Post-Arrival Screening for Malaria in Asymptomatic Refugees Using Real-Time PCR

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Abstract. Malaria is a significant health risk to refugee populations originating from endemic areas, but there is little consensus on screening and/or treatment approaches for malaria in this population. Furthermore, detection of malaria in semi-immune asymptomatic refugees is limited by the sensitivity of diagnostic tests used for screening. We determined the prevalence of malaria by microscopy and real-time polymerase chain reaction (PCR) in a consecutive population of 324 asymptomatic refugees examined in Edmonton, Canada, during 2009–2010. Although all thick and thin blood smear results were negative, 10 subjects (3.1%) tested PCR positive for *Plasmodium* DNA. Interestingly, 6 of 10 PCR positive subjects are at risk of malaria relapse by *P. vivax* or *P. ovale* infections. These results suggest that appropriate guidelines for malaria screening should consider the risk of relapsing infections, and they highlight the potential usefulness of real-time PCR in the diagnosis of asymptomatic malaria.

INTRODUCTION

According to the United Nations High Commission on Refugees, 43.3 million people were forcibly displaced in 2009.¹ Canada resettles the second highest number of refugees after the United States, accepting between 10,000 and 12,000 refugees each year.^{2,3} Refugees are a particularly vulnerable group with unique health risks, including those resulting from their varied exposure to infectious diseases both in their home and transit countries.⁴ The diagnosis of infectious diseases and appropriate screening or management strategies in refugees present a complex health challenge to clinicians and public health officials, particularly with exotic diseases that are not endemic to the resettlement country.

Malaria is a common disease of the tropics but may not be routinely considered by Canadian physicians in their differential diagnosis of acute illness.⁵ The potential for developing malaria among refugees resettling in Canada is high; in 2007, the top 10 source countries for refugees arriving in Canada, representing 9,663 refugees, were all endemic for malaria.² Approaches to mitigate the risk of malaria in refugee populations include mass antimalarial treatment pre-departure or on arrival and screen and treat strategies for targeted groups.^{6–10} The US Centers for Disease Control (CDC) issued new guidelines in 2008 for refugees arriving from sub-Saharan Africa that recommend presumptive treatment either pre-departure or on arrival.¹¹ Recent guidelines from the Australasian Society for Infectious Diseases include malaria testing and treatment at both pre-departure and post-arrival health assessments, regardless of the country of origin.¹² These national strategies aim to reduce the risk of clinical malaria in the individual as well as prevent the autochthonous transmission of malaria in areas where the disease has been eradicated,^{13–22} although the latter concern is of limited relevance in Canada. The effectiveness of strategies based on screening

at arrival depends heavily on the proportion of refugees that receive systematic assessment by a clinic with specific expertise in refugee health. If malaria infection is not identified on arrival, the diagnosis is likely to be missed or delayed if patients present to unselected emergency or primary health-care settings. In a study of imported malaria in the Toronto area, 59% of cases that presented to non-hospital facilities were missed.²³ Within the refugee population, a Quebec study reported failure to request a malaria smear in 19% of symptomatic cases.²⁴

The reported prevalence of asymptomatic malaria in refugees screened post-arrival ranges from 2.4% to 31.8%, depending on the target population and the method of diagnosis.^{5,25–30} Over the past decade, the risk of asymptomatic refugees developing clinical malaria has been underscored by the occurrence of two local outbreaks in North America. In 1992, an outbreak was reported in North Carolina in a group of 402 Montagnard refugees from Indochina¹⁰; subsequent screening showed a malaria prevalence of 58% by microscopy. The second major outbreak was reported in Quebec after the resettlement of 224 refugees who arrived from Tanzania in 2000–2001^{5,24}; malaria was detected by polymerase chain reaction (PCR) in 18.8% of asymptomatic refugees.⁵

The Quebec study compared the sensitivity and specificity of current diagnostic methods for the detection of parasites within the asymptomatic refugee population. Dramatic increases in sensitivity were observed with PCR, which detected two times as many infections as the OptiMAL Rapid Detection Tests (RDTs) and 25% more than microscopy.⁵ Similar problems with sensitivity were reported in the study by Stauffer and others³¹ using the Binax NOW ICT kit, whereas poor specificity and positive predictive values of RDTs in this population were described by Causer and others³² using the OptiMAL kit. These issues with the performance of diagnostic tests within semi-immune, asymptomatic populations suggest that the prevalence of *Plasmodium* infections may have been underestimated in studies that used microscopy or RDTs for screening.

In this study, we determined the prevalence of malaria in a consecutive population of 324 asymptomatic refugees who underwent routine medical examination at the New Canadians

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Clinic in Edmonton, Canada during 2009–2010. We compared the sensitivity of real-time PCR with microscopy.

MATERIALS AND METHODS

Population. All government-sponsored refugees arriving in Edmonton spend their first 2 weeks in Reception House, a temporary residence that provides resettlement assistance. During this time, they receive a free medical examination at the on-site New Canadians Clinic (NCC). The NCC provides culturally and linguistically accessible expert assessment with acute and preventive primary care services to government-sponsored refugees. Refugees who underwent a medical exam at the clinic between March 2009 and March 2010 were included in the study. Patient demographics and clinical and laboratory data were obtained through review of medical charts and results from electronic laboratory information systems. Two clinic nurses collected and recorded patient information in all medical charts, thus ensuring consistency and quality data. The same laboratories performed diagnostic tests for all study specimens. Patient information was deidentified before PCR testing. Ethical approval for this study was obtained from the Health Research Ethics Board of the University of Alberta.

Medical examination and laboratory testing. Routine assessment included a medical history, history of past malaria diagnoses, country of origin, and country of refugee residence. All refugees were asked whether they received pre-departure presumptive therapy. Assessment also included a complete physical examination, age- and origin-appropriate screening, and appropriate vaccination.

Microscopic examination. Peripheral blood samples were collected in ethylenediaminetetraacetic acid (EDTA) tubes. One thick smear and one thin smear were prepared per patient as per routine clinic policy and stained with Giemsa. Thin films were examined with 10× and 50× objectives to scan for other larger organisms (i.e., microfilaria and protozoa) and with the 100× objective to screen for smaller parasites, including *Plasmodium*. If no parasites were seen, 200–300 fields of the thin film were examined for a total of 15 minutes. Approximately 100 fields of the thick film were then examined for a total of 5 additional minutes before the specimen was reported as negative. All slides were read by two different laboratory technologists and reviewed by a pathologist. Microscopy was performed immediately after medical screening and served as the standard for patient care.

Real-time PCR. PCR was performed retrospectively on deidentified samples at the end of the collection year. Samples were stored frozen before processing. DNA was extracted from 40 µL of whole blood diluted in 160 µL of deionized water. DNA extraction was performed using the PSS GC12 instrument (Precision System Science Co. Ltd., Pleasanton, CA) using the DNA 200 extraction protocol and kits (E2003). DNA was eluted into a 100-µL volume. Real-time PCR was performed as described under universal conditions using the ABI Taqman 7500 platform.³³ Samples were initially screened for *Plasmodium* using genus-specific primers Plasmo1 and Plasmo2, and the FAM6-labeled Plasprobe was used to detect a region of the *Plasmodium* 18S rRNA gene that is conserved across all five species.³⁴ The *Plasmodium* species present in the sample was determined using species-specific forward primers with Plasmo2 and species-specific probes as described.³³ Quantitation of DNA copy numbers was calculated based

on a standard curve generated with a plasmid containing the *Plasmodium* target gene that was amplified using the *Plasmodium* screening reaction described above.

RESULTS

Study population. During the study period, 350 refugees were examined at the NCC. Blood samples were not available from 26 subjects because of unrelated laboratory reasons, and they were excluded from the study. These subjects originated from Iraq (9), Congo (1), Afghanistan (6), Somalia (8), Pakistan (1), and Sudan (1). Therefore, a total of 324 refugees was included in the study (Table 1). Nearly one-half of the refugees were either from Asia (47%) or Africa (49%), and the remainder were from South America (4%). Nine were from countries essentially without malaria transmission. There were slightly more males (169) than females (155). Ages ranged from 6 months to 77 years, with a mean age of 25 years. The majority reported no previous episodes of malaria (70%). Only 11 refugees gave a history of pre-departure malaria treatment, all of whom transited through Uganda.

Malaria screening results. Thin and thick blood smears were analyzed, and all 324 were negative by microscopy. However, analysis of the same blood samples by real-time PCR identified 10 samples that were positive for *Plasmodium* DNA (Table 2), giving an overall prevalence of 3.1%. Infections were identified in subjects originating from three countries: Democratic Republic of Congo (DRC; four positives) through Uganda or Tanzania, Burma (five positives) through Thailand, and Liberia (one positive) through Sierra Leone. The country-specific prevalence among refugees by PCR is 10.5%, 7.9%, and 20%, respectively.

Speciation by real-time PCR identified three cases of *P. falciparum*, five cases of *P. vivax*, and one case of *P. ovale*. The species could not be identified in one sample because of the low level of infection (462 gene copies/mL blood). However, because this person was from the same family as one of the refugees infected with *P. falciparum* who originated from the DRC

TABLE 1
Demographics of the refugee population in this study

Country of origin	Number of subjects	Mean age in years (range)	M/F	Malaria episodes (%)	Pre-departure prophylaxis (%)	Country prevalence (%)
Afghanistan	5	22 (3–33)	4/1	0	0	0
Bhutan	29	32 (1–73)	19/10	24	0	0
Burma	63	29 (1–77)	26/37	38	0	8
Burundi	5	19 (3–41)	3/2	60	0	0
Colombia	12	29 (1–54)	7/5	0	0	0
Cuba*	4	22 (7–35)	1/3	0	0	0
DRC	38	20 (3–54)	21/17	74	21	11
Eritrea	9	26 (18–37)	7/2	44	0	0
Ethiopia	8	24 (8–39)	3/5	63	0	0
Iran	9	24 (8–35)	7/2	0	0	0
Iraq†	39	27 (0.5–72)	18/21	0	0	0
Liberia	5	23 (5–50)	1/4	100	0	20
Pakistan	2	34 (34)	2/0	0	0	0
Russia*	1	23	0/1	0	0	0
Rwanda	6	14 (6–22)	3/3	0	0	0
Serbia*	4	22 (3–30)	2/2	0	0	0
Somalia	63	20 (2–50)	34/29	16	0	0
Sudan	20	21 (1–47)	10/10	45	15	0
Tadjikistan	1	36	0/1	0	0	0
Yemen	1	44	1/0	100	0	0

* No malaria.

† Limited malaria (*P. vivax* only).

and transited through Uganda, it is likely that the unidentified infection is also *P. falciparum*. One additional family member tested positive for malaria during the first screen, but the level of infection was too low to enable confirmation or speciation. The individual with *P. ovale* also originated from the DRC but transited through Tanzania. The other *P. falciparum* infection was in a Liberian who transited through Sierra Leone. Of the *P. vivax* infections, all five were Burmese refugees who transited through Thailand.

The level of infection was quantified by PCR and ranged from 462 to 5.2×10^5 copies of parasite DNA/mL blood. These low-level infections are consistent with the absence of symptoms and negative microscopy in these subjects. None were known to be pregnant, all were human immunodeficiency virus (HIV) seronegative, and none were noted to have a palpable spleen. Hemoglobin levels and platelet counts were normal in all the infected refugees (Table 2). Pre-departure treatment was self-reported in three of the PCR-positive refugees arriving from Uganda but not in any of the other infected individuals. Of the 10 positive subjects, 7 reported previous episodes of malaria (2 of whom reported many episodes).

Follow-up malaria testing. One of the key objectives in screening refugees from malaria-endemic countries is to prevent the development of clinical malaria post-arrival. Although retrospective testing of the blood samples by PCR identified 10 subjects with parasite DNA, these cases had not been treated on arrival, because the microscopy tests performed as part of the initial routine screening were all negative. Treatment was not provided when PCR results were obtained, because the specimens were anonymized. However, the development of clinical malaria in our study subjects was investigated by examining medical records during the post-arrival year before PCR testing. None of the 324 refugees were diagnosed with malaria in Alberta within 6 weeks after the initial screen at the NCC. For 267 refugees, no malaria diagnosis was reported at least 6 months after arrival. This group included the subjects infected with the relapsing *P. vivax* species. In 10 PCR-negative subjects, malaria was investigated in subsequent clinical examinations between 2 weeks and 11 months after arrival, but blood smears were negative in all cases.

DISCUSSION

In this study, we screened a population of refugees who arrived in Edmonton over the course of 1 year. The prevalence of asymptomatic malaria in our study was 3.1% by real-time PCR, much lower than the rates reported in other studies.^{5,27,30} However, those studies selected specific populations, often

with common demographic or geographic criteria and from groups thought to be at high risk of malaria. Our study represents a consecutive sample (except for the few missed because of administrative error) of all government-sponsored refugees coming to Edmonton during the study period. With the exception of nine refugees who arrived from Russia, Cuba, and Serbia, all other refugees (97%) originated in countries that are endemic for malaria. However, many refugees transited through non-malarious countries or regions; the length of time spent in transit is unknown and could impact the rate of asymptomatic malaria observed in this study.

Pre-departure treatment was self-reported in 11 of 324 subjects and only in refugees arriving from Uganda, suggesting that this intervention has not been widely implemented. Furthermore, protection from this strategy is not 100%, because three of our PCR-positive subjects had received pre-departure treatment. We do not know the specific medication prescribed to these subjects and whether it complied with the recommendation by the CDC to use artemisinin-based combination therapy (ACT) for refugees arriving from sub-Saharan Africa. Recently, three cases of *P. falciparum* malaria were reported in Burundian refugees who arrived in the United States from camps in Tanzania.³⁵ One case received presumptive treatment with sulfadoxine-pyrimethamine, which is known to be ineffective because of widespread drug resistance, whereas the other two were treated presumptively with artemether-lumefantrine in accordance with the CDC recommendation.³⁵ In light of these cases and others, the International Organization for Migration, which administers pre-departure treatment to refugees to the United States, is now considering other issues that can impact the efficacy of treatment, including compliance with the treatment regimen, absorption of the medication, and potential for reinfection before departure.

Nine of the positive subjects originated from two countries: four from the DRC and five from Burma. The risk of asymptomatic malaria in refugees from sub-Saharan Africa has been extensively documented and forms the basis of the US enhanced health assessments that target this population for pre-emptive malaria therapy before entry to the United States.¹¹ However, data are currently lacking on the specific risk of relapsing malaria, particularly in non-African populations. Given the prevalence of infections with relapsing malaria identified in our study and increasing recognition of the potential seriousness of disease caused by *P. vivax*, the current guidelines for malaria screening in refugees in the United States and Australia would potentially fail to prevent relapsing malaria in a substantial subset of the refugee population. US guidelines for refugees advocate pre-departure

TABLE 2
PCR-positive refugees

Country origin	Country transit	Age	Sex	<i>Plasmodium</i> species	Hb	Platelets	Pre-departure prophylaxis	DNA copies/mL blood
DRC	Uganda	11	M	<i>P. falciparum</i>	126	128	Y	1.8×10^5
DRC	Uganda	22	M	<i>P. falciparum</i>	172	167	Y	3.6×10^3
DRC	Uganda	16	M	Undetermined	130	203	Y	4.6×10^2
DRC	Tanzania	11	M	<i>P. ovale</i>	140	303	N	2.0×10^4
Liberia	Sierra Leone	15	F	<i>P. falciparum</i>	143	252	N	8.7×10^4
Burma	Thailand	2	F	<i>P. vivax</i>	108	422	N	1.4×10^5
Burma	Thailand	53	F	<i>P. vivax</i>	129	202	N	4.1×10^3
Burma	Thailand	22	M	<i>P. vivax</i>	166	207	N	5.2×10^5
Burma	Thailand	17	M	<i>P. vivax</i>	165	254	N	3.3×10^5
Burma	Thailand	27	M	<i>P. vivax</i>	158	175	N	6.1×10^4

treatment with artemether-lumefantrine (AL) but only for refugees arriving from sub-Saharan Africa.¹¹ Although the cost of presumptive treatment in Africa is significantly lower than diagnosis and treatment in the United States,³⁶ this treatment would eliminate blood-stage parasites; however, it would not prevent future relapses from *P. ovale* or address the risk of malaria in refugees arriving from areas endemic for *P. vivax*. Pre-departure treatment with primaquine in areas with relapsing species is not logistically and financially feasible because of the need for prior screening for G6PD deficiency and the multi-day treatment regimen, which makes adherence problematic. In Australia, the favored policy is for mass treatment on arrival with AL without laboratory diagnosis.¹² Again, this would not protect refugees from relapse infections.

In Canada, there are currently no guidelines for malaria screening of refugees, and a number of gaps in malaria surveillance have been identified.³⁷ The Canadian Collaboration for Immigrant and Refugee Health recently developed recommendations for clinical preventative care related to infectious diseases, mental health, non-communicable chronic diseases, and women's health in refugees and immigrants.³⁸ However, malaria screening was not included within these recommendations. Our study provides timely data to support the reevaluation of Canada's approach to refugee screening for malaria. In 2006, the Canadian government began accepting the first of 3,900 Karen refugees from the camps along the Thai-Burmese border.² The five cases of asymptomatic *P. vivax* in our study were included in this group, and two previous cases of symptomatic malaria were diagnosed in a subset of Karen refugees who settled in the Toronto area.³⁹ Furthermore, the government recently announced an increase in the number of refugees that would be accepted in Canada by 2,500 per year.² This includes an estimated 5,000 refugees from Bhutan, another region in Southeast Asia where *P. vivax* is endemic, expected to arrive between 2009 and 2012.²

In light of the data presented here and with the expectation of more refugees arriving from Southeast Asia, expanded multicenter studies across Canada should evaluate the malaria risk from species other than *P. falciparum* as well as the most appropriate method of diagnosis and best screening strategy within this population. We assume that any PCR-positive individual is at risk of relapse, but we did not observe any clinical cases of relapse during the course of our study. Moreover, PCR for blood-stage parasites would not be expected to identify all individuals with latent liver-stage infection. More systematic observation could clarify the frequency of symptomatic relapses and impact on the health system within 1 year after arrival. Experience could also improve targeting of malaria surveillance, because risk varies widely among endemic areas. Finally, more complete information of this type would clarify questions of cost effectiveness of possible strategies in the management of new arrivals. The preferred strategy might certainly be different in settings where refugees are systematically assessed on arrival, which we feel is ideal, than in those where refugees continue to lack specialized services. These findings could also impact the guidelines of other host nations who are accepting refugees from malaria-endemic countries.

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REFERENCES

1. Fatoumata L-K, 2010. *Number of Forcibly Displaced Rises to 43.3 Million Last Year, the Highest Level since Mid-1990s*. Available at: <http://www.unhcr.org/4c176c969.html>.
2. Citizenship and Immigration Canada, 2008. *Canada's Refugee Resettlement Program: Overview, Challenges, and Priorities*. Available at: http://www.metropolis2008.org/pdf/20081028/workshops/w028-28_sadiq-kareem.pdf.
3. Citizenship and Immigration Canada, 2010. *Facts and Figures 2009—Immigration Overview: Permanent and Temporary Residents*. Available at: <http://www.cic.gc.ca/english/resources/statistics/menu-fact.asp>.
4. Stauffer WM, Weinberg M, 2009. Emerging clinical issues in refugees. *Curr Opin Infect Dis* 22: 436–442.
5. Ndao M, Bandyayera E, Kokoskin E, Gyorkos TW, MacLean JD, Ward BJ, 2004. Comparison of blood smear, antigen detection, and nested-PCR methods for screening refugees from regions where malaria is endemic after a malaria outbreak in Quebec, Canada. *J Clin Microbiol* 42: 2694–2700.
6. Miller JM, Boyd HA, Ostrowski SR, Cookson ST, Parise ME, Gonzaga PS, Addiss DG, Wilson M, Nguyen-Dinh P, Wahlquist SP, Weld LH, Wainwright RB, Gushulak BD, Cetron MS, 2000. Malaria, intestinal parasites, and schistosomiasis among Barawan Somali refugees resettling to the United States: a strategy to reduce morbidity and decrease the risk of imported infections. *Am J Trop Med Hyg* 62: 115–121.
7. Stauffer WM, Kamat D, Walker PF, 2002. Screening of international immigrants, refugees, and adoptees. *Prim Care* 29: 879–905.
8. Sullivan P, 2000. Poor conditions in refugee camps make malaria screening difficult: expert. *CMAJ* 163: 1036.
9. Slutsker L, Tipples M, Keane V, McCance C, Campbell CC, 1995. Malaria in east African refugees resettling to the United States: development of strategies to reduce the risk of imported malaria. *J Infect Dis* 171: 489–493.
10. Paxton LA, Slutsker L, Schultz LJ, Luby SP, Meriwether R, Matson P, Sulzer AJ, 1996. Imported malaria in Montagnard refugees settling in North Carolina: implications for prevention and control. *Am J Trop Med Hyg* 54: 54–57.
11. Stauffer WM, Weinberg M, Newman RD, Causer LM, Hamel MJ, Slutsker L, Cetron MS, 2008. Pre-departure and post-arrival management of *P. falciparum* malaria in refugees relocating from sub-Saharan Africa to the United States. *Am J Trop Med Hyg* 79: 141–146.
12. Murray RJ, Davis JS, Burgner DP, Hansen-Knarhoi M, Krause V, Biggs BA, Lemoh C, Benson J, Cherian S, BATTERY J, Paxton G, 2009. The Australasian Society for Infectious Diseases guidelines for the diagnosis, management and prevention of infections in recently arrived refugees: an abridged outline. *Med J Aust* 190: 421–425.
13. Brookes DL, Ritchie SA, van den Hurk AF, Fielding JR, Loewenthal MR, 1997. *Plasmodium vivax* malaria acquired in far north Queensland. *Med J Aust* 166: 82–83.

14. Centers for Disease Control and Prevention, 2000. Probable locally acquired mosquito-transmitted *Plasmodium vivax* infection—Suffolk County, New York, 1999. *MMWR Morb Mortal Wkly Rep* 49: 495–498.
15. Centers for Disease Control and Prevention, 2003. Local transmission of *Plasmodium vivax* malaria: Palm Beach County, Florida, 2003. *MMWR Morb Mortal Wkly Rep* 52: 908–911.
16. Layton M, Parise ME, Campbell CC, Advani R, Sexton JD, Bosler EMZJR, 1995. Mosquito-transmitted malaria in New York City, 1993. *Lancet* 346: 729–731.
17. Macarthur JR, Holtz TH, Jenkins J, Newell JP, Koehler JE, Parise ME, Kachur SP, 2001. Probable locally acquired mosquito-transmitted malaria in Georgia, 1999. *Clin Infect Dis* 32: E124–E128.
18. Mantel CF, Klose C, Scheurer S, Vogel R, Wesirow AL, Bienze U, 1995. *Plasmodium falciparum* malaria acquired in Berlin, Germany. *Lancet* 346: 320–321.
19. Ooi PL, Goh KT, Lee KS, 1997. Local transmission of *Plasmodium vivax* malaria in Singapore. *Ann Acad Med Singapore* 26: 588–592.
20. Brook JH, Genese CA, Bloland PB, Zucker JR, Spitalny KC, 1994. Malaria probably locally acquired in New Jersey. *N Engl J Med* 331: 22–23.
21. Sunstrum J, Elliott LJ, Barat LM, Walker ED, Zucker J, 2001. Probable autochthonous *Plasmodium vivax* malaria transmission in Michigan: case report and epidemiological investigation. *Am J Trop Med Hyg* 65: 949–953.
22. Mundy SB, White AC Jr, Hines JS, Marino BJ, Young EJ, 1996. Mosquito-transmitted malaria acquired in Texas. *South Med J* 89: 616–618.
23. Kain KC, Harrington MA, Tennyson S, Keystone JS, 1998. Imported malaria: prospective analysis of problems in diagnosis and management. *Clin Infect Dis* 27: 142–149.
24. Ndao M, Bandyayera E, Kokoskin E, Diemert D, Gyorkos TW, MacLean JD, St. John R, Ward BJ, 2005. Malaria “epidemic” in Quebec: diagnosis and response to imported malaria. *CMAJ* 172: 46–50.
25. Cherian S, Fagan JM, Thambiran A, Geddes J, Burgner D, 2006. Severe *Plasmodium falciparum* malaria in refugee children despite reported predeparture antimalarial treatment. *Med J Aust* 185: 611.
26. Chih DT, Heath CH, Murray RJ, 2006. Outpatient treatment of malaria in recently arrived African migrants. *Med J Aust* 185: 598–601.
27. Maroushek SR, Aguilar EF, Stauffer W, Abd-Alla MD, 2005. Malaria among refugee children at arrival in the United States. *Pediatr Infect Dis J* 24: 450–452.
28. Sheikh M, Pal A, Wang S, MacIntyre CR, Wood NJ, Isaacs D, Gunasekera H, Raman S, Hale K, Howell A, 2009. The epidemiology of health conditions of newly arrived refugee children: a review of patients attending a specialist health clinic in Sydney. *J Paediatr Child Health* 45: 509–513.
29. Stauffer WM, Rothenberger M, 2007. Hearing hoofbeats, thinking zebras: five diseases common among refugees that Minnesota physicians need to know about. *Minn Med* 90: 42–46.
30. Marangi M, Di TR, Mens PF, Martinelli D, Fazio V, Angarano G, Schallig HD, Giangaspero A, Scotto G, 2009. Prevalence of *Plasmodium* spp. in malaria asymptomatic African migrants assessed by nucleic acid sequence based amplification. *Malar J* 8: 12.
31. Stauffer WM, Newberry AM, Cartwright CP, Rosenblatt JE, Hanson KL, Sloan L, Tsukayama DT, Taylor C, Juni BA, 2006. Evaluation of malaria screening in newly arrived refugees to the United States by microscopy and rapid antigen capture enzyme assay. *Pediatr Infect Dis J* 25: 948–950.
32. Causer LM, Bishop HS, Sharp DJ, Flagg EW, Calderon JF, Keane V, Shah JJ, Macarthur JR, Maloney SA, Cetron MS, Bloland PB, 2005. Rapid malaria screening and targeted treatment of United States-bound Montagnard refugees from Cambodia in 2002. *Am J Trop Med Hyg* 72: 688–693.
33. Shokoples SE, Ndao M, Kowalewska-Grochowska K, Yanow SK, 2009. Multiplexed real-time PCR assay for discrimination of *Plasmodium* species with improved sensitivity for mixed infections. *J Clin Microbiol* 47: 975–980.
34. Rougemont M, Van SM, Sahli R, Hinrikson HP, Bille J, Jatton K, 2004. Detection of four *Plasmodium* species in blood from humans by 18S rRNA gene subunit-based and species-specific real-time PCR assays. *J Clin Microbiol* 42: 5636–5643.
35. Centers for Disease Control and Prevention, 2008. Malaria in refugees from Tanzania—King County, Washington, 2007. *MMWR Morb Mortal Wkly Rep* 57: 869–872.
36. Collinet-Adler S, Stauffer WM, Boulware DR, Larsen KL, Rogers TB, Williams DN, 2007. Financial implications of refugee malaria: the impact of pre-departure presumptive treatment with anti-malarial drugs. *Am J Trop Med Hyg* 77: 458–463.
37. MacLean JD, Demers A, Ndao M, Kokoskin E, Ward BJ, Gyorkos TW, 2004. Malaria epidemics and surveillance systems in Canada. *Emerg Infect Dis* 10: 1195–1201.
38. Pottie K, Tugwell P, Feightner J, Welch V, Greenaway C, Swinkels H, Rashid M, Narasiah L, Kirmayer L, Ueffing E, Macdonald N, 2010. Summary of clinical preventive care recommendations for newly arriving immigrants and refugees to Canada. *CMAJ*, doi:10.1503/cmaj.090313.
39. Canadian Immunization Committee, 2007. Initial health screening results for Karen refugees: a retrospective review. *CCDR* 33: 16–22.