Transfusion Medicine and Hemotherapy

Review Article

Transfus Med Hemother 2022;49:205–216
DOI: 10.1159/000525414

Received: March 31, 2022
Accepted: June 7, 2022
Published online: July 15, 2022

Transfusion-Transmitted Malaria and Mitigation Strategies in Nonendemic Regions

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Keywords

Transfusion-transmitted malaria · *Plasmodium* spp. · Nonendemic countries · Donor screening strategies

Abstract

Background: Malaria is a mosquito-borne infectious disease caused by protozoan parasites of the genus *Plasmodium*. These parasites can be transmitted by blood transfusion especially through Red Cell Blood Concentrates collected from asymptomatic and parasitemic donors. As migration of populations from endemic areas to Europe and overseas recreational travel to endemic regions increase, there is growing risk of transfusion-transmitted malaria (TTM) in nonendemic regions of the world. The present work provides an overview of the mitigation strategies in nonendemic countries and their effectiveness and discusses possible approaches to evolve the strategies in order to maintain both a safe and adequate blood supply. ***Summary:*** The historical and current situation of malaria and TTM in Europe and on the North American continent are described. The infectivity of *Plasmodium* in blood components and the consequences of TTM are presented, along with the regulations and guidelines for TTM mitigation in Europe, USA, and Canada. The regulations/guidelines currently in place in Europe allow a certain amount of leeway for local policies. A questionnaire was used to survey European countries regarding their current strategies and recent TTM cases. From the questionnaire and published cases, approximately 20 cases of TTM were identified in the past 20 years in the USA and Europe. The vast majority of implicated donors have been former residents of malaria-endemic areas, particularly former residents of hyperendemic areas in Africa. The most recent TTM cases are discussed in detail to provide insight into the gaps in current strategies. The utility and uncertainties of pathogen reduction and serological and molecular testing methods are discussed. ***Key Messages:*** Overall, the risk of transfusion-associated malaria in nonendemic countries is considered to be low and very few TTM cases occurred in these regions in the last 20 years. The questionnaire-based strategy with questions about risk in relation to malaria exposure with or without selective testing based on questioning seems to be relatively effective, although rare and sometimes fatal transmissions still occur. An outstanding question is whether in the future molecular methods may further improve the safety of blood products and help constrain the loss of donors.

Introduction

Malaria is a vector-borne infection most commonly caused by five *Plasmodium* species, *P. falciparum*, *P. vivax*, *P. ovale*, *P. malariae*, and *P. knowlesi* [1]. *Plasmodium* parasites are transmitted by female *Anopheles* spp. mosquitoes. These mosquitoes are present throughout the world, including most European countries and the Americas [2, 3]. But in addition to vector-borne transmission, there are other important routes of transmission, such as by blood transfusion and organ or tissue transplantation; this is due to asymptomatic infections in donors with low-level parasitemia. All five species have been

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implicated in TTM events and, although rare, these continue to pose a risk to blood transfusion worldwide [4, 5].

Clinical malaria is reportable to public health in most nonendemic areas. Since the 1980s, *P. falciparum* has been the most frequently reported species of clinical malaria reported in countries where malaria is not endemic (e.g., USA, Canada, and European countries) [6]. In addition, *P. falciparum* has also been the most frequently implicated species in malaria associated with blood transfusion [7].

Epidemiology of Malaria in Europe, Canada, and the USA

In 2016, Europe became the first region in the world to be declared free of indigenous malaria by the WHO. The number of reported cases in Europe had fallen from 90,172 in the year 1995 to very few in 2015. In 2015, only seven cases were reported as acquired in the corresponding countries, five of them in Greece and one each in Belgium and The Netherlands. The five *P. vivax* cases in Greece occurred in regions where competent *Anopheles* vectors (*A. sacharovi*) are present and where malaria-infected migrants from endemic countries were also present. In 2018, according to the ECDC, 14 autochthonous malaria infections occurred in the European Union, ten in Greece (all *P. vivax*), one in Italy (*P. falciparum*), one in France (unknown plasmodial species), and two in Spain (*P. falciparum* and a *P. ovale*/*P. malariae* mixed infection) [6]. In the year 2019, there were nine confirmed autochthonous cases (two each in Germany, Greece, Spain, and France and one in The Netherlands) [8]. According to the European Centre for Disease Prevention and Control (ECDC), autochthonous cases occur sporadically in Europe, which can be either nosocomial or vector-borne origin [6, 9, 10]. There has also been observed an increasing number of so-called airport malaria in the period 2010–2020 in Europe [11].

In 2019, there were 8,638 confirmed malaria cases in the European Union/European Environment Agency (EEA), nearly all of which were imported, with a majority of the cases associated with travel. France reported the highest number of cases, followed by the UK and Germany [8].

In Canada, just under 500 malaria cases are reported annually, in former residents of or travelers to endemic areas [12]. In a review of cases from 2004 to 2014, about 75% of these cases were reported as acquired in sub-Saharan Africa and about 13% in South Central Asia [13]. In the USA, approximately 2,000 cases of malaria are reported annually. Essentially, all cases (with the exception of transfusion-transmitted cases) are associated with former residence in or travel to malaria-endemic areas, with the vast majority acquired in Africa [14]. Competent vectors may be found in some locations in the USA, but vector-borne transmission of malaria in the USA is rare [15].

Infectivity

The risk of malaria transmission through transfusions is due to the primary presence of the parasites in erythrocytes, particularly high in the case of transfusions. Parasites are primarily found in erythrocytes; the risk of malaria transmission by transfusion is particularly associated with whole blood or red blood cell concentrates (RBCs). However, platelets and leucocytes and even fresh nonfrozen plasma can also transmit malaria through contamination with infected residual erythrocytes [16–18]. Three TTM cases due to platelet concentrates in Canada suggest that even small numbers of infected erythrocytes are sufficient for the transmission of malaria to the recipient [19]. With *P. vivax*, it was shown that an infusion of ten parasites was sufficient for transmission of infection [16, 20, 21]. Therefore, it has been theorized that a minimum infectious dose of 10 or fewer parasites in a unit of donated blood could lead to a TTM [18]. Assuming a low parasitemia in the donor of 1-2 parasites per μl of blood, a donation of 450 mL of RBC would result in the transfusion of about 450,000–900,000 parasites [22, 23].

Because malaria parasites can persist in the human body for long periods of time, parasites can still be present in the blood years after exposure and transmit an infection to the recipient. In general, *P. vivax* and *P. ovale* typically do not persist for longer than 3 years, *P. falciparum* for about 1–2 years. However, long-term chronic asymptomatic infection can occur in individuals who previously lived in endemic areas. Recurrent *Plasmodium* infections that occur in individuals who reside for prolonged periods of time in malaria-endemic areas may result in a "semi-immune" state of chronic asymptomatic low-grade parasitemia [24]. Transfusion transmission has been documented many years after the donor left an endemic area. The longest periods described between a transmission by a blood donation and previous exposure to malaria in the donor were 13 years for a *P. falciparum* infection and 27 years for a *P. vivax* infection [25–27]. In the case of *P. malariae*, much longer intervals of up to 50 years and even longer have been described, which in very rare cases can lead to TTM [28]. In principle, a TTM can occur with a single preparation of a nonpathogen-inactivated blood component since all blood preparations have a residual content of erythrocytes. An exception is frozen plasma, where no TTM has been reported so far [15].

Storage/Survival

It has long been shown that plasmodia can survive in blood supplies for at least 10–12 days, possibly longer [23, 29]. Data generated more recently showed that all *Plasmodium* species can survive in blood components at temperatures from 2 to 6°C for some days or even weeks [30, 31].

Clinical Outcome: TTM

TTM can lead to severe cases more frequently than malaria transmitted by the vector. In vector-borne infection, parasites undergo the pre-erythrocytic stage during which innate immunity is activated to protect the host; however, parasites transmitted through blood products are released directly into the bloodstream, without the hepatic cycle [7]. TTM can be fatal, particularly in a nonimmune recipient and if not recognized and treated promptly [4, 32, 33].

Leucodepletion

Leucoreduction filters have not previously been considered as a viable method to remove RBCs infected with parasites from blood products because these filters are designed to allow RBCs to pass while trapping white blood cells and platelets. There are, however, reasons to suspect that changes which occur in malaria-infected RBCs may lead to adherence of infected RBCs within these filters. Schwartz and colleagues showed in a series of experiments that the exposure of phosphatidylcholine on the surface of malaria-infected RBCs and loss of deformability of RBCs due to infection would lead to the retention of those cells within the filter [34]. Whether or not leucoreduction filters are capable of reducing TTM is not clear from the abovementioned experiments, but indeed interesting observations on the incidence of TTM in the recent years can be made. Leucoreduction may have contributed to the decreasing number of TTM cases in addition to the declining numbers of autochthonous malaria since its widespread introduction in the mid to late 1980s [35].

Pathogen Reduction

Pathogen reduction technology (PRT) is a potentially valuable tool to reduce the risk of TTM. PRT is available for platelets, transfusable plasma, and, in some countries, for whole blood but not, to date, for RBCs. The use of pathogen-reduced whole blood is extremely rare. The INTERCEPT amotosalen-UV light system was demonstrated to reduce infectivity of *P. falciparum* by at least 6 logs in platelets and 6.9 logs in plasma [36]. In 2020, the US Food and Drug Administration (FDA) approved collection of platelet and/or plasma components from a select subset of donors with malaria risk who would otherwise have been deferred for 3 months, provided the components would be pathogen reduced with an FDA-approved system effective against *P. falciparum* (see Table 1) [15]. Currently, INTERCEPT treatment for platelets and plasma is the only FDA-approved PRT.

Two PRT methods have been demonstrated to reduce infectivity of *P. falciparum* in red blood cells or whole blood. Amustaline/GSH reduced *P. falciparum* infectivity at least 5.7 logs, and treatment with riboflavin plus UV light reduced infectivity at least 6.4 logs [40, 41]. The riboflavin system (Mirasol) was utilized in a prospective study in a hyperendemic area where 25% of donations were parasitemic as determined by *P. falciparum* DNA. The riboflavin/UV treatment of whole blood was reported to significantly reduce *P. falciparum* transmission from 22% to 4%. Thus, PRT has the potential to reduce transfusion transmission of malaria and perhaps eliminate transmission if the pathogen burden in the component is sufficiently low. However, the riboflavin study suggests that the efficacy of PRT may not be sufficient to completely eliminate transmission via red cell components with a high parasite content.

Donor Screening Strategies

Traditionally, the main strategy for reducing the risk of TTM in nonendemic areas has been to question potential donors about residence in or travel to malaria-endemic areas and about a history of malaria or a history of undiagnosed febrile illness during or soon after visiting a malarial area. Individuals meeting certain criteria have been excluded from donation (deferred). The specific questions and deferral periods have evolved over time, in an attempt to balance the safety of the blood supply against the loss of donors. In Europe and some other regions, testing can be used to shorten the deferral period [42]. Current European regulations and guidelines are shown in Table 1 [37, 38, 43]. In the USA and Canada, testing is not an available option [42]. The policies of the USA and Canada are also shown in Table 1.

Residents of nonendemic areas who travel to endemic areas represent the largest proportion of donors with potential risk, but these are also the least likely to be harboring infection [44]. Blood safety policies typically involve temporary deferral of travelers to malarial areas for a period of time intended to allow for manifestation of symptoms. This deferral period is currently 6–12 months in Europe, permitting use of testing to shorten the deferral

Table 1. Regulations/guidelines from several health authorities in nonendemic regions of the world

Guideline/country	Risk category	Donor management
European Commission Directive 2004/33/EC [37]	History of malaria	3 years after treatment and no symptoms; accept thereafter only if an immunologic or molecular genomic test is negative
	Lived in a malarial area within the first 5 years of life	3 years following last visit if symptom free, may be reduced to 4 months if an immunologic or molecular genomic test is negative at each donation
	Asymptomatic visitors to endemic areas	6 months after leaving the endemic area unless an immunologic or molecular genomic test is negative
	History of undiagnosed febrile illness during or within 6 months of a visit to an endemic area	3 years following resolution of symptoms; may be reduced to 4 months if an immunologic or molecular test is negative
EDQM [38]	History of malaria Undiagnosed febrile illness consistent with malaria during a visit to or within 6 months following departure from a malarial area Lived in a malaria-endemic area for a continuous period of 6 months or more at any time in their life at the time of their first donation and after each return from a malarial area	Defer for at least 4 months after departure and cessation of treatment/last symptoms; may then be accepted if the result of a validated immunologic test for antibodies to the malaria parasite is negative If the test is repeatedly reactive, the donor must be deferred and may be re-evaluated after a suitable period when the antibody test may have reverted to negative (a period of 3 years is suggested) If the test is not performed, the donor must be deferred until the test is performed and negative
EDQM [38]	All other individuals who visited a malarial area without reporting any clinical symptoms consistent with malaria	Defer for at least 4 months following departure from the malarial area; may then be accepted as blood donors if the result of a validated immunological test for antibodies to the malaria parasite is negative If the test is not performed, the donor may be accepted once a period of 12 months has elapsed following departure from the malarial area If the test is repeatedly reactive, the donor must be deferred and may be re-evaluated after a suitable period when the antibody test may have reverted to negative (a period of 3 years is suggested)
US [15]	History of malaria	Defer for 3 years, after which donor may be accepted if free of malaria during this period while residing in a non-endemic country
	Prior residence in a malaria-endemic country (continuous stay of 5 years or more)	Defer for 3 years, after which donor may be accepted unless they have traveled back to a malaria-endemic area
	Travel to a malaria-endemic area by a prior resident of a malaria-endemic country	Defer for 3 years after a visit to a malaria-endemic area if the donor has been a resident of nonendemic countries for less than 3 consecutive years
	Travel to malaria-endemic area by residents of nonendemic countries	Defer for 3 months after the last departure from a malaria-endemic area*
Canada [39]	History of malaria	Not eligible to donate whole blood or platelets
	Stayed in malaria-risk area 6 months or longer	Defer for 3 years
	Stayed in malaria-risk area less than 6 months	Defer for 3 months

* US FDA guidance permits collection of platelets and/or plasma from these donors provided the blood components are treated with an FDA-approved pathogen reduction device effective against *P. falciparum*.

to 4 months [37, 38]. In the USA, the deferral period for travelers was 12 months until recently; it was shortened to 3 months in 2020 in an attempt to balance blood supply considerations against the low risk of TTM from travelers (Table 1) [15]. This decision was based on an analysis of US malaria cases showing that 94% of nonresidents of endemic areas who acquired their infection from travel to endemic areas developed their symptoms within 3 months. Furthermore, the loss of donors from the 12-month travel deferral was felt to be disproportionate to the rarity of observed TTM linked to travelers who were not former residents of endemic areas [15, 35].

The most challenging question relates to management of donors who previously resided in endemic areas and

Table 2. Strategies in the corresponding countries

Country	Selective testing for donors at risk	Assays used	If tested negative, permission for donating blood		
			travelers	former residents of endemic areas	donors with a history of malaria
Austria	No	na	No	No	No
Belgium	Yes	Malaria EIA (Bio-Rad)	Yes	Yes	Yes
Croatia	Yes	Captia Malaria Total Ab EIA (Trinity Biotech) Malaria EIA (Bio-Rad) ELISA Malaria Ab (Diapro)	Yes	Yes	No
Denmark	No	na	No	No	Yes[1]
Estonia	Yes	Anti-*Plasmodium* (IgG) (Euroimmun)	Yes	Yes	Yes
Finland	Yes	S-PlasAb; EIA (performed in an external lab)	Yes	Yes	Yes
France	Yes	ELISA Malaria Ab (Diapro) 1th line ELISA Anti-*Plasmodium* (IgG) (Euroimmun) 2nd line	Yes	Yes	Yes
Germany	No/yes[2]	na	No/yes	No/yes	No
Greece	Yes	NovaLisa™ Malaria Elisa (NOVATEL Immundiagnostics GmbH)	No	Yes	Yes
Ireland	Currently not but is planned in the near future	Captia Malaria Total Ab EIA (Trinity Biotech) Planned to implement in the near future	No	No	No
Italy	Yes	Different assays	Yes	Yes	Yes
Malta	No	na	No	No	No
The Netherlands	Yes	Captia™ Malaria Total Antibody EIA (Trinity Biotech)	Yes	Yes	Yes
Norway	Yes	Malaria EIA (Bio-Rad)	Yes	Yes	No
Poland	No	na	No	No	No
Portugal	Yes	Malaria EIA (Bio-Rad)	Yes	Yes	Yes
Spain	Yes/no (dependent on the region)	Malaria EIA (Bio-Rad) Anti-*Plasmodium* (IgG) (Euroimmun) Captia™ Malaria Total Antibody EIA (Trinity Biotech)	Yes/no	Yes/no	Yes/no
Sweden	No	na	No	No	No
Switzerland	Yes	Anti-*Plasmodium* (IgG) (Euroimmun), Malaria EIA (Bio-Rad)	Yes	Yes	Yes
UK (England, Scotland, Wales, N. Ireland)	Yes	Captia Malaria Total Ab EIA (Trinity Biotech)	Yes	Yes	Yes

[1] Donors with a history of malaria but unsure of it being malaria. [2] Some blood banks in Germany test for *Plasmodium* antibodies according to the German Hemotherapy Guidelines.

who may have chronic low-level parasitemia. Although TTM cases are rare, the vast majority are from this population [35, 44]. Historically, many donor policies deferred prior residents for three or 5 years after departure from malarial areas, with automatic acceptance after this time. Some reported TTM cases have been related to prior residents who failed to disclose their history and donated within this deferral period; however, many of the TTM cases have been from prior residents who passed the deferral period yet remained infectious. For this reason, some blood authorities have implemented a requirement to test prior residents for *Plasmodium* spp. antibody as a condition for accepting them [24]. Former residents who travel back to endemic areas also may fail to demonstrate symptoms upon reinoculation with *Plasmodia*. Therefore, some blood policies have also required either testing or a longer deferral period for prior residents who travel back to endemic areas. The additional value of a donor

question about a history of malaria is somewhat questionable as many of the former residents who have transmitted infection did not report a history of malaria. Additionally, some individuals may answer yes to this question because they were given antimalarials for a febrile illness without a laboratory diagnosis of malaria. Nevertheless, some countries, such as Canada, permanently exclude individuals who report a history of malaria [42]. In the absence of an option for testing to reinstate such individuals, this can reduce the pool of donors available to support patients who need blood cell phenotypes from certain Asian or African populations. Selective screening of blood donors with risk for infections with *Plasmodium spp.* using serological assays has demonstrated to be a feasible and cost-effective strategy to minimize the number of rejected blood donations but keep a high blood safety [22, 42, 45–49].

Many western countries, including France, UK, Australia, Denmark, Finland, New Zealand, and Switzerland, have implemented selective malaria testing programs during the last 20 years [48–51]. These policies have been very successful as no or only very few TTM infections have been detected since then. Other European countries, including Norway and Spain, have slightly different strategies which comply with the European regulations but differ in fine details [48]. The USA and Canada rely solely on a deferral strategy to protect recipients from possible TTM infections [52, 53]. The deferral strategies rely on excluding donors for long enough after their travel to, or residence in, a malaria-endemic region to allow them to develop symptoms or resolve their infection [48]. The strategies in all nonendemic countries are dependent on donors providing accurate information concerning their potential malaria risk.

To assess current mitigation strategies and their efficacy in Europe, a short questionnaire was prepared and sent to European countries (see Additional Material). Table 2 displays the current strategies reported.

TTM Cases in Nonendemic Countries

In nonendemic malaria regions, the number of TTMs discovered and published has been relatively low over the last 20 years. In Europe, very few TTMs have been published in recent years, as exemplified by some European countries. In Germany, for example, in the last 40 years, a total of three cases of TTMs have been published. Two of them occurred in the 1980s and the last case in 1997 [54–56]. In France and England, a total of four cases were registered between 2002 and 2013. The donors all came from West Africa [42]. In France, four TTMs have been reported since 2002 [57]. The last TTM case in France occurred in 2015 [58]. In Italy, the first reported case dates to 1963 in Liguria and in 2005 a case occurred in Sicily [82]. The last TTM case in Italy occurred in the year 2019 with the transmission of *P. malariae* [60].

In the USA, 12 cases of TTM were reported for the period of 2000 through 2020 [9, 15, 42, 61]. All of the implicated donors were former residents of Africa. In six of the twelve cases, more than 3 years had elapsed since the donors had last been in an endemic area (i.e., the donors would be eligible by current criteria). The most recent US TTM case, in 2020, involved a *P. falciparum* transmission from a donor from Nigeria. The donor had malaria in childhood, had emigrated 4 years prior to donation, and had not subsequently visited an endemic country [61]. A donor sample was reportedly seropositive but polymerase chain reaction (PCR) negative; however, the PCR was performed on a retained segment from the donation that had undergone prolonged storage in the refrigerator (M. Hsiang, personal communication).

In Canada, the last reported case of TTM occurred in 1997 [19]. This *P. falciparum* transmission was associated with a donor from Ghana who had come to Canada 4 years prior to donation and did not report a history of malaria, i.e., met donor eligibility criteria.

Table 3 summarizes compiled responses from the European survey regarding identified TTM cases, as well as additional cases reported in a literature review published in 2018 [7]. Overall, ten cases were identified in the past 20 years for the countries represented in this table. In a majority of the countries, no TTMs were detected. Of course, it can never be excluded that other transfusion-related malaria cases in the last 20 years were undetected and therefore not notified and/or published. A detailed review of recent European cases provides insight into the efficacy and gaps of current mitigation strategies.

In 2011, *P. malariae* was transmitted by a Dutch traveler who had no history of residency in an endemic area and no history of malaria and whose last visit to an endemic area was more than 3 years prior to donation [62]. Stored plasma from the donation was negative for *Plasmodium* antibodies by enzyme-linked immunosorbent assay (ELISA) but positive by indirect immunofluorescent antibody test (IFAT). A freshly collected blood sample was negative by microscopy and antigen testing but positive by PCR.

France has identified four TTM cases in the past 20 years, all associated with former residents of Africa or islands off the coast of Africa [57]. In 2002, a fatal *P. falciparum* transmission was linked to a donor from Ghana who had been living in France for 4 years with no reported travel to Africa. The donation had not been tested but subsequent workup found the donor positive by microscopy, antibody, and PCR. The policy was changed to require antibody testing upon the first donation by individuals native of a malaria-endemic area or having lived

Table 3. Last TTM cases in the corresponding European countries**

Country	TTMs in the last 20 years reported by survey respondents	Last notified TTM case	Reference for last notified case
Belgium	None	None	na
Croatia	None	1964	na
Denmark	None	None	na
Estonia	None	None	na
Finland	None	None	na
France	4 cases	2015	[58]
Germany	None	1997	[56]
Greece	None*	1987	[81]
Ireland	None	None	na
Italy	3 cases*	2019	[60, 82]
Malta	None	None	na
The Netherlands	1 case	2011	[62]
Norway	None	None	na
Poland			
Portugal	None	None	na
Spain	1*	2002*	[83]
Sweden	None	1980	
Switzerland	None	1999	[84]
UK (England, Scotland, Wales, N. Ireland)	1 case	2003	[59]

In addition to cases reported by survey respondents, the table includes two additional cases in Italy (2005, 2008) and one case in Spain (2002) reported by [7]. * Respondent reported a 2006 case in Greece; this appears to have been a possible hospital acquired infection in which transfusion was not ruled out [85]. ** Note added in proof: An additional European TTM case (2019 case in Austria) is reported by Wagner et al. in this issue of *Transfusion Medicine and Hemotherapy*: Wagner T, Stadlbauer V, Stoeger K, Schorna K, Zink M. Criminal and civil responsibility of the donor in a case of transmission of malaria by a blood transfusion in a non-endemic country. Transfus Med Hemother. 2022. DOI: 10.1159/000525103.

there more than 6 months. Nevertheless, three additional TTM cases have occurred since then. In 2006, another fatal *P. falciparum* transmission occurred from a donor from the Ivory Coast who had been living in France for 5 years and had traveled to his native country 15 months prior to donation, but malaria risk was not identified in the medical interview and therefore the donation was not tested for *Plasmodium* antibodies. PCR and antibody testing on an archived plasma sample from the donation were positive. A 2012 fatal *P. falciparum* TTM involved a donor native of Benin who had lived in France for 12 years with no reported travel to malaria-endemic areas in that period. An antibody screen using the Captia malaria ELISA assay was negative. Retrospective retesting reconfirmed a negative ELISA result; however, an immunofluorescence antibody test (IFAT) was borderline and a PCR test was positive for *P. falciparum*. This case represented the first known TTM due to a false-negative result on the Captia malaria antibody screen [42]. In 2015, a second TTM due to a false-negative antibody test occurred. The donor was a native of the Comoro Islands and had lived in France for more than 3 years with no subsequent travel to malaria-endemic countries. Antibody testing of the donation by the malaria Lab 21 ELISA was negative. A follow-up sample was borderline by ELISA, positive by IFAT, and PCR positive for *P. malariae*.

The most recently reported TTM case in Europe, a transmission of *P. malariae*, occurred in 2019 in Italy [60]. The donor was a missionary who had traveled to endemic regions more than 10 years prior to donation. According to local policy, malaria antibody testing was not required for the donation. Follow-up evaluation of the donor showed positive results by microscopy and PCR.

The recent cases highlight that chronically infected former residents of endemic areas, most commonly Africa, are posing the greatest challenge for preventing TTM. The Netherlands case is unusual in that it reflects a donor without a history of residency in an endemic area but who nevertheless appeared to have developed a chronic asymptomatic infection. In several cases, the donors implicated in TTM had passed the deferral period or the period for which testing was required for acceptance. In three cases (The Netherlands case and the two most recent French cases), the donors tested negative on ELISA antibody tests, demonstrating the potential variability of antibody detection assays.

Testing Methods/Methodologies: Serology

To reduce cases of TTM, diverse screening strategies can be used, including pre-donation questionnaires and/or laboratory screening [63]. The use of serological and molecular tests could represent important tools in the prevention of diseases, but the sensitivity and specificity of screening tests in blood donors remain the object of study [35].

Serological testing for plasmodial antibodies is unsuitable for diagnosing acute malaria but is used in transfusion medicine for the screening of blood donors after a suitable deferral period to allow for the production of antibodies [22]. The aim of the serological testing is to demonstrate if a donor has been exposed to malaria parasites and not to demonstrate the infectivity of a potential blood component. Serological assays have been used in European countries to screen blood donors [7]. However, the lack of specific and sensitive test systems, suitable for processing a large number of samples, poses a challenge for the application of these protocols in the routine setting. In particular, the poor specificity of the tests can lead to the exclusion of donors who are otherwise eligible to donate blood, according to current guidelines. This in turn could have an impact on the adequacy of supply [47]. In order to be able to manage and counsel donors appropriately, these tests need to have a high specificity.

These serological tests often utilize parasite antigens from nonsexual blood stages, which are the main target of the immune response. Therefore, *Plasmodium*-specific antibodies are detected at the earliest 1 or 2 weeks after the initial infection [64].

Most of the commercially available ELISAs use antigens derived from *P. falciparum* and *P. vivax*. Only a few of the current commercial antibody tests include antigens from the five major species that infect humans [65]. Thus in many of the assays, the detection of antibodies against other malaria species (e.g., *P. ovale*, *P. malariae*, and *P. knowlesi*) relies on cross-reactivity of antibodies to the *P. falciparum* and *P. vivax* antigens or the detection of individuals having mixed-species infections. For example, a kit that has been used commonly by blood banks (Captia™ Malaria Total Antibody EIA, Trinity Biotech) contains recombinant antigens for *P. falciparum* and *P. vivax* but claims detection also for *P. malariae* and *P.ovale* [65]. Although in most cases there are indeed cross-reactive antibodies to these antigens, there are reports showing reduced sensitivity for the detection of antibodies generated against the other malaria species [48].

In a review of testing in the UK from 2010 to 2013, 138,782 donations were identified as malaria risk and screened for malarial antibodies using the Trinity Biotech malaria antibody EIA [66]. Of these, 4,302 (3.1%) were reactive and subjected to additional testing. A total of 1,955 of the reactive samples were reactive by an immunofluorescence antibody test (IFAT) or other confirmatory antibody tests and were subjected to DNA testing; 14 were positive for DNA. There was some variability in detection of the DNA-positive donations by the DiaPro, Cellabs, and Diamed EIAs with 6/14 samples nonreactive by the DiaPro assay. All five *P. vivax* DNA positive samples and one of three *P. ovale* samples were negative by the IFAT confirmatory assay (which contains *P. falciparum* parasites). All of the DNA-positive donations were from former residents of malarial areas; all were nonreactive on two malaria antigen assays, the Alere BinaxNOW rapid test, and the Cellabs Malaria Ag EIA.

In an Italian study, five commercial ELISA kits were evaluated using samples from 64 patients with malaria or a malaria history that were positive by IFAT [63]. The results showed a sensitivity below 65% for every assay, leading to a high proportion of false negatives, with poor agreement among the assays. A similar study in France of 108 samples from patients with well-documented malaria showed sensitivities ranging from 50% to 84% with different assays [67].

Two Australian "near miss" events provide further evidence of gaps in detection by antibody testing [42]. Two individuals made donations between 4 months and 13 months after travel to Papua New Guinea. The donors were diagnosed with *P. vivax* infections 1–2 months after their last donation, which had tested malaria antibody negative. The components from these donations had not been transfused.

In addition to questions about sensitivity of antibody screening tests, concerns have also been raised about specificity. In Switzerland, the specificity of the Malaria EIA used from 2007 until 2015 was 92.8% [47]. This highlights the low specificity of the tests used. After changing the EIA in the year 2016, the specificity increased but only to 95%. This low specificity poses a problem as false-positive results would prevent acceptance of the donors, unless the facility permits additional confirmatory testing to be used for final donor eligibility determination.

Another consideration in the use of antibody testing is that serological tests are indirect tests; they do not necessarily indicate parasitemia and could lead to the exclusion of uninfected donors [68, 69]. In Europe, assays from five different manufacturers are used in the countries that have a selective testing strategy in place (Table 2).

Testing Methods/Methodologies: Molecular Methods

Recently, molecular methods for *Plasmodium* detection have gained attention. For decades, microscopy and antigen tests have served as the mainstay for diagnosis of

symptomatic malaria infection [70]. These two methods have similar limits of detection of approximately 100 parasites per μL (100,000 parasites per mL) [70, 71]. It is well established, however, that these assays miss many *Plasmodium* infections that are detectable by molecular methods [71, 72]. A recent literature review by Wu et al. of studies in asymptomatic populations found that on average, rapid antigen tests detected slightly more infections than microscopy but only 41% of PCR-detectable infections. A variety of targets have been used for molecular assays. Most have targeted *Plasmodium* genomic DNA. To improve sensitivity, researchers have often targeted sequences present in the genome in multiple copies, such as the genes for 18S RNA, which are reportedly present in 5–8 copies per genome [71]. Such an assay for 18S RNA genes was used in the UK study described above that detected *Plasmodium* DNA in 14 donors not reactive by antigen tests [66]. A research PCR for 18S rRNA gene was reported to detect *Plasmodium* DNA in samples from 19/101 (18.81%) asymptomatic adults in Ghana who tested negative by microscopy [73]. The prototype of an automated multiplex NAT method was recently described, using the 18S rRNA gene target [74]. In a pilot study of 4,745 blood donations, three donors infected with *P. vivax* were identified with parasitemia concentrations ranging from 13 to 1,410 copies/μL. The authors concluded that the system could lead to an improvement in the safety of blood donations in endemic countries.

More recently, ribosomal RNA (rRNA) has gained interest as a more sensitive target for molecular screening because it is reportedly present in as many as 10,000 copies per parasite [75]. The two largest manufacturers of donor screening NAT assays have described prototype assays targeting *Plasmodium* ribosomal RNA [76, 77]. The assays would be performed on the automated systems currently used for routine donor infectious disease testing. These malaria assays utilize the same concept as their *Babesia* assays that are in routine use in the USA to screen donors for *Babesia*, another intraerythrocytic parasite. The *Babesia* assays detecting ribosomal RNA are reported to have 95% limits of detection for *B. microti* of approximately three parasites per mL of whole blood [78, 79]. If this sensitivity is achieved also for plasmodia, this would comprise an analytic sensitivity more than 30,000-fold improved in comparison to microscopy or antigen testing. No reliable data are available on parasite concentrations in former residents of malaria-endemic areas who have long-term chronic infections and whether the new molecular methods would be sufficiently sensitive to detect them.

Very few cases of TTM in nonendemic areas have been studied to evaluate whether the donors would have been detected by molecular methods; in these few reports, the molecular methods were not detailed. In one US case of transfusion-transmitted *P. falciparum* from a former resident of Benin, the donor's infection was detected by PCR and antibody but not antigen [80]. In the two French TTM cases described above that were missed by antibody screening, both donors were detectable by PCR [57]. In some cases, molecular testing was only performed on samples that had been stored in the refrigerator for several weeks, which likely caused degradation of molecular targets. It would be important to test donors linked to TTM using fresh samples to continue to evaluate the question of detectability. It is a hopeful sign for the efficacy of molecular screening that in the USA there have been no cases of transfusion-transmitted *Babesia* reported for donations screened by the rRNA molecular assays described above, which are in most cases being performed in pools of 6 or 16. According to the evaluation of the questionnaire in the European countries, molecular biological methods are only used currently in very few countries as additional testing for the confirmation of screening-reactive donation samples.

Conclusion

TTM in nonendemic areas is rare but may be fatal. Approximately 20 cases have occurred in the USA and Europe in the past 20 years. The vast majority of implicated donors have been former residents of malaria-endemic areas, particularly former residents of hyperendemic areas in Africa. Over the last decades, donor management guidelines have evolved in an attempt to maximize blood safety while containing donor loss.

All strategies to prevent TTM in nonendemic areas are dependent on identification of donors with exposure risk during the pre-donation interview. Some countries, such as the USA and Canada, use deferral of at-risk donors as their sole TTM mitigation strategy. To minimize donor loss in the face of blood supply challenges, some countries have recently shortened the deferral period for residents of nonendemic areas who travel to endemic areas, given the low risk of TTM from such donors. Other countries permit shortening of the deferral period through the use of antibody testing. To reduce the risk of TTM from former residents of endemic areas, some countries require an antibody screen to qualify these donors after a prescribed deferral period. Causes of breakthrough malaria transmissions may include failures during the donor interview to elicit the malaria risk information from the donor or to correctly characterize the risk (e.g., travel vs. former residence), donation by individuals with asymptomatic infection whose deferral period had passed, and failure of the antibody assay to identify infected donors.

Anti-*Plasmodium* antibody screening of former residents to exclude those with evidence of malaria infection has provided an option in some countries to reduce do-

nor loss while maintaining a high level of safety. However, this method is not perfect. Studies of current antibody tests demonstrate suboptimal sensitivity, and negative EIA antibody results have been reported for at least 3 donations implicated in TTM [7, 63, 67].

The potential role of molecular tests for mitigation of TTM remains to be defined. There is a concern that testing of a donor sample may be inadequate to exclude the presence of a small number of parasites in a unit of donated blood when considering the theoretical minimum infectious dose (i.e., 1–10 parasites in a unit of blood). It is possible, however, that the typical concentration of parasites in the blood of chronically infected individuals may be sufficient to be detected by the new highly sensitive molecular tests. Investigations of TTM cases have reported positive PCR results on fresh samples from the implicated donors, using assays that targeted relatively low-copy number genomic DNA targets. This provides some cause for optimism that the new, high-sensitivity molecular tests that target high-copy number ribosomal RNA targets may be able to detect the chronically infected donors that are causing TTM. Thus, molecular assays could provide another tool for use in TTM mitigation strategies. Future studies would be useful to comparatively estimate the frequency of detection of asymptomatic parasitemic donors with serological and molecular assays.

As an additional option, PRT may be used to enable collection of low red-cell content blood components (platelets and plasma) from travelers with a low risk of infection, as recently permitted in the USA. Currently, RBCs cannot yet be processed with PRT. Even when available, however, PRT may not be sufficiently potent to completely prevent transmission of malaria through RBCs, given the potentially high pathogen burden in these components. In summary, TTM is a rare but serious transfusion complication, and mitigation strategies will continue to evolve in the ongoing efforts to maintain both a safe and adequate blood supply.

Acknowledgments

We thank the following individuals for filling out the short Malaria questionnaire: Austria: Christof Jungbauer; Belgium: Marie-Pierre Rodenbach; Croatia: Daniel Grubesci; Denmark: Lene Holm Harritshoe; Estonia: Riin Kullaste; Finland: Susanne Ekblom-Kullberg; France: Pierre Gallian; Germany: RKI (Ruth Offergeld); Greece: Constantina Politis; Ireland: Niamh O'Flaherty; Italy: Vincenzo De Angelis; Malta: Monique Debattista and Sandra Cutajar; The Netherlands: Ryanne Lieshout-Krikke; Norway: Henning Øfsteng; Poland: Piotr Grabarczyk; Portugal: Mario Chin; Spain: Salvador Oyonarte and Silvia Sauleda; Sweden: Jonas Nordberg; Switzerland: Peter Gowland; and UK: Lisa Jarvis, Ines Ushiro-Lumb.

Conflict of Interest Statement

Dr. Niederhauser has no conflict of interest. Dr. Galel is an employee and shareholder of Roche Diagnostics. The conclusions, findings, and opinions expressed in this manuscript are solely those of the author and do not reflect or represent those of Roche Diagnostics.

Funding Sources

There was no funding.

Author Contributions

Both authors provided substantial contributions in the conception of the work writing and editing of the manuscript.

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