Investigation of a case of suspected transfusion-transmitted malaria

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BACKGROUND: Transfusion-transmitted malaria (TTM) is a rare occurrence with serious consequences for the recipient. A case study is presented as an example of best practices for conducting a TTM investigation.

CASE REPORT: A 15-year-old male with a history of sickle cell disease developed fever after a blood transfusion. He was diagnosed with *Plasmodium falciparum* malaria and was successfully treated. The American Red Cross, New York State Department of Health, and the Centers for Disease Control and Prevention investigated the eight donors who provided components to the transfusion. The investigation to identify a malaria-positive donor included trace back of donors, serologic methods to identify donor(s) with a history of malaria exposure, polymerase chain reaction (PCR) testing, microsatellite analysis to identify the parasite in a donor and match its genotype to the parasite in the recipient, and reinterview of all donors to clarify malaria risk factors.

RESULTS: One donor had evidence of infection with *P. falciparum* by PCR, elevated antibody titers, and previously undisclosed malaria risk factors. Reinterview revealed that the donor immigrated to the United States from Togo just short of 3 years before the blood donation. The donor was treated for asymptomatic low parasitemia infection.

CONCLUSION: This investigation used standard procedures for investigating TTM but also demonstrated the importance of applying sensitive laboratory techniques to identify the infected donor, especially a donor with asymptomatic infection with low parasitemia. Repeat interview of all donors identified as having contributed to the transfused component provides complementary epidemiologic information to confirm the infected donor.

Malaria is a vector-borne disease wherein *Plasmodium* parasites infect and lyse red blood cells (RBCs) resulting in an acute febrile illness.[1] In 2016, the estimated global burden of malaria was 216 million cases worldwide, with the majority of malaria deaths due to *Plasmodium falciparum*.[2] Increases in immigration from, and travel to, endemic areas facilitate importation of malaria to nonendemic countries.[3] In the United States there are approximately 1700 cases of imported malaria per year, an increase since the 1970s.[4] Typically, two-thirds of these cases are *P. falciparum* while the second most common species identified is *P. vivax*. The vast majority of cases are imported, with 99% of all infections presenting within 1 year of return travel to, or arrival in, the United States from a malaria-endemic region.[4] A small number are congenital, transfusion-related, needlestick-associated, or otherwise undetermined.[5–7] The estimated incidence of

ABBREVIATIONS: ARC = American Red Cross; DHQ = donor history questionnaire; IFA(s) = immunofluorescence assay(s); NYSDOH = New York State Department of Health; PET = photo-induced electron transfer; TTM = transfusion-transmitted malaria.

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transfusion-transmitted malaria (TTM) in the United States is less than one case per million units of blood collected.[8] From 2000 to 2017 there were 11 cases total, eight of which were due to *P. falciparum* (National Malaria Surveillance System. Division of Parasitic Diseases and Malaria, unpublished data, 2017).[5,6,9–14]

To protect the US blood supply from malaria, blood centers rely on screening questionnaires and deferral of donors who have had possible exposure to malaria within a specified timeframe. The Food and Drug Administration (FDA) recommends a 3-year deferral for donors who are former residents of malaria-endemic countries and for donors who have ever had malaria. Residents of the United States who have traveled to malaria-endemic countries are deferred for 1 year following their return (Table S1, available as supporting information in the online version of this paper).[8,15,16] Early malariotherapy studies demonstrated that the majority of malaria infections among this nonimmune patient population cleared within 1 year despite nontherapeutic doses of an antimalarial medication. Among those who had been inoculated with the relapsing parasite *P. ovale*, there were no patients with parasitemia at 3 years. This forms the rationale for the 3-year deferral period.[17,18] While rare, there is evidence that parasites of all species can persist beyond 3 years.[8,19] Donor deferral policies must balance the need to limit exposure to transmissible organisms against the need to maintain a large enough pool of donors to meet the transfusion needs of the population. The number of annual travel deferrals in the United States under the current screening guidelines is estimated to be greater than 150,000, whereas an estimated 6.8 million volunteers successfully donate blood each year.[20,21] Approximately 70% of cases of TTM occur due to failure to defer a donor during the screening interview, often because the donor incorrectly completes the questionnaire.[22]

Although it is a rare event, TTM has potentially deadly consequences for recipients, and it is important to have clear procedures in place to investigate and identify malaria-positive donors. A case is presented of a transfusion-transmitted *P. falciparum* infection and the ensuing investigation.

CASE REPORT

A 15-year-old male with a history of sickle cell disease and no history of travel presented to the emergency department with chest pain and malaise. He was receiving monthly erythrocytopheresis and was last transfused 24 days prior. After a negative evaluation for acute chest syndrome, he was discharged. Four days later, he developed a fever of 37.7°C and back pain. Preerythrocytopheresis samples were collected at that time that showed 118×10^9/L platelets, 22×10^9/L white blood cells, with ring trophozoites observed in the RBCs. Blood smear microscopy identified *P. falciparum* with parasitemia of 0.5%. A whole blood sample was sent to the state public health laboratory for real-time polymerase chain reaction (RT-PCR) testing which confirmed infection with *P. falciparum* and was negative for *Babesia microti*. The patient had no symptoms of severe malaria and was successfully treated with an oral regimen of atovaquone-proguanil.

Suspecting TTM, the health care facility notified the blood provider (American Red Cross [ARC]) and the New York State Department of Health (NYSDOH). For assistance with the investigation, ARC contacted the Centers for Disease Control and Prevention (CDC). The three agencies coordinated the following investigation that included identification of donors who contributed to the transfused component, quarantine of donated blood products, collection and testing of samples, and repeat interviews of the involved donors (Table 1).

The ARC identified eight donors (Donors A-H) who provided the transfused blood products. To protect the blood supply from products related to the donors under investigation, ARC placed a deferral on all involved donors for the duration of the investigation and traced any remaining in-date cellular blood components from these donors for retrieval, per FDA guidelines.[15] Only one distributed cellular cocomponent, from Donor H, was unexpired (Table S2, available as supporting information in the online version of this paper). ARC notified the facility as part of its investigation, but the component had already been transfused. All eight donors contributed additional acellular products that were distributed, and these did not require quarantine or retrieval.

RBC component segments were available from five of eight transfused units, according to the date of transfusion and the retention-time policy for the facility. Segments had undergone processing to include filtration and addition of stabilizing agents, minimizing the volume of donor plasma, and diluting the residual antibody. ARC contacted all donors of whom five consented to the collection of a follow-up sample. Altogether, three donors had both segments and follow-up samples available for testing. Two donors had only segments available, one of which contained insufficient volume to complete testing. Two donors had only follow-up samples available. One donor was lost to follow-up (Table 2).

Immunofluorescence assays (IFA) against *P. falciparum, P. vivax*, and *P. malariae* parasites were performed on samples from the six donors with adequate samples (Table 2).[23] Antibody titers of 1:64 or more was defined as a positive reaction. Donor A had multiple positive samples. Titers for Donor A were 1:64 for *P. falciparum* in the segment sample and 1:1024 for *P. falciparum* in the subsequent follow-up samples; the difference in magnitude of the results was likely due to antibody dilution in the segment. A follow-up sample from Donor A was positive for *P. vivax* (1:256). This was most likely due to cross-reactivity related to elevated *P. falciparum*

TABLE 1. Actions, roles, and responsibilities during TTM case investigation*

Actions	Responsible agencies
Confirm recipient infection and TTM event	
Diagnose malaria in recipient (blood smear and/or PCR)	HCF, PHL, or CDC if assistance needed
Report the case of malaria	HCF to ARC and SHD; HCF or SHD to CDC
Confirm recipient's infection (PCR)	HCF, PHL, CDC
Confirm recipient's travel history	HCF, ARC, SHD
Secure malaria treatment for the recipient	HCF
Secure blood products from involved donors	
Trace back blood products to identify donors	ARC
Implement deferrals of donors involved in TTM event	ARC
Quarantine remaining blood products from involved donors	ARC
Conduct TTM investigation	
Collect any immediately available donor specimens	ARC
Review initial screening questionnaires and donor information	ARC
Contact donors: • Request follow-up specimens • Conduct in-depth interviews	ARC
Forward samples for testing	From ARC to PHL to CDC
Perform testing of donor specimens (e.g., RT-PCR, nested-PCR, microsatellite, serology)	PHL, CDC
Close TTM investigation	
Coordinate and provide malaria treatment for positive donor	ARC, SHD
Disseminate test results to all partners	ARC, SHD, PHL, CDC
Clear deferrals and quarantined products from confirmed negative donors	ARC

* State Health Department, CDC, and ARC jointly determine which agency is in the best position to coordinate efforts and manage results database.
HCF = health care facility; PHL = public health laboratory; SHD = state health department.

titers rather than a positive *P. vivax* reaction (see PCR testing results below). All other donors tested with IFA were negative.

Five segments and five follow-up samples were tested using RT-PCR at the NYSDOH public health laboratory. All samples, including those from Donor A, were negative. Samples were forwarded to the CDC for additional testing. CDC uses photo-induced electron transfer (PET)-RT-PCR; cycle threshold values of 40 and below indicate a positive PET-PCR result.[24] Segments for Donor A resulted in a borderline value of 40.9, suggesting either a very low parasite-level infection or a negative result. All donor samples were tested using the more sensitive nested-PCR, which was positive for *P. falciparum* in Donor A only.[25] A follow-up sample for Donor A was also tested by PET-PCR and nested-PCR, and results were negative. Analysis using seven neutral microsatellite markers was attempted to match the recipient's parasite genotype with that of Donor A, but none of these markers were amplifiable in the Donor A samples, likely due to low-level parasitemia. An alternative genotyping method was performed which involves the amplification of three loci in the MSP-1 and two loci in the MSP-2 genes using nested-PCR. One marker at MSP-1 and one at MSP-2 were shown to be of similar size in the recipient and Donor A samples. The results suggest that parasites found in the recipient were similar to those from Donor A (Table 2). However, amplification of more than one loci per gene is preferable to indicate a definitive match between donor and recipient.

In parallel to the laboratory investigation, ARC reinterviewed five donors with specific questions regarding malaria risk factors (Table 3). The three donors who did not provide follow-up samples were unavailable for reinterview. On the initial donor history questionnaire (DHQ), all donors denied transfusion or transplants in the past 12 months, accidental needlestick or other needle use, and history of past malaria infection. Donor A had denied being outside of the United States or Canada in the past 3 years and ever having had malaria on the DHQ, which was administered in March 2017. Upon reinterview, Donor A reported having been born in Togo, a malaria-endemic country, and immigrating to the United States in May 2014, which was within the 3-year deferral period. The donor reported three previous episodes of malaria but could not remember the dates; history of malaria infection is also subject to a 3-year deferral. Donor A reported a history of a blood transfusion in infancy and denied needle sharing or recent hospital or laboratory exposures. Reasons for Donor A's nondisclosure on the DHQ were not obtained during the interview, but it was believed that they responded to the DHQ truthfully at the time of donation.

After the completion of the investigation, ARC removed deferrals from those donors who had no laboratory evidence of current malaria infection. Donors who could not be followed up remain deferred with the ARC system. ARC and the NYSDOH coordinated case management for Donor A to receive appropriate treatment in accordance with CDC guidelines.[27]

TABLE 2. Investigation findings and results of tested donors and recipient by RT-PCR, PET-PCR, nested-PCR, genetic marker analysis, IFA, and reinterview*

	RBC component segments						Follow-up sample					
	RT-PCR	PET-PCR	Nested-PCR	Genetic marker MSP-2	Genetic marker MSP-1	IFA	RT-PCR	PET-PCR	Nested-PCR	IFA	Donor status (last donation)	Reinterview findings
Recipient	23.9	22.78	*P. falciparum* positive	ICI ~300 bp	MAD20 ~180 bp							
Donor A	Negative	40.9	*P. falciparum* positive	ICI ~300 bp	MAD20 ~180 bp	ratios: 1:64 <1:64 1:1024				ratios: 1:64 <1:64 1:1024†	First time	Born in West Africa, moved to United States less than 3 years before donation; multiple malaria infections as a child; history of childhood transfusion
							Negative§	43.65‡ Negative§	Negative‡	ratios: 1:64 <1:64 1:1024§		
Donor B											Repeat (01/2017)	
Donor C							Negative			ratios: 1:64 <1:64 1:1024	Repeat (01/2014)	Last travel with potential malaria exposure 1 + years before donation; no past history of malaria
Donor D							Negative			ratios: 1:64 <1:64 1:1024	Repeat (01/2017)	Last travel with potential malaria exposure 10 + years before donation; no past history of malaria
Donor E	Negative	Negative	Negative			ratios: 1:64 <1:64 1:1024					Repeat (11/2016)	
Donor F	Negative										First time	
Donor G	Negative	Negative	Negative			ratios: 1:64 <1:64 1:1024	Negative	Negative	Negative	ratios: 1:64 <1:64 1:1024	Repeat (01/2017)	No history of travel; no past history of malaria
Donor H‖	Negative	Negative	Negative			ratios: 1:64 <1:64 1:1024	Negative			ratios: 1:64 <1:64 1:1024	Repeat (09/2016)	Last travel with potential malaria exposure 10 + years before donation; no past history of malaria

* Blood products under review were donated in March 2017. Threshold for positive PET-PCR result < 40.
† Donor A pretreatment follow-up Sample 1.
‡ Donor A pretreatment follow-up Sample 2.
§ Donor A, posttreatment follow-up sample.
‖ Additional components from Donor H were transfused to another recipient before the start of the investigation. A follow-up sample tested negative for malaria, and ARC informed the facility at the close of the investigation that the recipient of those blood products did not require further monitoring.
MSP-1/2 = merozoite surface protein-1/2; Pf = *P. faliciparum*; Pm = *P. malariae;* Pv = *P. vivax.*

TABLE 3. Example topics and questions to include during in-depth interview to determine extended travel history and malaria exposures for all donors during investigation

What is the donor's extended travel history?
Consider including travel history beyond 1 year before donation.
Potentially review the donor passport, if possible, to verify travel history and dates.
Sample questions:
Where outside of the United States have you *ever* traveled before the date of donation?
Has the donor ever lived in a malaria-endemic country?
"Lived in" is defined as 5 or more years, but investigator can consider shorter periods of time.
Sample questions:
Where were you born?
Did you grow up or spend more than 1 year outside the United States? Where and for how long?
Has the donor had malaria infection before?
If yes, consider when and where it was acquired, what were the treatment details, and was primaquine treatment taken to prevent relapse if indicated.
Sample questions:
Have you ever had malaria?
Have you ever had an undiagnosed febrile illness before date of donation?
Has the donor had any unusual exposures to malaria?
Unusual exposures may include transfusion, needle sharing, and hospital or laboratory exposures.
Sample questions:
Have you recently been hospitalized or undergone a transfusion?
Are you currently employed? What is your profession?
Have you ever shared needles for tattooing or substance use?

DISCUSSION

In a TTM investigation, the first step is to identify all donors who contributed transfused component, defer those donors, and identify in-date products for retrieval. While retention segments from the time of donation and other remaining products could be tested for evidence of malaria, the processing of blood product can dilute parasite or antibody content. Although parasitemia can decrease over time, collecting follow-up samples can still be useful.

Donors with asymptomatic low parasitemia have been most frequently associated with TTM; therefore, molecular diagnostic techniques (e.g., PCR) and serology (detection of antibody responses) are the best methods due to their high sensitivities for detecting malaria parasites and exposure to malaria, respectively. Availability of malaria-specific PCR can vary by laboratory, and it is less sensitive than serology when the levels of parasitemia are very low; among the 11 TTM cases since the year 2000, only four implicated donors were PCR positive (malaria surveillance–United States, 2017, unpublished raw data).[9,11] Nonetheless, PCR testing for a TTM investigation should be performed at a qualified public health reference laboratory. As seen in this investigation, the sensitivity of PCR also varies by the method used. Nested-PCR is more sensitive than RT-PCR, but it is more laborious, time-consuming, and more susceptible to false positives due to DNA contamination. However, nested-PCR should be attempted in TTM investigations, even when RT-PCR results are negative. A match by microsatellite analysis is the most definitive way to confirm the source of the infection, but its usefulness can be impaired by low parasitemia. If no donor sample is positive by PCR in a TTM investigation, then serologic tests to identify previous exposure in all donors should be considered to identify the most likely source(s) of the infection. Identifying multiple donors with negative results and one donor with a positive serology result can provide sufficient evidence to indirectly implicate a donor. This approach is not sufficient if there are many untested donors and no donor with a positive PCR result. In this investigation, RBC component segments were the most easily obtained for initial testing and successfully identified the parasite by PCR; follow-up samples identified the antibody-positive donor.

In terms of preventing TTM, the DHQ is an imperfect tool, and the applied deferral periods are based on the natural history of the disease, specifically the duration of infection in nonimmune individuals.[17,18] Questionnaire and deferral approaches might be less reliable when the infected donor is a former resident of a malaria endemic area who has been living in the United States longer than the 3-year postimmigration deferral period. Such donors are typically asymptomatic and have partial immunity to malaria with low-level parasitemia that is difficult to detect.[8,22] Reinterviewing donors provides investigators an opportunity to obtain potentially more accurate information about country of origin, travel outside of the United States, and past malaria history, which a donor might not have previously recalled or disclosed in the DHQ. In this investigation, the epidemiologic information obtained by repeat questioning matched the laboratory results and further strengthened the case for a single infected donor. In some nonendemic countries, donated blood from those with a history of residency in a malaria-endemic area are tested for malaria antibodies before being accepted.[28] Serologic screening could have captured this particular donor, but there are currently no recommended screening tests, nor guidelines for their use in the United States.

Finally, recipient monitoring, while not part of a TTM investigation per se, is an essential part of the follow-up process in TTM investigations. For cellular blood components that had been transfused from a donor identified with a "history of malaria," FDA recommends 3 months of posttransfusion monitoring for the recipient.[15] Unfortunately, this guideline was challenging to implement in a timely manner because to accurately confirm which donor had history of malaria, laboratory testing was required, which takes time, especially among asymptomatic donors. Thus, there may be a delay between identifying which donor(s) have had a history of malaria and the initiation of recipient monitoring.

In conclusion, although TTM is rare, malaria is a time-sensitive, life-threatening condition; having established methods for the prompt investigation of such cases will help

to limit exposure through the blood supply and assess the ongoing residual risk of TTM. The case presented is an example of a best practice approach for TTM investigations, which included: 1) prompt tracing of donors; 2) using sensitive serologic methods to identify donor(s) with a history of malaria exposure; this approach was applied to all samples available among all donors under investigation; 3) PCR testing to directly identify parasites in donor blood; 4) microsatellite analysis in an attempt to match parasites from the donor with those found in the recipient; and 5) using epidemiologic data from the DHQ and reinterview to complement the more robust laboratory data.

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CONFLICT OF INTEREST

The authors have disclosed no conflicts of interest.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article.

Table S1. (A) FDA Guidance for Industry, recommendations for the deferral of donors currently residing in nonendemic areas related to malaria exposure risk and (B) quarantine of components under investigation.

Table S2. Additional donated product cocomponents by donors under investigation. Donor H contributed blood products requiring quarantine, but the components were transfused prior to the start of the investigation.