Transfusion-transmitted malaria (TTM) has been an important clinical and public health problem since at least 1911 [1]. Worldwide, around 3000 cases of TTM were reported between 1950 and 1980. These were predominantly from nonendemic countries, so this is believed to be a significant underestimate of the global burden [2]. TTM is rare in nonendemic countries, occurring in <1 per million blood units in the United States [3]. Only 2 confirmed cases have occurred in England since 1996 [4]. In recipients with no immunity to malaria, TTM can be rapidly fatal if it is not recognized and treated quickly [5]. In malaria-endemic countries, asymptomatic carriage of malaria parasites is common, so parasitemia detected in a transfusion recipient could have been acquired from a mosquito bite rather than from the transfused blood. TTM can therefore only be confirmed by genotyping to demonstrate that the parasite in the recipient is identical to the one in the transfused blood.

The incidence of TTM in residents in endemic areas is not known. Nevertheless, the World Health Organization (WHO) recommends that all blood donations should be screened for malaria. This policy has significant resource implications and has not been widely implemented by transfusion services in sub-Saharan Africa [6, 7]. The prevalence of malaria parasitemia in

Transfusion-Transmitted Malaria in Ghana

Alex K. Owusu-Ofori,1 Martha Betson,2 Christopher M. Parry,3 J. Russell Stothard,4 and Imelda Bates4

1Department of Clinical Microbiology, Komfo Anokye Teaching Hospital, Kumasi, Ghana; 2Department of Production and Population Health, Royal Veterinary College, Herts, United Kingdom; 3Wellcome Trust Major Overseas Programme, Mahidol-Oxford Tropical Medicine Research Unit, Angkor Hospital for Children, Siem Reap, Cambodia and 4Disease Control Strategy Group, Liverpool School of Tropical Medicine, United Kingdom

Background. In sub-Saharan Africa, the prevalence of malaria parasitemia in blood donors varies from 0.6% to 50%. Although the burden of TTM in malaria-endemic countries is unknown, it is recommended that all donated blood is screened for malaria parasites. This study aimed to establish the incidence of TTM and identify a suitable screening test.

Methods. Pregnant women, children, and immunocompromised malaria-negative transfusion recipients in a teaching hospital in Ghana were recruited over the course of 1 year. Parasites detected in recipients within 14 days of the transfusion were genotyped and compared to parasites in the transfused blood. The presence of genotypically identical parasites in the recipient and the transfused blood confirmed transfusion-transmitted malaria. Four malaria screening tests were compared to assess their usefulness in the context of African blood banks.

Results. Of the 50 patients who received transfusions that were positive for Plasmodium falciparum by polymerase chain reaction (PCR), 7 recipients developed PCR-detectable parasitemia. In only 1 of the 50 recipients (2%) was the parasite identical to that in the transfused blood. The prevalence of P. falciparum malaria in transfused blood was 4.7% (21/445) by microscopy, 13.7% (60/440) by rapid diagnostic test, 18% (78/436) by PCR, and 22.2% (98/442) by enzyme immunoassay.

Conclusions. Although malaria parasites are commonly detected in blood donors in malaria-endemic areas, transfusion-transmitted malaria occurs infrequently. Policies recommend screening blood donors for malaria, but none of the commonly used methods is sufficiently sensitive to be used by blood banks in malaria-endemic countries.

Keywords. Transfusion-transmitted malaria; blood transfusion; screening; Plasmodium falciparum; blood donor.
African donors depends on the local endemicity and transmission season and varies from 0.6% in Nairobi, Kenya, which is not endemic for malaria, to over 50% in highly endemic northern Nigeria [8]. Transfusion service directors, policy makers, and practitioners from across Africa have identified a lack of information about the clinical impact of TTM and about suitable malaria screening methods as critical knowledge gaps [9]. It is possible that the paucity of reports of TTM from endemic countries accurately reflects a true low prevalence and is not due to under-reporting of TTM. This is because most residents of endemic areas are semi-immune and can persistently harbor a few parasites, and so a new, low-level infection from a healthy blood donor may have limited, if any, clinical consequences [10]. However, pregnant women, immunocompromised patients, and young children are particularly susceptible to malaria; in Africa they are the biggest users of blood transfusions, so they may be at particular risk of TTM. Our study aimed to determine whether TTM occurs in these vulnerable groups in endemic areas and to identify which test is most appropriate for screening donated blood for malaria.

**METHODS**

### Participants and Recruitment

This study was conducted between January and December 2010 at the Komfo Anokye Teaching Hospital (KATH), Kumasi, in malaria-endemic Ghana. The blood bank at KATH issues 14,500 units of blood a year. Pregnant women, children (aged between 1 and 15 years), immunocompromised patients with cancer, human immunodeficiency virus (HIV), or malnutrition, and patients with chronic diseases such as sickle cell anemia, chronic renal failure, and liver disease (ie, recipients most “at risk” of malaria) who were to receive a blood transfusion were eligible for this study. Patients were excluded if they were considered too ill (unconscious, shocked, or delirious) for signs and symptoms of malaria to be elicited. Patients were also excluded if they were to be transfused in the operating theatre because they were not accessible for monitoring by the study team, and they may have been unaware of events during transfusion. Patients who were parasitemic by microscopy prior to their transfusion or who had taken antimalarials within 5 days prior to transfusion were also excluded. Each day, eligible patients were initially identified using details on the transfusion request form, and their status was confirmed by scrutinizing their clinical notes and by direct questioning of the patient.

### Patient Monitoring and Follow-up

Patients who consented to participate were assigned unique study numbers. Patients were followed up for 14 days post-transfusion, and any blood unit they received during this period was uniquely identified. Patients’ vital signs, blood pressure, temperature, pulse, and respiratory rate were measured at the start of the transfusion, 15 and 30 minutes after the start of the transfusion, and then hourly until the transfusion had been completed. After the transfusion, and for the next 3 days, patients were examined daily and their vital signs recorded every 8 hours. Scheduled follow-up of the patients took place on days 7 and 14 after the transfusion. Patients were encouraged to come back to the study team if they felt unwell. At each follow-up visit a blood sample was examined by microscopy, and any patient found to be parasitemic was treated with antimalarials according to local guidelines. Patients’ physicians were free to give patients any other medications they deemed necessary.

### Investigations for TTM

Samples of blood were taken from the transfusion recipient before transfusion and at each follow-up visit. Samples were also taken from all blood units transfused to the study patients during their admission. Polymerase chain reaction (PCR) for malaria was performed on all transfused blood samples and on samples from recipients of malaria-positive blood. Blood samples were spotted on 3M filter paper; air dried, and sealed in a plastic envelope with a desiccant. The blood spots were transported to Liverpool School of Tropical Medicine, United Kingdom for *Plasmodium* PCR detection and parasite genotyping. Whenever parasitemia was detected by PCR in samples from both the transfused unit and the patient, the paired samples were genotyped to determine whether the parasites were the same.

For PCR, parasite DNA was extracted from blood spots using the chelex/saponin method as previously described [11]. A SYBR-Green real-time PCR assay was employed to detect *Plasmodium falciparum* in DNA extracted from all blood spots, using Brilliant III SYBR Green PCR mix (Agilent Technologies, Santa Clara, CA) and 1 µL of template DNA in a total reaction volume of 20 µL [12]. The amplification was performed on a Stratagene MX 3005P real-time PCR system (Agilent Technologies) using the following cycling program: 95°C for 3 minutes; 40 cycles of 95°C for 10 seconds, 55°C for 10 seconds, and 60°C for 1 minute. The PCR product identity was confirmed as previously described using a melt curve analysis [12]. Serial dilutions of the International Standard ranging from 1 × 10⁶ to 1 × 10³ IU/µL were included in duplicate in each real-time PCR run, allowing an approximate quantification of the samples to be carried out.

Genetic analysis of *P. falciparum* in patients and the corresponding transfused unit of blood were carried out using
nested PCR for the detection of allelic diversity at the merozoite surface protein 2 (msp2) loci, as described elsewhere [13]. Following the nested PCR reactions, electrophoresis of products was carried out on 2% agarose gel. When DNA products were detected in both the transfused blood and in the patient’s sample, the paired samples were run side-by-side on a 10% polyacrylamide gel, stained with ethidium bromide, and visualized using a Gel Doc EZ imaging system (Biorad, Hercules, CA). Automatic band detection and fragment sizing was carried out using Image Lab software (Biorad). For both the 3D7 and FC27 families, alleles were considered to be the same if molecular weights were within 10 base pairs of each other. If alleles from the blood bag and patient were the same, TTM was said to have occurred. To increase the discrimination power of the msp2 genotyping, the products of nested PCR amplification for the 3D7 family were subjected to digestion with the ScrF I restriction enzyme (Fermentas, Germany) as described elsewhere [13]. PCR fragments were visualized and sized as described above.

Evaluation of Malaria Screening Methods of Blood Units

A sample from each transfused unit of blood was screened for *P. falciparum* by microscopy, rapid diagnostic test (RDT), enzyme immunoassay (EIA), and PCR. RDT was performed immediately, and microscopy was performed within 24 hours of sample collection. And 1 mL of blood was placed in an eppendorf tube and stored immediately at −20°C for batched malaria EIA testing.

*Microscopy:* Slides were only declared negative after 100 high-power fields had been examined. Slides were examined by 2 microscopists and reviewed by an expert microscopist when there was disagreement; 10% of slides were also reviewed by the expert microscopist.

*RDT:* The test was carried out using the First Response Malaria Antigen test kit (Premier Med Ltd, India) according to the manufacturer’s instructions. This test is based on the detection of histidine-rich protein-2 (HRP-2).

*EIA:* The Malaria Antigen CELISA (Cellabs, Sydney, Australia) test kit used for this study is based on the detection of HRP2 and was performed according to the manufacturer’s instructions. In sum, 2 positive and 2 negative controls were included on each plate. The cutoff level was calculated as the sum of the optical density of the negative control and 0.1. Any sample with an absorbance value above the determined cutoff level was considered positive for *P. falciparum* antigen.

To compare the accuracy of the 4 different test methods used to detect *P. falciparum* parasitemia, a composite reference standard method was used [14]. A reference gold standard test was constructed by combining the results of PCR and microscopy such that a positive result by either test in combination or singly (ie, +PCR/−microscopy, −PCR/+microscopy and +PCR/+microscopy) was considered a reference positive. The reference test was negative when both PCR and microscopy were negative. Using the constructed gold standard, the sensitivity, specificity, negative predictive, and positive predictive value of PCR, microscopy, EIA, and RDT was calculated. This method was used because though microscopy remains the gold standard for parasite detection, it is not as sensitive as PCR.

**Statistical Analyses**

All statistical analyses were performed with the Predictive Analytics Software (PASW) statistical package 18 (SPSS Inc). Descriptive statistics including measures of central tendency and dispersion were used where appropriate. Parasite densities of *P. falciparum* identified by microscopy and DNA copies determined by qPCR were converted into logarithm values and correlation assessed by Pearson’s coefficient *r*.

Ethical approval was obtained from the ethics committee of the Liverpool School of Tropical Medicine in Liverpool, United Kingdom, and the Kwame Nkrumah University of Science and Technology, Kumasi, Ghana.

**RESULTS**

In sum, 372 patients were eligible for the study, but 136 were excluded for the following reasons: 30 patients withdrew their consent, 45 were lost to follow up, 20 were too ill or refused venesection, and 41 died. The remaining 236 patients completed the study schedule. Female patients (69.5%) exceeded males due to recruitment of pregnant women. There were 13 children (5.5%) in the study; the majority of children in the hospital who received blood transfusions were ineligible for the study because they had received malaria treatment on admission. The median age for study participants was 35.0 years (interquartile range, 25–48). One month prior to their enrollment, 27.1% (64/236) of patients had received antimalarial treatment. They had, however, completed treatment at least 5 days before enrollment and so they were not excluded. During the study, 45 of 236 (19.0%) patients were treated with antimalarials, although only a quarter of them had a laboratory confirmation for malaria.

**Malaria in Transfusion Recipients**

Of all the enrolled patients, 18.2% (68/372) received a blood transfusion that was positive for *P. falciparum* by PCR, but only 50 could be evaluated for transfusion-transmitted malaria (Figure 1). None of these 50 recipients had malaria parasitemia prior to transfusion, but 7 of the 50 recipients (14%) became positive for *P. falciparum* by PCR within 14 days of their transfusion. In one patient, recipient ID 223, genotyping demonstrated that the parasite in the recipient was identical to that in the transfused blood (Table 1). This recipient was afebrile and...
asymptomatic but was treated with antimalarials on day 1 because malaria parasites had also been detected by microscopy. For the other 6 PCR-positive recipients, the parasites in the recipients and the blood bag were not genotypically identical, indicating that they had not acquired their infection from the blood transfusion (Table 1). Parasites from patient ID 356 were of the same family (3D7) as those in their transfused blood, but the parasites were distinguishable on digestion with Scr1F. For patients ID 184, ID 301, and ID 369, the P. falciparum in the recipient and the transfused blood were from the same 3D7 family of msp2 allele but were different clones. For patients ID 9 and ID 319 the P. falciparum parasites in the patient samples and the corresponding blood bag were from different families of the msp2 alleles (Table 1). Genotypically confirmed TTM therefore occurred in only 1 (2.0%, 95% confidence interval [CI], −2%–6%) of 50 patients who received a blood transfusion positive for P. falciparum by PCR. The parasite density in the blood bag that caused the TTM was 280/µL.

### Malaria Screening Tests

In the study period 445 units of blood were transfused into 372 patients. The units had been stored for a median duration of 2 days (interquartile range, 1–6 days). In the blood bags with Plasmodium parasites, the highest density found was 3520/µL but overall, the geometric mean density was low (251/µL). The prevalence of P. falciparum in the transfused blood units was 4.7% (N = 445) by microscopy, 13.7% (N = 440) by RDT, 18% (N = 436) by PCR, and 22.2% (N = 442) by EIA.

Table 2 shows the accuracy of each of the 4 test methods used to detect parasitemia. PCR has the highest sensitivity (93.4%; 95% CI, 84.77%–97.6%), whereas microscopy had the lowest sensitivity (23.7%; 14.9%–35.1%). Specificity for all tests were high, the lowest being 86.5% (95% CI, 82.4%–89.8%) for the EIA method and the highest being 100% for PCR and microscopy (Table 2).

Of the 431 transfused units that were tested by all 4 malaria screening methods, 67.9% (293) were negative by all 4 tests, and 12 were positive by all 4 tests (Table 3). In the 13 samples
that were positive by both PCR and microscopy (ie, quantitative tests) the parasite densities of transfused blood determined by microscopy were positively correlated with the *P. falciparum* DNA concentration determined by real-time PCR (Pearson’s correlation coefficient $r = 0.528, P = .043$).

**DISCUSSION**

Our study has found that recipients of blood transfusions who live in malaria-endemic regions are at risk of TTM but that the incidence of transmission, even among high-risk patients, was low. Only 1 of 50 patients (2%) who received blood that was positive for malaria by PCR contracted malaria from the blood transfusion. The other 49 patients who received a PCR malaria-positive transfusion had either no molecular evidence of infection (43 patients) or the malaria parasite detected post-transfusion was not the same as in the transfused blood (6 patients). In a study from Sudan that did not perform PCR testing and genotyping, all 12 patients who received malaria-positive blood developed microscopically confirmed malaria, and 2 patients (0.52%) developed parasitemia after transfusion despite receiving microscopy-negative blood [15]. It is therefore clear

| Table 1. Genotyping of Positive Paired Samples of Patients and Transfused Blood Unit |
|----------------------------------|----------------------------------|----------------------------------|----------------------------------|
| **Screening Results of Blood Bag** | **Patient Results** | **Presence in Sample** | **Comparison of Fragments** | **TTM Confirmed?** |
| Pt/blood ID | Micro | RDT | EIA | PCR | day of +ve ff-up PCR | 3D7 | FC27 | 3D7 family | FC27 | 3D7 digest | Yes/No |
| 9 | BU-9 | – | – | + | + | D3 | + | – | ND | ND | ND | No |
| 184 | – | – | – | + | + | D1 | + | – | ND | ND | ND | No |
| 223 | BU-184 | + | + | – | + | + | D1 | + | – | Same | Same | Same | Yes |
| 301 | BU-223 | + | + | + | + | + | + | + | ND | ND | ND | No |
| 319 | BU-301 | – | – | + | + | + | + | + | ND | ND | ND | No |
| 356 | BU-319 | + | + | + | + | + | + | + | ND | ND | ND | No |
| 369 | BU-356 | – | + | + | + | + | + | + | ND | ND | ND | No |
| BU-369 | + | + | + | + | + | + | + | + | ND | ND | ND | No |

**Table 2. Sensitivity and Specificity of Malaria Screening Tests Using a Composite Gold Standard as the Reference Test**

<table>
<thead>
<tr>
<th>Screening test</th>
<th>Gold standard result</th>
<th>Sensitivity % (95% CI)</th>
<th>Specificity % (95% CI)</th>
<th>PPV % (95% CI)</th>
<th>NPV % (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PCR +ve</td>
<td>71</td>
<td>0</td>
<td>93.4 (84.7–97.6)</td>
<td>100 (98.7–100)</td>
<td>100 (93.6–100)</td>
</tr>
<tr>
<td>PCR –ve</td>
<td>5</td>
<td>355</td>
<td>100</td>
<td>100 (93.6–100)</td>
<td>98.6 (96.6–99.5)</td>
</tr>
<tr>
<td>Microscopy +ve</td>
<td>18</td>
<td>0</td>
<td>23.7 (14.9–35.1)</td>
<td>100 (98.7–100)</td>
<td>100 (93.6–100)</td>
</tr>
<tr>
<td>Microscopy –ve</td>
<td>58</td>
<td>355</td>
<td>100</td>
<td>100 (93.6–100)</td>
<td>98.6 (96.6–99.5)</td>
</tr>
<tr>
<td>RDT +ve</td>
<td>32</td>
<td>25</td>
<td>42.1 (31.0–53.9)</td>
<td>92.9 (89.6–95.3)</td>
<td>56.1 (42.4–69.0)</td>
</tr>
<tr>
<td>RDT –ve</td>
<td>44</td>
<td>330</td>
<td>42.1 (31.0–53.9)</td>
<td>92.9 (89.6–95.3)</td>
<td>56.1 (42.4–69.0)</td>
</tr>
<tr>
<td>EIA +ve</td>
<td>47</td>
<td>48</td>
<td>61.8 (49.9–72.5)</td>
<td>86.5 (82.4–89.8)</td>
<td>49.5 (39.1–59.8)</td>
</tr>
<tr>
<td>EIA –ve</td>
<td>29</td>
<td>307</td>
<td>61.8 (49.9–72.5)</td>
<td>86.5 (82.4–89.8)</td>
<td>49.5 (39.1–59.8)</td>
</tr>
</tbody>
</table>

**Abbreviations:** CI, confidence interval; EIA, enzyme immunoassay; Micro, microscopy; ND, not done; PCR, polymerase chain reaction; Pt ID, patient identification; RDT, rapid detection test; TTM, transfusion transmitted malaria.
that not all malaria occurring after transfusion is transmitted by that transfusion.

The surprising low incidence (2%) of TTM in our study raises concern about whether policies which recommend that transfusion recipients in endemic regions should routinely receive anti-malarials [16] are justified. Although we specifically selected high-risk recipients, malaria transmission occurred infrequently, suggesting that routine treatment for all recipients of blood transfusion is probably unnecessary.

None of the 4 malaria screening tests evaluated in this study would be ideal for African blood banks to use to prevent TTM, primarily because they were either insufficiently sensitive or too sensitive. Residents of malaria-endemic regions generally have clinical but not parasitological immunity to malaria, so healthy blood donors may harbor low levels of parasitemia. The different prevalence rates of malaria seen in blood donors are due to different test sensitivity of the diagnostic methods. The limits of sensitivity of microscopy and RDT are 10–100 parasites/μL [17–19], which is equivalent to (5–50) × 10⁶ parasites per unit of transfused blood and therefore adequate to be used to detect low-level infections. The HRP2 antigen in the EIA and RDT tests potentially cross-reacts with antibodies against rheumatoid factor and may persist for weeks after treatment; both these factors result in false-positive results [20–22]. Real-time PCR is highly sensitive with a detection limit of 0.7–5 parasites/μL [23, 24]. PCR is, however, not a practical method for routine use. Microscopy, despite not being highly sensitive, remains the gold standard for Plasmodium detection [25].

Msp1, msp2, and glutamate-rich protein (glurp) are the most commonly used molecular markers for P. falciparum [26], and we used the msp2 locus as it is the marker with the most discriminatory power [27–29]. A limitation of this study is that we may have failed to detect low-level P. falciparum clones in the transfused blood. Semi-immune adults living in malaria-endemic sub-Saharan Africa can have subclinical infections with up to 10 genetically distinct P. falciparum variants or clones [30] simultaneously, and nested PCR methods may not be sensitive enough to detect low abundance variants, especially if mixed infections are present [27, 31].

The widespread practice of treating transfusion recipients presumptively for malaria—and the assumption that transfused blood is the source of malaria occurring post-transfusion—are based on misperceptions about the true burden of TTM. There is also insufficient evidence to support policies that advocate screening donated blood for malaria. Major gaps exist in knowledge concerning the risks of TTM and its clinical impact in semi-immune populations, and there are no screening tools for malaria that are practical, affordable, and suitably sensitive for use by blood banks in Africa. The prevalence of malaria in blood donors is variable but can reach 50% in some parts of West Africa. Implementation of any policy that advocates rejection of all such donors will have a major impact on the availability of blood for transfusion and undoubtedly increase mortality, particularly among pregnant women and children, and must be underpinned by robust evidence.

Notes

Acknowledgments. We wish to acknowledge the following who worked on the project in Kumasi: David Sambian, David Ntiamoah, Elliot Dogbe, Mhort Attan Ayibo, Maxwell Owusu, Mary Osei Wusu, Deborah Otchere-Darko and Bernard Arhin. We also acknowledge Prof Hilary Ranson and Dr Chris Jones for their support in our PCR work. We thank Dr Shirley Owusu-Ofori and all staff of the Transfusion Medicine Unit for their cooperation and express our appreciation to all patients and their families for their participation in the study.

Financial support. This work was supported by the Commonwealth Scholarship Commission, UK, through the British Council and Liverpool School of Tropical Medicine.

Potential conflicts of interest. All authors: No reported conflicts. All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

References


10. Hassig A, Ambroise-Thomas P, Bruce-Chwatt LJ. Which are the appropriate modifications of existing regulations designed to prevent the transmission of malaria by blood transfusion, in view of the increasing frequency of travel to endemic areas? Vox Sang 1987; 52:138–48.


