to the PCR results during the interview. As a result, any misclassification would be nondifferential and bias the association toward the null. In addition, gravidity was not associated with infection prevalence (Table 1), which suggests primigravida mothers were not subject to increased recall bias. This lack of association between parity and malaria infection prevalence may have resulted from the disproportionate number of primigravida mothers in the study (82.6%). This high number of primigravidae women in our study may limit its generalizability.

Although it is unlikely that maternal breast milk IgG antibodies are systemically absorbed into the circulation where malaria parasites reside,[16] EBF protected infants in this study from developing clinical malaria. This observed effect may be a byproduct of EBF reducing overall disease burden,[10,11] allowing for increased resource allocation to malaria defenses, or the role of EBF in training and modulating the infant's immune system.[15] It is possible that some of the fever episodes among the malaria-infected children were due to other etiologic agents. As such, our definition of clinical malaria is lenient. In addition, antimalarial use was associated with increased risk of malaria infection, possibly due to detection of biologically inactive parasites. However, the point estimates of both malaria infection and clinical malaria in the presence and absence of bed nets suggest that EBF has a protective effect. In addition, EBF reduced fever risk among infants with malaria infection more than those without a malaria infection (OR = 0.08 versus OR = 0.74, respectively). Given these indications, our findings need to be replicated in a larger prospective study.

Received January 4, 2016. Accepted for publication June 6, 2016.

Published online August 22, 2016.

Financial support: The study was supported by a grant from the Bill & Melinda Gates Foundation to FHI 360, through the Alive and Thrive Small Grants Program (University of California, Davis, CA) via a subaward (no. 09-000076-AT11-123-UNC-DRC) to the University of North Carolina (Chapel Hill, NC).

Authors' addresses: Nicholas F. Brazeau, Department of Epidemiology, University of North Carolina, Chapel Hill, NC, E-mail: nbrazeau@med.unc.edu. Martine Tabala, Landry Kiketa, Dyna Kayembe, Jean Lambert Chalachala, and Bienvenu Kawende, School of Public Health, University of Kinshasa, Kinshasa, Democratic Republic of the Congo, E-mails: martabala@yahoo.fr, landrykiketa@yahoo.fr, dirchkayembe@yahoo.fr, chalachalav@yahoo.com, and bienvenukawende@yahoo.fr. Bruno Lapika, Department of Anthropology, School of Public Health, University of Kinshasa, Kinshasa, Democratic Republic of the Congo, E-mail: lapikadi@yahoo.fr. Steven R. Meshnick, Department of Epidemiology, University of North Carolina, Chapel Hill, NC, E-mail: meshnick@email.unc.edu. Marcel Yotebieng, College of Public Health, Ohio State University, Columbus, OH, E-mail: yotebieng.2@osu.edu.

Reprint requests: Nicholas F. Brazeau, Department of Epidemiology, University of North Carolina, Chapel Hill, NC 27516, E-mail: nbrazeau@med.unc.edu, Tel: 269-929-2537, Fax: 919-962-8331.

REFERENCES

1. World Health Organization (WHO), 2014. *World Malaria Report 2014*. Geneva, Switzerland: WHO Press.
2. Doolan DL, Dobano C, Baird JK, 2009. Acquired immunity to malaria. *Clin Microbiol Rev 22:* 13–36.
3. Riley EM, Wagner GE, Ofori MF, Wheeler JG, Akanmori BD, Tetteh K, McGuinness D, Bennett S, Nkrumah FK, Anders RF, Koram KA, 2000. Lack of association between maternal antibody and protection of African infants from malaria infection. *Infect Immun 68:* 5856–5863.
4. Wilson PT, Malhotra I, Mungai P, King CL, Dent AE, 2013. Transplacentally transferred functional antibodies against *Plasmodium falciparum* decrease with age. *Acta Trop 128:* 149–153.
5. Nankabirwa V, Tylleskar T, Nankunda J, Engebretsen IMS, Sommerfelt H, Tumwine JK; PROMISE EBF Research Consortium, 2011. Malaria parasitaemia among infants and its association with breastfeeding peer counselling and vitamin A supplementation: a secondary analysis of a cluster randomized trial. *PLoS One 6:* e21862.
6. Kalanda BF, Verhoeff FH, Brabin BJ, 2006. Breast and complementary feeding practices in relation to morbidity and growth in Malawian infants. *Eur J Clin Nutr 60:* 401–407.
7. Ibadin O, Ofili N, Morrison O, Nkwuo E, 2012. Exclusive breastfeeding and malaria in early infancy: experience from Benin City, Nigeria. *J Med Biomed Res 11:* 116–122.
8. Kalanda BF, Verhoeff FH, Brabin BJ, 2006. Breast and complementary feeding practices in relation to morbidity and growth in Malawian infants. *Eur J Clin Nutr 60:* 401–407.
9. World Health Organization (WHO), 2013. *WHO Recommendations on Postnatal Care of the Mother and Newborn*. Geneva, Switzerland: WHO Press.
10. Bhutta ZA, Das JK, Walker N, Rizvi A, Campbell H, Rudan I, Black RE; Lancet Diarrhoea and Pneumonia Interventions Study Group, 2013. Interventions to address deaths from childhood pneumonia and diarrhoea equitably: what works and at what cost? *Lancet 381:* 1417–1429.
11. Roberts TJ, Carnahan E, Gakidou E, 2013. Can breastfeeding promote child health equity? A comprehensive analysis of breastfeeding patterns across the developing world and what we can learn from them. *BMC Med 11:* 12.
12. Yotebieng M, Labbok M, Soeters H, Chalachala J, Lapika B, Vitta B, Behets F, 2015. Ten Steps to Successful Breastfeeding programme to promote early initiation and exclusive breastfeeding in DR Congo: a cluster-randomized controlled trial. *Lancet 9:* e546–e555.
13. Plowe CV, Djimde A, Bouare M, Doumbo O, Wellems TE, 1995. Pyrimethamine and proguanil resistance-conferring mutations in *Plasmodium falciparum* dihydrofolate reductase: polymerase chain reaction methods for surveillance in Africa. *Am J Trop Med Hyg 52:* 565–568.
14. World Health Organization (WHO), 2007. *Indicators for Assessing Infant and Young Child Feeding Practices*. Available at: http://www.who.int/maternal_child_adolescent/documents/9789241596664/en/. Accessed June 15, 2014.
15. Ballard O, Morrow AL, 2013. Human milk composition nutrients and bioactive factors. *Pediatr Clin North Am 60:* 49.
16. Van de Perre P, 2003. Transfer of antibody via mother's milk. *Vaccine 21:* 3374–3376.