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MICROBIOLOGY

Immunochromatographic antigen testing alone is sufficient to identify asymptomatic refugees at risk of severe malaria presenting to a single health service in Victoria

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Summary

Current screening guidelines for malaria in new refugees include a combination of thick and thin film examination and immunochromatographic antigen test (ICT). However, as the prevalence of malaria in our population has decreased due to changing refugee demographics, we sought to determine if an ICT alone can reliably exclude malaria in our asymptomatic refugee population. A retrospective analysis was conducted of all investigations for malaria performed from 1 August 2011 to 31 July 2013, including thick and thin blood film examination, BinaxNOW ICT, and external morphological and polymerase chain reaction (PCR) validation where applicable. Malaria was diagnosed in 45 of 1248 (3.6%) patients investigated, all of whom were symptomatic and the majority (71.1%) returned travellers. All 599 asymptomatic refugees screened were negative. Overall, 42 of 45 malaria cases were detected by the ICT; sensitivity 93.3% (95% CI 80.7–98.3%) and negative predictive value (NPV) 99.8% (99.2–99.9%). All 21 cases of *Plasmodium falciparum* and 20 of 22 cases of *Plasmodium vivax* were detected, giving a sensitivity of 100% (80.8–100%) and 90.9% (69.4–98.4%) respectively. Too few cases of *Plasmodium malariae* and no cases of *Plasmodium ovale* or *Plasmodium knowlesi* were diagnosed for adequate assessment to be carried out. These data suggest that full malaria screening in all asymptomatic refugees with the combination of thick and thin blood films and rapid antigen test may not be warranted. Alternative screening approaches should be considered, including the use of ICT alone, or limiting screening of asymptomatic refugees to only those originating from countries with high incidence of malaria.

Key words: Malaria, mass screening, refugees.

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INTRODUCTION

Malaria, a potentially fatal disease caused by infection by *Plasmodium* species, remains a significant cause of morbidity and mortality in many parts of the world, with hundreds of millions of infections and over one million deaths per year worldwide.[1] Five species of *Plasmodium* cause disease in humans: *P. falciparum, P. vivax, P. ovale, P. malariae* and *P. knowlesi*. Of these *P. falciparum* is responsible for nearly all malaria deaths.

The prevalence of disease reflects the distribution of its vector: the female *Anopheles* mosquito that transmits *Plasmodium* during a blood meal, predominantly found in tropical and subtropical areas. There are on going large-scale international efforts aimed at the worldwide eradication of malaria.

Australia has been free from endemic malaria since 1981,[1,2] however *Anopheles* mosquitoes persist in the Northern parts of the country (above latitude 19°S) and several locally acquired cases have been reported including a small outbreak of *Plasmodium vivax* in far Northern Queensland in 2002.[3,4]

While the majority of cases of malaria diagnosed in Australia are in returned travellers,[5] immigrants and refugees from malaria endemic regions are also recognised as important high-risk groups. In particular, cases of symptomatic *P. falciparum* malaria have occurred in recently-arrived refugees, particularly children, thus posing that risk of life-threatening illness might develop in people unfamiliar with the Australian health care system.[2] In this context, guidelines for the appropriate screening for new refugee arrivals have been developed.[1,6] These recommend that all refugees should be offered screening for malaria with both examination of thick and thin blood films and an antigen-based rapid detection test, as part of a comprehensive health assessment, ideally within one month of arrival in Australia. These guidelines were written primarily to address issues for refugees originating in sub-Saharan Africa, where malaria is a leading cause of mortality and morbidity in many countries; however, they included refugees from all regions.

According to the Department of Immigration and Citizenship (DIAC) settlement database,[7] 23,178 of the 74,859 (30.96%) people granted a humanitarian visa between 1 August 2008 and 31 July 2013 were settled in Victoria. Approximately 30% of Victorian refugees are settled in the southeast metropolitan region of Melbourne, particularly the City of Greater Dandenong and the City of Casey, which fall in the Monash Health catchment area.[8] In 2012 the number of refugees settling in Victoria increased from 4000 to around 7400 people per annum.[9] The demographic profile of the refugee population settling in Australia has also changed dramatically in recent years, with the largest proportion currently from Afghanistan and Iraq (15.4% and 18.9%, respectively[7]), countries with a lower burden of malaria compared with sub-Saharan Africa. World Health Organization data indicate in 2011 there were eight confirmed cases of malaria in Iraq, all of which were imported, with no cases of local transmission.[10] The incidence of malaria in Afghanistan in 2012 was 3.5 cases/1000 population, compared with >140 cases/1000 population in Ghana, >70 cases/1000 population in Uganda and 14.8 cases/1000

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population in Sudan over the same period.[10] *Plasmodium vivax* accounts for 98% of malaria infections in Afghanistan with *P. falciparum* accounting for just 2%. In contrast, *P. falciparum* represents nearly 95–100% of malaria infections in sub-Saharan African countries.[11]

In the setting of changing refugee demographics and increasing refugee numbers and subsequent increased demand on pathology services, we sought to review our current practices of performing a thick and thin film as well as an immunochromatographic test (ICT) on all refugees who have resettled within the Dandenong–Casey district. Specifically, we sought to establish the overall prevalence of malaria within our screened population, particularly asymptomatic refugees settled in our catchment area, and determine whether ICT alone (without the labour-intensive performance of thick and thin blood film examination) had a negative predictive value adequate to reliably exclude malaria in this latter group.

MATERIALS AND METHODS

A retrospective analysis was conducted of all investigations for malaria performed at Monash Medical Centre over a 2 year period from 1 August 2011 to 31 July 2013, including rapid antigen testing, thick and thin blood film examination as well as the results of external morphological and polymerase chain reaction (PCR) validation carried out at the Victorian Infectious Diseases Reference Laboratory (VIDRL) where applicable. Rapid antigen testing was performed using the BinaxNOW ICT assay (Alere, USA), which targets the histidine-rich protein II (HRPII) antigen specific to *Plasmodium falciparum* and a pan-malarial antigen, common to all malaria species capable of infecting humans (BinaxNOW product information sheet). Venous blood, 15 μL collected in an EDTA blood tube, is added to the sample pad (capillary whole blood can be used, however is not in our laboratory). The test is read 15 min after closing the device (and following the addition of reagent on two occasions). A positive result is depicted visually as either a T1 line indicating *P. falciparum*, a T2 line indicating non-*P. falciparum* or both in mixed infections. The control line (C) will appear on all valid tests. Thin and thick blood films were prepared from blood collected in EDTA tubes, with May-Grunwald-Giemsa stain used for the thin film and commercially obtained 1% polychrome methylene blue in 0.15 M phosphate buffer and 0.2% eosin in 0.15 M phosphate buffer stains (Field's stains A and B, respectively; Histolab) for thick films. Both were reviewed by a laboratory scientist and a haematologist or haematology registrar (requires approximately 30 min in total for adequate assessment). The medical histories of patients who returned a positive antigen screen and/or thick and thin film result were reviewed to determine the nature of presentation (symptomatic versus asymptomatic screening investigation), the demographic details of the patient (refugee, returned traveller or other) and to confirm the presence or absence of disease. The request form alone was reviewed in patients negative for malaria to determine the nature of presentation. Patients were determined to be asymptomatic refugees only if the request clearly identified them as such (patients were considered symptomatic if the requesting clinician had documented any symptoms that could be consistent with malaria, even if the form stated 'routine health assessment/screening').

Patients were deemed to be malaria positive according to the presence of malarial parasites on the thick and thin film (and PCR validation when available). Subjects were considered malaria negative if the thick and thin films were negative and the patient did not represent for testing to our institution(s) in the following 3 months.

The sensitivity and specificity, negative and positive predictive values, and their corresponding 95% confidence intervals (95% CI) were calculated.

RESULTS

A total of 1524 malaria investigation encounters (consisting of the combination of rapid antigen test plus thick and thin blood film examination) were identified between 1 August 2011 and 31 July 2013. Two hundred and seventy-six were found to be duplicates/triplicates, leaving 1248 patients investigated with rapid antigen test and thick and thin films. Of these, 599 patients (48%) were asymptomatic refugees routinely screened, while 649 patients (52%) were symptomatic. The mean age of subjects was 30.8 years and 68% were male. A final diagnosis of malaria was made in 45 patients (3.6%) (Tables 1 and 2); 21 patients (46.7%) with *P. falciparum*, 22 patients (48.9%) with *P. vivax* and two patients with *P. malariae*. No cases of *P. ovale* or *P. knowlesi* were diagnosed during the time period. All 45 cases of malaria diagnosed were in symptomatic patients and 43 (95.6%) of these were from bloods drawn in the emergency department. The majority of cases (71.1% overall) were in returned travellers. This proportion was higher in patients with *P. falciparum* (90.5%), all of whom had returned from sub-Saharan Africa, than *P. vivax* (54.5%), with 40.9% of cases of the latter diagnosed in recent immigrants and the majority of cases acquired in the Indian subcontinent (predominantly India and Pakistan). One diagnosis of *P. vivax* was confirmed in a refugee from Pakistan who had been granted humanitarian entry six months earlier: no previous testing had been performed. The remaining 1195 (95.8%) patients were found to be negative for both rapid antigen test and thick and thin film examination, and did not subsequently represent for testing within the specified 3 months. No cases of malaria were diagnosed in the 599 routine (asymptomatic) refugee screening episodes.

Rapid antigen test was positive in 47 patients, of whom malaria was confirmed in 42, with five false positive *P. falciparum* results (negativity confirmed by external morphological and PCR evaluation). Four of the five instances were in asymptomatic refugees with no documentation of previous/recent malaria infection or treatment on the pathology request form. One false positive result was in a returned traveller from Malaysia presenting to the emergency department with fever and malaise. Three cases of malaria were not detected by the rapid antigen test; two cases of *P. vivax* in symptomatic returned travellers (Cambodia and India) presenting to the emergency department, and one case of *P. malariae* in a symptomatic patient who had immigrated from Sierra Leone with a past history of malaria treated on several occasions. All three cases were associated with a low parasite count: 0.05%, 0.01% and <0.01%, respectively. The overall sensitivity, specificity and negative predictive value for the rapid antigen test for all malaria species were 93.3% (95% CI 80.7–98.3%), 99.6% (99–99.8%) and 99.8% (99.2–99.9%), respectively (Table 3). Sensitivity was slightly lower for *P. vivax* (90.9%); however, the ICT did not miss a single case of *P. falciparum,* resulting in high accuracy indices for this species (Table 3). Too few cases of *P. malariae* and no cases of *P. ovale* or *P. knowlesi* were diagnosed for adequate assessment to be carried out.

Table 1 Breakdown of malaria diagnoses from thick and thin films

	Symptomatic	Asymptomatic refugees	Total
No. of patients	649	599	1248
P. falciparum	21	0	21
P. vivax	22	0	22
P. malariae	2	0	2
Total malaria	45	0	45
Negative	604	599	1203