Relapsing vivax malaria despite chemoprophylaxis in two blood donors who had travelled to Papua New Guinea

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Two Australian blood donors were diagnosed with relapsing Plasmodium vivax *malaria 5 and 15 months, respectively, after their most recent travel to a malaria-endemic country. Common features included travel to Papua New Guinea (specifically, the Kokoda Trail); full compliance with recommended malaria chemoprophylaxis; and negative results on malaria antibody testing at the time of donation. Although all fresh blood components from the two donors issued on the basis of these negative results were recalled before transfusion, these cases underscore the increased potential for relapse of* P. vivax *in donors returning from malaria-endemic countries, as well as the inability to identify the potential for relapse using current malarial screening tests.* **(MJA 2010; 192: 471-473)**

Clinical record

Patient 1

A 63-year-old man donated blood to the Australian Red Cross Blood Service (the Blood Service) 127 days after returning from an organised trek on the Kokoda Trail, Papua New Guinea (PNG). An enzyme immunoassay (EIA) for *Plasmodium falciparum* and *Plasmodium vivax* antibodies was non-reactive at donation. Twenty-six days later, the donor's wife notified the Blood Service that her husband had been admitted to hospital with fever and rigors; he was subsequently diagnosed with *P. vivax* malaria based on visible *P. vivax* parasites in a blood film (13 800 parasites/μL), and a positive result on a (non-*P. falciparum*) malarial antigen test (Box).

He was successfully treated with primaquine. After his discharge from hospital, he was interviewed by a Blood Service medical officer and reported that he had no history of malaria; complied fully with malarial prophylaxis (doxycycline 100 mg daily, starting 2 days before entering PNG and finishing 14 days after returning home); had 2 months of lethargy after his return and developed febrile symptoms 20 days after donation; and had not travelled outside Australia after his donation. He also reported that seven of his 15 trekking companions were diagnosed with malaria after the trek.

Patient 2

A 39-year-old man donated blood 13 months after returning from PNG. He had made three previous donations, the first 5 months after returning from PNG, all testing negative for malarial antibodies. During the trip, the donor walked the Kokoda Trail and complied fully with prophylaxis (atovaquone–proguanil, 250 mg/100 mg daily, starting 1 day before and finishing 7 days after travel). He did not recall any recognisable malarial symptoms during the trek, but noted that a trekking companion had malaria on return to Australia. Approximately 66 days after his latest donation, the donor notified the Blood Service that he had recently been admitted to hospital with a febrile illness subsequently diagnosed as non-*P. falciparum* malaria; *P. vivax* was later confirmed from the blood film (0.5% parasitaemia) (Box). He was treated with atovaquone–proguanil (250 mg/100 mg four times daily for 3 days), made a full recovery and was discharged from hospital. He later confirmed that he had not travelled outside Australia after his return from PNG.

Results of diagnostic testing of the two donors are summarised in the Box.

Discussion

Malaria is transmitted predominantly through the bite of an infected female *Anopheles* mosquito, but, because the parasite invades and multiplies in red blood cells (RBCs), it can also be transmitted by transfusion of any blood component containing RBCs.[1] Although malaria is not endemic in Australia, between 500 and 900 cases are notified annually, constituting an ongoing risk of transfusion-transmitted malaria (TTM).[2] However, this risk is well controlled — the most recent recorded case of TTM occurred in 1991, involving a donor infected with *P. falciparum*.[3] Notably, the transfusion recipient died, an outcome observed in about 10% of TTM cases caused by *P. falciparum*.[4]

To minimise TTM risk in Australia, each potential donor is asked questions to elicit if he or she has spent time in malaria-endemic countries or is at risk of having had malaria. Those identified at risk of infection are tested with an EIA for *P. falciparum* and *P. vivax* antibodies (Malaria EIA, NewLabs, Newmarket, United Kingdom). When the EIA is negative, the RBC component of the donation is considered for transfusion if at least 4 months have elapsed since the donor's risk exposure. The 4-month waiting period minimises the possibility of false-negative test results that arise from testing within the putative 7–14-day "window period" before a complete antibody response is detectable.

The Blood Service implemented serological testing of donors for malaria in 2005, replacing the previous strategy of restricting manufacture of fresh blood components from at-risk donations (ie, donations from people who had visited malaria-endemic countries in the previous 12 months or from those who had resided in an endemic country for a cumulative total of 6 months or more in the previous 3 years).[5] While effectively minimising the risk of TTM, the older strategy resulted in significant loss of transfusible components (estimated in 2001 at about 5% of the Blood Service's annual RBC production). This loss was considered unacceptable in the face of mounting demands on supplies of blood and blood products. The feasibility of serologically testing at-risk donors to reduce the period of restriction and consequent component loss had been established in Europe, where serum tests had been implemented in France[6] and the UK.[7] Furthermore, the use of a validated antibody test to reinstate donors after a minimum of 4 months is permitted by the applicable regulatory standard used by Australia.[8]

The predominant TTM risk is associated with so-called "semi-immune" individuals born or resident for extended periods in

Blood testing for relapsing *Plasmodium vivax* in the two patients

	Place	Time	Pf/Pv antibody EIA*	Pf/Pv antigen ICT†	PCR‡	Blood film
Patient 1						
Sample 1	Blood Service	At donation (127 days after return from PNG)	Non-reactive (S/Co 0.28)	Not tested	Not tested	
Sample 2	Blood Service	At hospital admission (26 days after donation)	Reactive (S/Co 4.2, 3.6§)	Positive Pf band negative Pan malaria positive	Plasmodial DNA detected (4415 parasites/μL)	Pv parasites visible (13 800 parasites/μL)
Sample 3	Blood Service	In hospital after treatment (33 days after donation)	Reactive (S/Co 4.8, 3.7§)	Negative	DNA not detected	
Patient 2						
Sample 1	Blood Service	At donation (13 months after return from PNG)	Non-reactive (S/Co 0.27)	Not tested	Not tested	
Sample 2	Hospital	At hospital admission (66 days after donation)	Reactive (S/Co > 19, 18.8§)	Positive Pf band negative Pan malaria positive	Plasmodial DNA detected (1861 parasites/μL)	Pv parasites visible (0.5% parasitaemia)

Blood Service = Australian Red Cross Blood Service. EIA = enzyme immunoassay. ICT = immuno-chromatographic test. PCR = polymerase chain reaction. PNG = Papua New Guinea. Pf = *Plasmodium falciparum*. Pv = *Plasmodium vivax*. S/Co = sample-to-cut-off ratio (this test relates to the level of antibodies in each sample compared with a predetermined cut-off level).
* Malaria EIA, NewLabs, Newmarket, United Kingdom. † Binax NOW Malaria assay, Inverness Medical, United States. ‡ artus malaria RG PCR, Qiagen, Hilden, Germany.
§ Two values were reported because repeat testing usually requires a double check to ensure accuracy. ◆

malaria-endemic countries.[9] In the semi-immune person, the infection may take the form of an "equilibrium" in which very low parasite loads (generally undetectable by microscopy, and even polymerase chain reaction [PCR] testing) coexist with malarial antibodies without producing overt symptoms. Most recently recorded cases of TTM have resulted from the failure to detect and exclude the RBC-containing components of donations from semi-immune donors infected with *P. falciparum*.[4,6] When parasite loads are extremely low, even the best plasmodial PCR assay is unable to interdict all potentially infectious donations, given that a transfusion contaminated with as few as 10 parasites can transmit infection.[1] This underpins the rationale for antibody-based testing as the optimum donor-screening test, underscored by the Australian regulatory standard's explicit exclusion of the use of molecular tests to screen donors.[8]

Another potential TTM risk is that both *P. vivax* and *Plasmodium ovale* have a hypnozoite form that can persist in the liver and lead to relapses after successful treatment of the primary infection.[10] The interval from primary infection to relapse ranges from 1 month to 4 years.[11,12] Chemoprophylactic agents are prescribed based on their efficacy against blood-stage parasites, but they are, with the exception of terminal (ie, postexposure) primaquine prophylaxis, ineffective against hypnozoites.[13] Thus, they cannot prevent relapse but may delay its onset.[11]

Our two cases were strikingly similar, and the evidence strongly implicates PNG (specifically, the Kokoda Trail) as the site of primary infection for both. This is consistent with published evidence showing that, among non-immune travellers and soldiers returning to Australia, those from PNG and neighbouring countries were more likely to have relapsing malaria.[13-15]

These two cases of apparent relapse associated with *P. vivax* malaria in non-immune donors are, to our knowledge, the first reported cases detected by antibody testing. Further, they were unexpected because the perceived TTM risk is predominantly associated with *P. falciparum* infected semi-immune individuals. This either indicates that malarial antibody titres in individuals harbouring hypnozoites decline to undetectable levels 4 months or more after infection or, alternatively, that levels of parasitaemia during a "suppressed" primary infection may be too low to stimulate a significant antibody response. Thus, the current testing strategy cannot be relied on to discriminate donors at risk of relapse. This should not be seen as a reason to reject antibody testing per se, as no other available laboratory test for parasitaemia can reliably identify these individuals. Notably, the strongly positive EIA results in samples taken from the two donors at the time of admission to hospital support a robust antibody response and are consistent with the high sensitivity of the Newmarket EIA observed in samples taken from patients with acute disease.[1]

The TTM risk posed by relapsing *P. vivax* infection occurs during the asymptomatic period because symptomatic individuals would be prevented from donating. Although not precisely known, this period is expected to be short, perhaps several days. The TTM risk posed by our two patients was contained. One RBC component had been issued (Patient 1), based on its non-reactive malarial antibody test result, 20 days before symptom onset. This was successfully recalled, avoiding any potential risk to recipients. Considering the 20-day period between donation and symptom onset, it is highly likely that the donation was made before the onset of parasitaemia and, therefore, the RBC component would not have been infectious. No fresh blood components from Patient 2 were issued.

What do these two cases suggest about the safety of the current Blood Service testing strategy? They certainly raise concern given that their late detection could have resulted in transfusion of potentially infectious blood components. However, such cases appear to be exceedingly rare — these are the only two reported in

Australia in more than 4 years of testing. Furthermore, the contribution of such cases to overall TTM risk appears to be minute, as no TTM cases have been reported since testing began We recently published a comprehensive review that supports the existing strategy — it concluded that the current TTM risk was less than 1 in 3.3 million and had not measurably increased after implementing the testing strategy.[16] Importantly, the Blood Service achieved this level of safety while recovering over 70 000 fresh blood components that would otherwise have been unavailable annually. Nonetheless, recognising the limitations of the testing strategy and the imperative to reduce recipient risk where possible, the Blood Service is considering mitigation options. As these two cases suggest travel to PNG carries a disproportionately high risk, the Blood Service is considering the feasibility of excluding donors returning from PNG from the testing protocol, and restricting fresh component production from their donations for appropriate periods of time.

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Competing interests

None identified.

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