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Residual risk of transfusion-transmitted malaria infection in a malaria endemic sub-Saharan African setting

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Abstract

Background: In Cameroon, as in many malaria endemic countries in Africa, blood donors are not routinely screened for *Plasmodium* infection that potentially could lead to severe malaria in some recipients. This study aimed at defining the prevalence of malaria among blood donors in Cameroon, and determining the risk of transfusion-transmitted malaria (TTM) following a single unit of blood transfusion.

Methods: A total of 250 blood donors were recruited at the Douala General Hospital in Cameroon. Blood samples were tested for the presence of *Plasmodium* by using a rapid diagnostic test (RDT), and by thin and thick blood smear microscopy. A mathematical model was performed to calculate individual risk and to estimate population incidence rates of TTM per year. Different data sources were used in the sensitivity analysis prior to estimation of malaria transfusion risk.

Results: More than half (96.8%) of all blood donors were men, and the mean age of the donors was 28.5 (SD = 8.9) years. Infected volunteer donors represented 2.80% while infected family-replacement donors comprised 97.20%. The prevalence of *P. falciparum* infection was 12.0%, and the population mean parasite density was 6,056 (95% CI: 4,542–8,076) parasites/ μ L. The individual median residual risk of TTM was 5.59 per 10,000 or 2.64 per 1,000 units of blood transfused every year in Douala or Cameroon, respectively.

Conclusions: This study confirms the presence of *P. falciparum* as one of the most prevalent TTIs in the region. The residual risk of TTM is high among blood recipients, urging to conduct in malaria-endemic areas a cost-benefit analysis of systematically screening blood units for malaria parasites before transfusion versus systematically treating the recipient after transfusion.

Keywords: Blood transfusion, Residual risk, *Plasmodium falciparum*, Cameroon

Background

A blood transfusion is needed for severe anemia, an indication of one or more causes, medical, traumatic, surgical, obstetric or pediatric [1]. Blood transfusion is common worldwide and represents a safe therapeutic procedure when performed in compliance with immunological and hygienic standards, as well as following the strict

screening of donors for transfusion-transmissible infections (TTIs) [1].

Between 1992 and 2002, health services in Cameroon witnessed an exponential rise in blood donors from 75,000 to 130,000, following the introduction of blood safety guidelines [2]. Despite recent improvements in blood safety in Cameroon following adoption of the 2003 law on blood donation and transfusion [3], the risk of TTI remains high due to the increased demand for blood transfusion. Beside the immunological risk, micro-organisms including bacteria, viruses, and parasites could be transmitted during blood transfusion, and bacterial contamination remains a major potential risk of

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infection during blood transfusion [4–11]. As in many countries worldwide, efforts to minimize blood transfusion risks in Cameroon focus on performing serological tests for human immunodeficiency virus (HIV), hepatitis B virus (HBV), hepatitis C virus (HCV), and *Treponema pallidum* (*T. pallidum*) due to the fact that these infections are the most feared by patients and prescribers [12–15] and because of the potentially chronic clinical sequelae associated with these agents [16, 17]. Although recommended by the WHO [12], blood donors are not routinely screened for the detection of *Plasmodium* infection, which is a treatable infectious disease. This represents a real problem in non-endemic areas where blood recipients may be at increased risk of developing severe malaria due to *Plasmodium falciparum* (*P. falciparum*) [18, 19]. In endemic areas, the risk of symptomatic malaria infection after transfusion is of particular importance in non-immune children, pregnant women and in immunocompromised patients, the reason for which antimalarial treatment is systematically prescribed in some hospitals following blood transfusion [20]. Studies carried out in different African countries have shown that *Plasmodium* parasites can survive in blood stored at 4 °C for up to 18 days, and for extended periods when frozen [12, 21]. Thus, some African countries have implemented the obligatory screening of blood units destined for transfusion in pregnant women and young children [6]. In Cameroon, where malaria is endemic, asymptomatic infected blood donors can potentially transmit malaria. However, very few studies have been undertaken to assess the prevalence of malaria infection in blood donors and to investigate the residual risk of TTM in Cameroon and Africa to date [5, 6, 22]. This study aimed to determine the prevalence of malaria, and to estimate the residual malaria risk among blood donors in Cameroon.

Methods

Study areas and overall study design

We conducted a cross-sectional study among voluntary and family blood donors attending the blood transfusion center of the Douala general hospital (DGH) over a period of 3 months. The DGH is a reference public health structure with 320 beds, and its blood transfusion center collects over 3500 bags per year that are distributed in all wards of the hospital and other health facilities in the Littoral Region. Blood donors with signs or symptoms associated with any disease, or with a recent history that constitutes a risk for malaria parasite carriage were excluded. Standard questionnaires and data collection sheets were used to collect anthropometric (age, sex, weight), socio-demographic (occupation, place of residence), and clinical (symptoms, blood pressure) and biological data.

Detection of malaria infection and malaria transfusion risk estimation model

P. falciparum infection was diagnosed by rapid diagnosis tests (RDTs) and microscopic examination of thick and thin blood smears from peripheral venous blood collected in EDTA tubes. The ACON Malaria *P. falciparum*® RDT, which detects the *P. falciparum* Histidine Rich Protein 2 (PfHRP-2) was performed according to the manufacturer's instructions using whole blood. The parasitological results were interpreted as positive or negative according to kit instructions. Thick and thin blood smears were performed according to WHO standards [23]. Briefly, 10 µL of blood were spread on microscope slides and slides were dried and stained with 5% Giemsa. Parasite density (expressed in parasites per microliter (p/µL) of blood) was determined by counting the number of asexual parasites against 300 white blood cells with 1 parasite for every 3–4 white blood cells deemed 200 asexual parasites/µL based on the assumption that the white blood cell count is approximately 8000/µL). Slides were considered negative when parasites were not detected following examination of microscopy fields containing at least a total of 1000 white blood cells. Parasitemia level $\geq 20\%$ was chosen as the cutoff because it is the definition of hyperparasitaemia by the WHO (WHO 2000) criteria in an endemic area [24]. When thick films were positive, thin films were read for species determination.

A model proposed by Kane et al., which is based on a generalized simple linear equation, was used to calculate different risk probabilities associated with transmission of bloodborne pathogens [25]. This model allows the calculation of average individual risk in a particular geographical region as well as estimating population incidence of TTIs per year. Data sources provided by the BUCREP (Central Bureau of Census and Population Studies) and the 3rd GPHC (General Population and Household Census) were used to estimate population sizes [26]. In the absence of reliable national and uncertainty data on the number of blood components transfused each year in Cameroon, we used different data sources including WHO, CDC/PEPFAR program, journal articles, and other academic publications to estimate the number of transfused blood units per year [7, 27–29]. Malaria prevalence rates were also obtained from diverse sources [30, 31]. For malaria transmission risk estimation (probability that an infectious blood bag with *Plasmodium* can be either transmitted to the recipients), we carried out a sensitivity analysis to identify the most contributing input parameters [32].

Statistical analyses of data

Categorical variables were expressed as frequencies, whereas numerical variables were presented as means \pm SDs or 95% CI (95% confidence interval instead of), if normally distributed. Parasitemia, a numerical variable

found to be highly skewed in its distribution, was log-transformed. To compare proportions, we used Chi-square test or Fisher's exact test. Numerical values were compared using the U-test of Wilcoxon. All statistical analyses were done using Stata software package (version 11 SE) and R software (version 3.1.1) [33]. Only p -values < 0.05 were considered significant in all analyses.

Results

Characteristics of blood donor population

For a duration of 3 months in 2011, TTI screening testing was performed on 250 blood donors. The characteristics of the donor groups are compared in Table 1. Seven (2.8%) blood donors were volunteers whereas 243 (97.2%) were family-replacement donors. Overall, the prevalence of male donors was 96.8%, and the male/female sex ratio was 30/1. Most of the donors were laborers (47.3%), with pupils and students representing 30.4% of all donors. The majority of donors (68.4%; 171/250) were less than 31 years old, with the highest frequency recorded in the 21–26 years age group. The mean age of male donors was 28.4 (SD = 8.9), whereas that of the female donors was 30.2 (SD = 7.8). None of the donors had previously received a transfusion or a history of fever, malaria or antibiotics drug use within the week before recruitment.

Malaria infection among blood donors and residual transmission risk

RDT was positive for *Plasmodium* in 31 (12.4%) of 250 analyzed blood donors, and 30 of these were also positive by thick blood smear microscopy. *P. falciparum* was the only identified species by thin blood smear. One positive thick blood smear (0.4%) with *Loa loa* microfilaria was also found. The parasitemia geometric mean was 6,056.82 (95% CI: 4542.0–8076.9) p/μL and most of *P. falciparum* positive blood donors (87.1%) had a mean parasitemia $\leq 4\%$. The average parasite carriage per 450 ml donated

blood was 3 parasites. The frequency of positive blood donors according to the age group are shown in Fig. 1. It was highest (25.9%) in the 15–21 years age group and decreased thereafter. It was 11.0% among 21–31 years age group and 4.3% above 31 years. There was a significant difference ($p = 0.002$) in the frequency of *Plasmodium* infected blood donors according to age groups.

Malaria transfusion residual risk estimation is presented in Table 2. Regardless of the hypothesis formulated (through the different transmission level: low, medium and high) for the sensitivity analysis, it was estimated that approximately 8,559 [5,150–12,838] or 69,734 [52,396–87,072] units of blood were transfused in Douala or Cameroon each year, respectively. Among these, the most likelihood hypothesis is that 1,061 or 20,049 units of blood could be considered contaminated in Douala or Cameroon, respectively. Based on our deterministic model, the individual median risk of acquiring TTM was estimated to be high (5.59 infections per 10,000 and 2.64 per 1,000 units of blood transfused in Douala or Cameroon, respectively). Regardless of our sensitivity analysis, the risk of receiving *Plasmodium* infected blood unit depended more on the malaria prevalence than the number of transfused blood units.

Co-infection

At baseline screening, 30 (12.0%) HBsAg, 2 (0.8%) anti-HCV, and 3 (1.2%) anti-HIV positive results were recorded. *T. pallidum* was found in 16 (6.4%) blood donors. Among the donors, one had a triple infection (*P. falciparum* + HIV + *T. pallidum*), whereas seven cases of double infection were identified; three for *P. falciparum* + *T. pallidum*, three for *P. falciparum* + HBV, and one for *P. falciparum* + HCV.

Discussion

Malaria parasites are generally recognized risk of blood transfusion in malaria endemic countries worldwide, but

Table 1 Characteristics of blood donors population

	Volunteers donors	Family-replacement donors	Total	p
Number	7 (2.8)	243 (97.2)	250	
Age mean (SD), years	28.6 (6.31)	28.5 (8.9)	28.5 (8.9)	0.527
Men	7 (100.0)	235 (96.7)	242 (96.8)	0.238
Thick and thin blood smear	1 (14.3)	29 (11.9)	30 (12.0)	/
Parasitemia geometric mean (IC95), parasites/μl	4500.0 (1.0–1.0)	6115.2 (4544.0–8234.7)	6056.8 (4542.0–8076.9)	/
Occupation				
Staff members	1 (14.3)	53 (21.8)	54 (21.6)	0.019
Laborers	4 (57.1)	114 (46.9)	118 (47.2)	
Pupils/Students	2 (28.6)	74 (30.5)	76 (30.4)	
Unemployment	0 (0.0)	2 (0.8)	2 (0.8)	

Data are number and proportion (%) of blood donors, unless otherwise indicated. Variables were compared by Fischer's exact test (categorical variables) or U-test of Wilcoxon (continuous variables); Statistical significance: P -value < 0.05

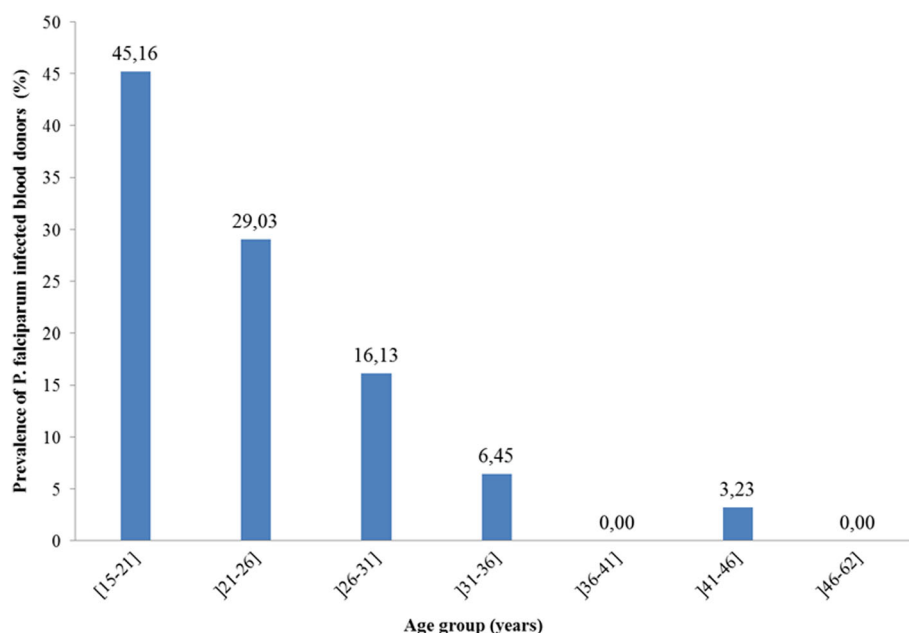


Fig. 1 Distribution of *Plasmodium* infectious status according to blood donor's age groups

the incidence of TTM remains unknown in many African countries. To our knowledge, this study is the first to investigate the residual risk of TTM in Cameroon, and among the few studies conducted in Africa till date.

During a 3-month high malaria transmission period in Douala, Cameroon, we recruited 250 blood donors at the DGH, with family-replacement donors representing 97.2% of all blood donors, the majority of whom were male (male/female sex ration of 30:1). Indeed, despite the high blood transfusion needs in sub-Saharan Africa regions, and the increased awareness of its populations,

family relatives or acquaintances remain the principal blood donors in the region [4, 8, 34–36]. This is different in Western countries and in North Africa, where blood supply is mainly based on volunteer donation [15, 37, 38]. The trend of high male/female ratio has been reported in other studies in Cameroon [4, 34, 39] and elsewhere [37, 40–42]. This could be explained by physiological factors such as pregnancy, menstruation, and feeding which exclude some women from blood donation.

Blood transfusion practice remains a major challenge in malaria-endemic areas because many potential blood

Table 2 Individual risk of transfusion-transmitted malaria infection according to the transmission level hypothesis (low, medium or high)

Variables	Douala			Cameroon		
	Low	Medium	High	Low	Medium	High
Population (estimation in 2010)		1 907 479		19 406 100		
Estimated proportion of transfusion (u)	0.0027	0.0045	0.0067	0.0027	0.0036	0.0045
Estimated N° of transfusion per year	5,150	8,559	12, 838	52,396	69,734	87,072
Estimated Prevalence among donor- P (vi)	0.121	0.124	0.143	0.13	0.29	0.45
Estimated N° of infections per year	623	1,061	1,836	6,550	20,049	39,182
Unsafe transfusion proportion (not screened in an malaria risk context)-P (u)	0.97	0.98	0.99	0.97	0.98	0.99
Probability for exposure to infected blood units-P (E) = P (vi)*P (u)	0.1173	0.1215	0.1416	0.1212	0.2818	0.4455
Probability for malaria transmission following blood transfusion-P (t)	0.98	0.99	0.99	0.98	0.99	0.99
Partially immune population	0	0.025	0.05	0	0.025	0.05
Probability for recipient susceptibility to malaria-P (s)	0.95	0.975	1	0.975	1	0.95
Individual risk of receiving a transfused infected blood unit every year-P (I); P (I) = 1-[1-P (S)*P (t)*P (e)] u	3.1238×10^{-4}	5.5965×10^{-4}	1.0268×10^{-3}	1.1399×10^{-3}	2.6423×10^{-3}	3.2335×10^{-4}

donors are infected. Unfortunately, malaria screening is not routinely done in these areas for reasons that include the unavailability of rapid diagnostic tools in local blood banks, and the lack of high performance assays to detect submicroscopic parasite loads in asymptomatic individuals. Our screening of blood donors at the DGH resulted in an incidence rate of 12.0%. This incidence is similar to that found in other countries [29, 43, 44]. It was higher than the 0.7% in Nairobi a non-endemic area, the 6.5% in Yaoundé, the 7.8% in Ibadan, and the 6.5% in Sudan [5, 29, 45–47]. In contrast to our study, a higher prevalence of malaria was reported during the rainy season in other countries; 26.5% in Lagos, 33.5% in Cotonou and over 55% in highly endemic northern Nigeria [29, 46, 48]. Together, these findings confirm that the prevalence of malaria parasitaemia in African donors depends on the local endemicity and the transmission season [4, 5, 29, 44, 49].

P. falciparum is the most widespread species in Cameroon [50] and was the only *Plasmodium* species that was found by RDT and thin smear microscopy in our study. Parasite densities (geometric mean: 6,056.82 p/μL) were high and seemed to be sufficient to cause TTM. Indeed, a study conducted in Ghana revealed that the parasite density in blood units that caused TTM was 280 p/μL [49], which could lead to severe and often fatal disease, especially for non-immune recipients [51, 52]. Additionally, a study conducted in a hyperendemic area in Ghana showed that as few as ten parasites are sufficient to initiate fulminant malaria in humans [53], and various studies have reported the detection of *P. falciparum* by PCR or microscopy in some patients soon after blood transfusion despite receiving microscopy-negative blood [45, 49]. It is worth-noting that microscopy is very less sensitive in asymptomatic malaria cases wherein PCR-based methods are most preferred [45, 49, 54]. Together, these findings suggest that despite the absence of clinical signs, donors living in endemic areas are very likely to transmit malaria through blood transfusion.

By including estimates of blood recipient population, the risks of TTI in screened or unscreened blood units, and by conducting probabilistic sensitivity analysis, we estimated that the median risk of acquiring *P. falciparum* from a single unit of blood in Douala or Cameroon is 5.59 infections per 10,000 or 2.64 infections per 1,000 units of transfused blood, respectively. Regardless of our sensitivity analysis, the risk of receiving *Plasmodium* infected blood unit depends more on the malaria prevalence than the number of transfused blood units. Transfusions alone would be responsible for 1,061 and 20,049 malaria infections respectively in Douala or Cameroon each year.

The proposed Kane model takes into account the immunity or semi-immunity of the donors, and therefore a variable sensitivity to infection, and a partial infectivity

to any pathogen [25]. The data on infectivity are difficult to access, and the few available data cannot be applied to all countries given the differences in malaria transmission endemicity levels. We used different values covering a wide range of prevalence (low and high hypothesis) in Cameroon by using the sensitivity analysis. Probabilistic sensitivity analysis indicates that the true risks may even be higher considering the number of individuals with low and undetectable blood parasitaemia, and the ability of these parasites to remain viable for long periods in stored blood units [12, 21].

A review of studies conducted in sub-Saharan Africa indicates that malaria is one of the most significant post-transfusional infections [29]. The risk is quite real, and this high residual risk of malaria raises once again two major concerns with respect to blood safety in malaria endemic areas: i) the introduction of systematic RDT-based screening and destruction of infected blood units as clinical criteria remains unreliable [55–57], or ii) the systematic provision of anti-malarial prophylaxis to blood recipients as recommended by the WHO and other stakeholders [58–61]. Eliminating all *Plasmodium* infected blood units is however inconceivable as many patients do not have access to blood when needed; 38% of the estimated 80 million units of annually donated blood worldwide come from the developing world [58]. Which of the 1st or the 2nd strategies is the most cost-effective to lessen the risk of TTM in sub-Saharan Africa? Some possible answers are presented in the review by Nansseu et al. [62]. In either case, there is an urgent need to conduct cost-benefit studies to evaluate each of these two TTM preventive methods. In our context, *Plasmodium* positive blood units could be transfused under cover of appropriate and effective malaria treatment of recipients, particularly for fragile subjects (neonates, pregnant women).

Limitations and strengths

A limitation of this study was in the small sample size of donors ($n = 250$) and the non-use of PCR to detect submicroscopic malaria. Compared to previous reports, our study is the first to investigate the residual risk of TTM in Cameroon. Although our findings clearly cannot be applied to all regions, this work has important implications for understanding the burden of malaria that may be attributable to contaminated blood products in Cameroon.

Conclusions

This study confirms the presence of TTIs in blood donors at the DGH in Cameroon, identifying *P. falciparum* as one of the most prevalent TTIs in the region. Our findings suggest a high risk of TTM among blood recipients in malaria endemic countries, requiring the implementation

of appropriate preventive measures such as systematic pre-screening of blood donors or systematic anti-malarial prophylaxis to blood recipients in high-risk populations. A comparative cost-benefit analysis is however needed to identify the most efficient preventive strategy for each affected region.

Abbreviations

BUCREP: Bureau Central de Recensement et des Etudes de Population/Central Bureau of Census and Population Studies; **CDC:** Centers for disease control and prevention; **CI:** Confidence interval; **DGH:** Douala General Hospital; **GPNC:** General Population and Household census; **HBV:** Hepatitis B virus; **HCV:** Hepatitis C virus; **HIV:** Human immunodeficiency virus; **PEPFAR:** President's Emergency Plan for AIDS Relief; **PIHRP-2:** *P. falciparum* Histidine Rich Protein 2; **RD1:** Rapid diagnostic test; **T. pallidum:** *Treponema pallidum*; **TTIs:** Transfusion-transmissible infections; **TTM:** Transfusion-transmitted malaria; **WHO:** World Health Organization

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Availability of data and materials

All data supporting these findings can be found in the blood transfusion center of the clinical biology unit of the DGH, a reference public health structure located in Douala, Cameroon. Data could not be shared, they are the property of the hospital which is only to decide their disclosure or not.

Authors' contributions

COE and CEEM coordinated the study and drafted the manuscript. COE, END and JPNM collected data and participated in its design. COE, GT and CEEM performed the statistical analysis. All authors read and approved the final manuscript.

Competing interests

The authors declare that they have no competing interests.

Consent for publication

Consent to publish has been obtained from all included persons in the study.

Ethics approval and consent to participate

This study was conducted in accordance with ethics directives related to research on humans in Cameroon. The DGH institutional review board approved the study. Before enrollment, subjects were informed on the purpose and process of the investigation (goals, methodology, study constraints, data confidentiality, and rights to opt out from the study), and an oral informed consent was obtained from all participants.

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