1-18-2021

Prevalence of Malaria Parasite Infections among U.S.-Bound Congolese Refugees with and without Splenomegaly.

Moses Mwesigwa
International Organization for Migration

Jessica L. Webster
Centers for Disease Control and Prevention; Oak Ridge Institute for Science and Education

Sam Lubwama Nsobya
Makerere University

Alexander Rowan
Thomas Jefferson University

Mukunda Singh Basnet
International Organization for Migration

See next page for additional authors

Am. J. Trop. Med. Hyg., 104(3), 2021, pp. 996–999
doi:10.4269/ajtmh.20-0924

Prevalence of Malaria Parasite Infections among U.S.-Bound Congolese Refugees with and without Splenomegaly

Moses Mwesigwa,[1] Jessica L. Webster,[2,3] Sam Lubwama Nsobya,[4] Alexander Rowan,[5] Mukunda Singh Basnet,[1] Christina R. Phares,[2] Michelle Weinberg,[2] Alexander Klosovsky,[1] Marwan Naoum,[1] Philip J. Rosenthal,[6] and William M. Stauffer[2,7]*

[1]*International Organization for Migration, Kampala, Uganda;* [2]*Centers for Disease Control and Prevention, Atlanta, Georgia;* [3]*Oak Ridge Institute for Science and Education, Oak Ridge, Tennessee;* [4]*Department of Pathology, School of Biomedical Sciences, Makerere University, Kampala, Uganda;* [5]*Sidney Kimmel Medical College, Thomas Jefferson University, Philadelphia, Pennsylvania;* [6]*Department of Medicine, University of California, San Francisco, California;* [7]*Departments of Medicine and Pediatrics, University of Minnesota, Minneapolis, Minnesota*

Abstract. All U.S.-bound refugees from sub-Saharan Africa receive presumptive antimalarial treatment before departing for the United States. Among U.S.-bound Congolese refugees, breakthrough malaria cases and persistent splenomegaly have been reported. In response, an enhanced malaria diagnostic program was instituted. Here, we report the prevalence of plasmodial infection among 803 U.S.-bound Congolese refugees who received enhanced diagnostics. Infections by either rapid diagnostic test (RDT) or PCR were detected in 187 (23%) refugees, with 78 (10%) by RDT only, 35 (4%) by PCR only, and 74 (9%) by both. Infections identified by PCR included 103 monoinfections (87 *Plasmodium falciparum*, eight *Plasmodium ovale*, seven *Plasmodium vivax*, and one *Plasmodium malariae*) and six mixed infections. Splenomegaly was associated with malaria detectable by RDT (odds ratio: 1.8, 95% CI: 1.0–3.0), but not by PCR. Splenomegaly was not strongly associated with parasitemia, indicating that active malaria parasitemia is not necessary for splenomegaly.

During 2013–2019, more than 50,000 Congolese refugees living in east Africa resettled to the United States. During 2014–2015, 14% of Congolese refugees resettling to the United States from the Kyangwali Refugee Settlement in the Kikuube district of Uganda were noted to have splenomegaly.[1] A subsequent investigation of splenomegaly in Congolese refugees resettled to the United States highlighted malaria infection as a relatively common condition, although the definitive etiology of splenomegaly in this population was not determined.[2] These observations led to enhanced diagnostics for malaria infection testing for U.S.-bound Congolese refugees from Kikuube district, Uganda, to better manage malaria by detecting additional cases and by providing species-specific treatment. We determined the prevalence of plasmodial infection among 803 U.S.-bound Congolese refugees evaluated under this enhanced diagnostics program.

As part of the U.S. Refugee Resettlement Program, all U.S.-bound refugees originating in sub-Saharan Africa receive presumptive predeparture treatment with artemether/lumefantrine (AL), unless contraindicated, as recommended by the U.S. CDC to prevent symptomatic *Plasmodium falciparum* malaria disease during travel or following arrival. Treatment is administered in a standard 3-day course under direct observation and should be completed no sooner than 5 days before departure.

In 2015, in response to the observed high prevalence of splenomegaly in U.S.-bound Congolese refugees, the CDC added diagnosis and management recommendations, stating that all refugees with clinical splenomegaly (enlargement of the spleen) identified at their initial medical examination (occurring 3–6 months before departure to the United States) should receive a malaria rapid diagnostic test (RDT; SD Bioline Malaria Ag *P.f/Pan* test, Abbott Diagnostics Korea Inc., Gyeonggido, Republic of Korea) and, if positive, receive antimalarial treatment with AL. In addition, the CDC recommended that all refugees with splenomegaly receive primaquine after arrival in the United States, if glucose-6-phosphate dehydrogenase activity is normal, to presumptively eliminate *Plasmodium vivax* and *Plasmodium ovale* hypnozoites.

Despite these recommendations, many refugees had persistent splenomegaly months to years after arrival in the United States.[2] In addition, there were increasing reports of breakthrough malaria, particularly *Plasmodium malariae* despite predeparture treatment with AL,[2] which was of concern because of increasing reports of AL treatment failure for *P. malariae* infections.[3] Because *P. malariae* has been well associated with splenomegaly,[4–6] the reports of *P. malariae* in Congolese refugees raised additional concern that the documented persistent splenomegaly could be related to inadequate malaria presumptive treatment, especially if baseline prevalence of *P. malariae* infection was higher than expected.

In response to these concerns, and given the continuing high burden of malaria in U.S.-bound Congolese refugees from Uganda, the CDC expanded its guidance for malaria testing and treatment in 2018 to include the RDT for *P. falciparum* for all Congolese refugees (with or without splenomegaly) plus PCR. Refugees with a positive RDT and those diagnosed with splenomegaly (with or without a positive RDT) were treated with AL at the initial examination (approximately 6 months before departure).[7] In addition, when PCR results became available (at a later date), refugees with positive results who had not previously received appropriate treatment based on species, or had a newly identified infection, were offered treatment.[8] It should be noted that in addition to enhanced diagnostics and treatment at initial medical evaluation, all refugees without a contraindication (including those who received previous treatment based on a positive RDT, positive PCR, or diagnosis of splenomegaly) received AL treatment completed no sooner than 5 days before departure for the United States, consistent with previous guidance.[9] An outline of this guidance is provided in Table 1.

* Address correspondence to William Stauffer, Infectious Diseases and International Medicine, Departments of Medicine and Pediatrics, Human Migration and Health, Center for Global Health and Social Responsibility, University of Minnesota, 420 DE St. SE, MMC 250, Minneapolis, MN 55455. E-mail: stauff005@mn.edu

TABLE 1

Overview of the CDC's guidance for malaria and splenomegaly in U.S.-bound refugees, 2007–2018

Year of implementation	Population	Recommendation
2007	All U.S.-bound refugees originating in sub-Saharan Africa	Predeparture treatment with artemether/lumefantrine (AL) unless contraindicated Standard 3-day course under direct observation Completed no sooner than 5 days before departure
2015	All refugees with splenomegaly identified at initial medical examination	Receive a malaria RDT (SD Bioline Malaria Ag *P.f/Pan* test) If positive, antimalarial treatment with AL Clinicians providing care for refugees with splenomegaly directed to treat with primaquine (PQ), if glucose-6-phosphate dehydrogenase (G6PD) activity is normal
2018	All Congolese refugees (with or without splenomegaly)	Testing for malaria at initial examination expanded to include the RDT for *Plasmodium falciparum* plus PCR
	Refugees with positive RDT and all refugees with splenomegaly (with or without positive RDT)	Treatment with AL at initial examination
	Refugees with positive PCR results who were not treated previously	Treatment with AL for blood-stage infection followed by a 2-week course of PQ (if G6PD normal) for those who had *Plasmodium ovale* or *Plasmodium vivax* infections

RDT = rapid diagnostic test.

The program was instituted to enhance the diagnosis and management of malaria in U.S.-bound Congolese refugees. The goal of this evaluation is to retrospectively report prevalence of malaria based on enhanced testing and to correlate the malaria findings with detection of splenomegaly. The proposed evaluation was reviewed by a CDC human subject advisor and determined to be a non-research evaluation of program activities.

Malaria RDT results were interpreted by International Organization for Migration laboratory personnel. DNA was extracted from blood dried on Whatman 3-MM filter paper with Chelex (Cetiva, Marlborough, MA),[7] with nested PCR amplification of species-specific sequences of 18S subunit ribosomal RNA genes and discrimination of species by electrophoresis of amplicons, as described previously.[8] Clinical data, including the presence of splenomegaly and RDT results, were collected retrospectively from medical records.

During February–March 2018, 803 refugees had a physical examination including an abdominal examination and tested for malaria parasites by RDT and PCR. Demographic characteristics were compared between individuals with and without detectable malaria infections using chi-squared tests. Adjusted odds ratios and 95% CIs were calculated using multivariable logistic regression models. All models were adjusted for age, gender, family size, and birth country.

This cohort consisted of an approximately equal number of women (51%) and men (49%), with age largely less than 55 (median = 14, interquartile range = 6–28) years; 41% were born in Uganda to Congolese parents, and 59% were born in the Democratic Republic of the Congo (DRC). A total of 187 (23%) refugees were positive by either RDT or PCR, 78 (10%) were positive by malaria RDT only, 35 (4%) were positive by malaria PCR only, and 74 (9%) were positive by both RDT and PCR. In the 109 infections identified by PCR, 87 (80%) had monoinfections with *P. falciparum*, eight (7.3%) with *P. ovale*, seven (6.4%) with *P. vivax*, one (0.9%) with *P. malariae*, and six (5.5%) mixed infections (Table 2).

Most (75%) malaria infections detected by PCR were among children aged 14 years and younger. The highest prevalence of infection was in those aged 6–14 years (24%) and the lowest in those aged 25–54 years (5%). In the adjusted regression analysis (Table 3), odds of malaria infection detected by PCR were more than four times higher among children aged 6–14 years than adults aged 25–54 years (OR = 4.6, 95% CI = 1.3–15.6) and almost seven times higher among adults aged 55 years or older than that in the same reference group (OR = 6.6, 95% CI = 2.1–20.5).

TABLE 2

Demographic characteristics and screening results of U.S.-bound Congolese refugees screened for malaria and splenomegaly, Uganda, 2018

Variable	*n* (%), (*N* = 803)
Age (years)	
0–2	94 (12%)
3–5	94 (12%)
6–14	227 (28%)
15–24	151 (19%)
25–54	201 (25%)
55+	36 (4%)
Median (interquartile range)	14 (6–28)
Gender	
Male	393 (49%)
Female	410 (51%)
Family size	
1–3	131 (17%)
4–7	319 (40%)
8+	353 (44%)
Nationality	
DRC	800 (99.6%)
Rwanda	3 (0.4%)
Birth country	
DRC	328 (41%)
Rwanda	2 (0.3%)
Uganda	473 (59%)
Malaria rapid diagnostic test results	
Negative	651 (81%)
Positive	152 (19%)
Malaria PCR results	
Negative	694 (86%)
Positive	109 (14%)
P. falciparum	87 (80%)
P. malariae	1 (1%)
P. ovale	8 (7%)
P. vivax	7 (6%)
P. falciparum and *P. malariae*	1 (1%)
P. falciparum and *P. ovale*	3 (3%)
P. falciparum and *P. vivax*	1 (1%)
P. falciparum, *P. ovale*, and *P. malariae*	1 (1%)
Splenomegaly	
Negative	711 (89%)
Positive	92 (11%)

DRC = Democratic Republic of the Congo; *P. falciparum* = *Plasmodium falciparum*; *P. malariae* = *Plasmodium malariae*; *P. ovale* = *Plasmodium ovale*; *P. vivax* = *Plasmodium vivax*.

TABLE 3

Multivariable logistic regression analyses for splenomegaly and for malaria infection detectable by PCR among U.S.-bound Congolese refugees, Uganda, 2018

	Splenomegaly				Malaria infection			
	Unadjusted odds ratio		Adjusted odds ratio*		Unadjusted odds ratio		Adjusted odds ratio†	
Variable	95% CI	*P*-value	95% CI	*P*-value	95% CI	*P*-value	95% CI	*P*-value
Age (years)								
0–2	–	–	–	–	2.27 (0.90–5.74)	0.078	1.81 (0.44–7.21)	0.398
3–5	0.30 (0.09–1.03)	0.056	0.17 (0.04–0.75)	0.02	4.52 (2.03–10.61)	< 0.001	3.45 (0.93–12.81)	0.065
6–14	2.18 (1.23–3.84)	0.007	1.16 (0.42–3.23)	0.774	5.96 (3.07–12.76)	< 0.001	4.57 (1.33–15.63)	0.016
15–24	1.54 (0.81–2.95)	0.188	1.00 (0.44–2.28)	0.994	1.35 (0.54–3.39)	0.51	1.36 (0.46–4.05)	0.579
25–54	Ref	–	Ref	–	Ref	–	Ref	–
55+	0.82 (0.23–2.93)	0.763	0.56 (0.15–2.10)	0.389	4.61 (1.57–12.99)	0.004	6.58 (2.11–20.54)	0.001
Gender								
Male	Ref	–	Ref	–	Ref	–	Ref	–
Female	0.75 (0.48–1.15)	0.187	0.74 (0.47–1.17)	0.195	0.86 (0.57–1.28)		0.86 (0.56–1.30)	0.463
Malaria by RDT								
Yes	1.95 (1.19–3.17)	0.008	1.75 (1.00–3.04)	0.049	–	–	–	–
No	Ref	–	Ref	–	–	–	–	–
Malaria by PCR								
Yes	1.66 (0.95–2.90)	0.077	1.52 (0.83–2.78)	0.175	–	–	–	–
No	Ref	–	Ref	–	–	–	–	–
Malaria by PCR or RDT								
Yes	2.02 (1.27–3.21)	0.003	1.84 (1.09–3.09)	0.021	–	–	–	–
No	Ref	–	Ref	–	–	–	–	–
Malaria by PCR and RDT								
Yes	1.57 (0.81–3.04)	0.181	1.41 (0.69–2.88)	0.348	–	–	–	–
No	Ref	–	Ref	–	–	–	–	–

RDT = rapid diagnostic test.

* Four separate models were run for malaria infection detectable by RDT, by PCR, by PCR or RDT, and by PCR and RDT, each adjusted for age, gender, family size, and birth country.

† Model adjusted for age, gender, family size, and birth country.

Splenomegaly was observed in 92 refugees (11%), with the highest prevalence among those aged 6–14 years (19%), similar to previous reports in this population.[1] Of the 92 refugees with splenomegaly, malaria parasite infections were detected by RDT in 27 (29%) and by PCR in 18 (20%). Adjusting for age, gender, family size, and birth country, odds of splenomegaly were higher among those with malaria infection detected by PCR than those without (OR = 1.5, 95% CI = 0.8–2.8) and among those who were RDT positive as compared with those RDT negative (OR = 1.8, 95% CI = 1.0–3.0), but the association was significant only for the RDT comparison (Table 3). Of note, among those positive by RDT, an unusually high proportion (51%) were negative by PCR.

This analysis has limitations. This was a retrospective analysis of data collected during routine care and not designed as a prospective study. Malaria prevalence may vary as transmission intensity changes over time, and this cross-sectional investigation offers only a snapshot of the evaluation period. Furthermore, the high rate of PCR negativity among those who tested positive by RDT was unexpected because PCR offers a more sensitive test for parasitemia. Expert microscopy was not included in the enhanced program evaluation. In the operational setting, either RDT or PCR may have been inaccurate because of undetermined factors. Potential contributors to discrepancies between RDT and PCR results include persistence of circulating histidine-rich protein beyond that of plasmodial DNA in those recently treated for malaria (including self-treatment in advance of their medical examination for resettlement), errors in reading of RDTs in the field setting, false-negative PCR assays due to polymerase inhibitors in samples, and false-positive PCR assays due to contamination from other samples.[9,10]

In conclusion, malaria was prevalent in U.S.-bound Congolese refugees living in Kikuube district, Uganda, in February–March 2018. Splenomegaly was prevalent in this population at rates similar to those found in 2014.[1] Efforts to delineate the optimal treatment regimen for malaria in the population before migration to the United States would be beneficial, particularly as 18% of PCR-positive samples had *P. ovale* or *P. vivax*. Traditionally, presumptive treatment has not focused on relapsing (*P. vivax* and *P. ovale*) forms of malaria because U.S.-bound refugees have mostly not originated in areas with high prevalence of these parasites. In light of the prevalence of *P. ovale* and *P. vivax* that is higher than that expected in Congolese refugees, presumptive treatment for relapsing malaria in some refugee populations should be reconsidered. There was no clear direct association between malaria parasitemia and splenomegaly, indicating that active malaria parasitemia is not necessary for splenomegaly or that splenomegaly persists over time despite parasite clearance. Nonetheless, the background high prevalence of malaria infection suggests a likely role for malaria infection in the etiology of splenomegaly, as previously suggested.[11,12] Despite anecdotal reports of high prevalence of *P. malariae* infection in Congolese refugees, < 1% of detected species were *P. malariae* in this program. The small number of *P. malariae* cases identified suggests this species is not a driver of splenomegaly. Etiologies other than malaria that could account for or contribute to splenomegaly should be further explored.

Received July 29, 2020. Accepted for publication October 30, 2020.

Published online January 18, 2021.

Disclaimer: The findings and conclusions in this report are those of the authors and do not necessarily represent the official position of the CDC.

Authors' addresses: Moses Mwesigwa and Mukunda Singh Basnet, International Organization for Migration; Kampala, Uganda, E-mails: mmwesigwa@iom.int and mbasnet@iom.int. Jessica L. Webster, Centers

for Disease Control and Prevention, Oak Ridge Institute for Science and Education; Atlanta, GA, E-mail: jlw494@drexel.edu. Sam Lubwama Nsobya, Department of Pathology, School of Biomedical Sciences, Makerere University, Kampala, Uganda, E-mail: samnsobya@yahoo.co.uk. Alexander Rowan, Sidney Kimmel Medical College, Thomas Jefferson University, Philadelphia, PA, E-mail: arr011@jefferson.edu. Christina R. Phares and Michelle Weinberg, Centers for Disease Control and Prevention; Atlanta, GA, E-mails: ctp7@cdc.gov and mpw5@cdc.gov. Alexander Klosovsky, International Organization for Migration, Washington, DC, E-mail: aklosovsky@iom.int. Marwan Naoum, International Organization for Migration, Nairobi, Kenya, E-mail: nmarwan2@iom.int. Philip J. Rosenthal, Department of Medicine, University of California, San Francisco, San Francisco, CA, E-mail: philip.rosenthal@ucsf.edu. William Stauffer, Centers for Disease Control and Prevention, University of Minnesota, Departments of Medicine and Pediatrics, Minneapolis, MN, E-mail: stauf005@umn.edu.

REFERENCES

1. Goers M et al., 2016. Notes from the field: splenomegaly of unknown etiology in Congolese refugees applying for resettlement to the United States–Uganda, 2015. *MMWR Morb Mortal Wkly Rep 65:* 943–944.
2. Zambrano LD et al., 2018. Unresolved splenomegaly in recently resettled congolese refugees–multiple States, 2015–2018. *MMWR Morb Mortal Wkly Rep 67:* 1358–1362.
3. Smith A, Denholm J, Shortt J, Spelman D, 2011. *Plasmodium* species co-infection as a cause of treatment failure. *Travel Med Infect Dis 9:* 306–309.
4. Marsden PD, Hutt MS, Wilks NE, Voller A, Blackman V, Shah KK, Connor DH, Hamilton PJ, Banwell JG, Lunn HF, 1965. An investigation of tropical splenomegaly at mulago hospital, Kampala, Uganda. *Br Med J 1:* 89–92.
5. Vinetz JM, Li J, McCutchan TF, Kaslow DC, 1998. *Plasmodium malariae* infection in an asymptomatic 74-year-old Greek woman with splenomegaly. *N Engl J Med 338:* 367–371.
6. Yadav RS, Sharma VP, Ghosh SK, Kumar A, 1990. Quartan malaria–an investigation on the incidence of *Plasmodium malariae* in Bisra PHC, district Sundargarh, Orissa. *Indian J Malariol 27:* 85–94.
7. Plowe CV, Wellems TE, 1995. Molecular approaches to the spreading problem of drug resistant malaria. *Adv Exp Med Biol 390:* 197–209.
8. Snounou G, Viriyakosol S, Jarra W, Thaithong S, Brown KN, 1993. Identification of the four human malaria parasite species in field samples by the polymerase chain reaction and detection of a high prevalence of mixed infections. *Mol Biochem Parasitol 58:* 283–292.
9. World Health Organization, 2019. *Rapid Diagnostic Tests*. Available at: http://www.who.int/malaria/areas/diagnosis/rapid_diagnostic_tests/en/. Accessed January 5, 2021.
10. Wu L, van den Hoogen LL, Slater H, Walker PG, Ghani AC, Drakeley CJ, Okell LC, 2015. Comparison of diagnostics for the detection of asymptomatic *Plasmodium falciparum* infections to inform control and elimination strategies. *Nature 528:* S86–S93.
11. Adekile AD et al., 1993. Spleen in sickle cell anemia: comparative studies of Nigerian and U.S. patients. *Am J Hematol 42:* 316–321.
12. Tantravahi SK, Williams LB, Digre KB, Creel DJ, Smock KJ, DeAngelis MM, Clayton FC, Vitale AT, Rodgers GM, 2012. An inherited disorder with splenomegaly, cytopenias, and vision loss. *Am J Med Genet A 158A:* 475–481.