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Selective malaria antibody screening among eligible blood donors in Jiangsu, China

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ABSTRACT

The risk of transfusion-transmitted malaria is a major concern in many countries. This study investigated the prevalence of malaria antibodies and parasitemia in eligible blood donors in Jiangsu, in Eastern China. Malaria antibodies were detected in 2.13% of the 704 plasma samples studied. We found that the prevalence of malaria antibodies was not significantly correlated with gender, occupation and frequency of donation, but it increased with age. No *Plasmodium* was observed in red blood cells and no *Plasmodium* DNA was detected in any of the antibody-positive samples. The prevalence of malaria antibodies was not higher than expected in Eastern China.

KEYWORDS: Malaria antibodies. Eligible blood donor. *Plasmodium* DNA.

Malaria is a major cause of morbidity and mortality worldwide and is caused by parasites of the *Plasmodium* species, transmitted to humans through the bite of infected female *Anopheles* mosquitoes. It is one of the most common diseases in the world; more than half of the world's population lives in regions where malaria is endemic. Each year, between 300 and 500 million cases are reported globally, resulting in more than 1 million deaths, most of them among the younger population[1]. In 1955, it was estimated that malaria was endemic to 70-80% of the counties in China; then malaria infection was inhibited by more than 80% after the launch of a national malaria control program[2]. China has implemented a national malaria elimination strategy to achieve complete elimination by 2020[3]. In 2010, it was calculated that approximately half of the Chinese population lives in areas with no risk of malaria, 1% lives in high-risk areas, such as the Yunnan Province, and the remaining lives in low-risk areas, such as the Jiangsu province[4].

However, the risk of malaria transmission from other endemic settings continues to increase due to the population mobility for travel, business and work. This constitutes a major challenge for malaria elimination in China. Between 2010 and 2014, a number of cases of imported *Plasmodium falciparum* were reported from all the provinces of China, largely due to import from other countries, especially from countries in Africa where *P. falciparum* is very prevalent[5]. A number of Chinese workers also travel as laborers to Africa, where many countries are endemic for malaria; this trend has further increased the number of potential malaria-infected donors in China. No autochthonous cases of malaria have been reported in the Jiangsu province since 1998[6]; sporadic cases of imported malaria, mostly from Africa and Southeast Asia, have been reported in recent years. This has led to an increase in the proportion of blood donors at risk for malaria. In August 2013, a transfusion-transmitted malaria (TTM) case caused by *P. falciparum* was reported in

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Jiangsu Province Blood Center for the first time. The blood donor was a worker who recently returned from Kenya and once had malaria. He later admitted to concealing his medical history in order to know whether he had recovered enough to donate blood.

This study was designed to determine the prevalence of malarial antibodies among blood donors, aiming to alert the appropriate authorities to the risk of TTM.

The present study was performed from July to September 2015. Prospective donors were examined and given a questionnaire including their travel history. They were then screened for alanine aminotransferase (ALT), syphilis, and hepatitis B surface antigens (HBsAg) at different collection sites. The collected blood samples were screened by two different reagents for ALT, HIV, HBsAg, HCV, and syphilis; the negative samples were further subjected to a nucleic acid test (NAT) for HIV, HBV and HCV. We selected plasma samples from eligible blood donors (negative for the routine screening above and no self-reported malaise), whose blood samples could be used clinically for malaria antibodies screening.

All samples were tested for malaria antibodies using the Pan Malaria Antibody CELISA kit (CeLLabs, Sydney, Australia), as recommended by the manufacturer. The kit uses a sandwich ELISA for the detection of specific IgG antibodies against *P. falciparum, P. vivax, P. malariae,* and *P. ovale* in serum and plasma samples. Results were defined as sample-to-cut-off (S/CO) ratios. The test package insert indicated a gray zone between 0.8 and 1.0, which was extended to between 0.5 and 1.0 by our testing laboratory. All initially reactive samples were retested in duplicate to determine repeated reactivity.

Whole blood samples from malaria antibody-reactive samples were delivered to a clinical lab in a local hospital. Thick and thin blood films stained with Giemsa were prepared and microscopy readings were performed by an expert to detect parasitemia. DNA was extracted from 0.2 mL of whole blood of all the reactive samples using a QIAamp® DNA Blood Mini Kit (Qiagen Inc. Hilden, Germany), according to the manufacturer's instructions. A genus-specific primer pair and four species-specific primer pairs (targeting *P. falciparum, P. vivax, P. malariae,* and *P. ovale*) for nested PCR assay were designed based on the genes for the small-subunit ribosomal RNA of *Plasmodium*; the thermal cycler conditions used were as described previously[7].

Informed consents were obtained before blood donation. The study was approved by the Ethical Committee of Jiangsu Province Blood Center (serial number 2014-11).

Among the 704 blood samples studied, 44 were from foreign citizens or workers in foreign companies, 270 from workers returned from malaria-endemic provinces, 206 from university students from tropical or subtropical regions of China, and 184 from local Jiangsu citizens. Malaria antibodies were detected in 2.84% (20/704) of - blood samples, including seven gray zone samples. Positive samples were retested showing that 15 (2.13% of total) were confirmed positive for malaria antibodies. The S/CO values of 12 of these positive samples were between 1.03 and 1.79, while one had a value of 2.37 and two had 0.9 in the gray zone. Eight of them were mobile workers, five were local citizens, one worked in a foreign company, and one was a foreign university student from Africa. The prevalence of malaria antibodies in subjects from foreign countries and companies was the highest one (4.55%).

We found no significant correlation between the expression of malaria antibodies and several parameters, including genders (χ^2 = 0.01, P = 0.9203), occupation (χ^2 = 2.75, P = 0.0973), or donation frequencies (χ^2 = 0.22, P = 0.6390); however, the prevalence of malaria antibodies was found to increase with the element of age. Blood donors aged 46-60 years had the highest prevalence of malaria antibodies, and this prevalence was significantly different from that in other age groups (χ^2 = 10.29, P = 0.0013) (Table 1).

Table 1 - Prevalence of malaria antibodies and demographic characteristics of blood donors

Donor characteristics	Blood donors (N)	Malarial antibodies (N)	Percent (%)
Gender			
Male	502	10	1.99
Female	202	5	2.48
Age(years)			
18- 45	602	8	1.33
18- 25	224	2	0.89
26- 35	205	2	0.98
36- 45	173	4	2.31
46-60	102	7	6.86
Occupation			
University student	206	1 (foreigner)	0.49
Worker	498	14	2.81
Foreign countries	44	2	4.55
Other provinces	270	8	2.96
Local province	184	4	2.17
Donation Frequency			
First	552	13	2.36
Repeat	152	2	1.32
Total	704	15	2.13

No *Plasmodium* parasites were detected in red blood cells by microscopy and none of the samples tested positive for *Plasmodium* DNA as assessed by nested PCR.

TTM cases were often reported worldwide. Blood donors are not routinely tested for malaria before blood donation in China. Here, we reported that the prevalence of malaria antibodies was 2.13% among blood donors in Eastern China. We also showed the significant relationship between the prevalence of malaria antibodies and donor's age. Individuals aging 46-60 years had the highest prevalence of malaria antibodies (6.86%), while individuals aging 18-25 years had a much lower prevalence (0.89%). Donors who were mobile workers had higher prevalence of malaria antibodies (2.81%) than university students (0.49%). This could be attributed to their work environment with higher exposure to mosquitoes. We recorded the lowest prevalence of malaria antibodies in Chinese university students; most of these subjects had never even heard of malaria or *Plasmodium*. In Jiangsu, the first-time blood donors are predominant (> 70%) and, thus, it was not surprising that the prevalence of malaria antibodies in first-time donors was higher than that in those who had donated before.

The risk of TTM differs widely in non-endemic countries, where imported infection occurs in individuals who have traveled to or migrated from endemic regions[8]. Therefore, blood donors were screened for malaria antibodies to identify those at risk for malaria in many non-endemic areas. The English transfusion service[9] has been screening blood donors for over 10 years. From 2010 to 2013, 138,782 donations were identified as being at risk for malaria and screened for malaria antibodies. Of these, 4,302 (3.1%) were reactive to the primary malarial antibody, and malaria DNA was found in 14 of 1,955 samples investigated. In Australia[10], of 154,804 samples screened, 7,055 (4.56%) were initially reactive and 6,786 (4.38%) were reactive in the repeated tests. One tested positive in the PCR assay and showed a very low level of parasitemia. In Switzerland[11], 12,887 donors, who had traveled to regions at risk for malaria, were screened for antibodies against the *Plasmodium* species. Of these samples, 1,011 were reactive and a further 152 fell within the gray zone of the assay. The prevalence of malaria antibodies was 9.02%. Between 2005 and 2011 in the USA[12], 103 (1.84%) samples were found to be initially reactive and 88 (1.57%) were reactive in the repeated test for malaria antibodies in 5,610 donors at risk for malaria; none was tested positive for malaria DNA. In India[13], the prevalence of malaria antibodies in eligible donors was 16.9%. None of the donors was positive for malaria on microscopic examination. In our investigation, blood donors were selected according to their ID numbers from different provinces. Most of them came from historically endemic provinces. The donors did not report their medical histories, and most of them were not at risk for malaria. This is the reason for the low prevalence (2.13%) of malaria antibodies in our subjects, compared to those of other countries. Other reasons for the prevalence variation might be differences in climate, environmental factors, endemic status of the region, and the EIA kits used.

There are different strategies for reducing the risk of TTM. These include screening the blood donors using questionnaires or by direct parasite detection, antibody/antigen testing, and nucleic acid testing. Microscopy is a reference method to diagnose malaria parasitemia in endemic areas, but it cannot identify "semi-immune" donors with a very low level of parasitemia. Moreover, microscopic techniques are inappropriate for universal screening of blood donors because it is time-consuming and requires significant expertise and specialized equipment. In endemic countries, the detection of plasmodial DNA by PCR has been suggested as a method to screen infectious donations with low parasitemia doses[14,15]. Retrospective analyses of implicated donors have confirmed the presence of high titers of antibodies in these individuals[16]. The method used in this study demonstrated sufficiently high sensitivity and specificity to screen at-risk donors in non-endemic areas, but cannot be used to diagnose acute malarial infection. Moreover, a negative result in the malaria antibody test cannot guarantee that the donor is not infected. It may also result in the unnecessary rejection of donors.

Our results illustrated that the prevalence of malaria antibodies was no higher than expected, even in donors from regions where malaria is endemic. Additionally, parasitemia was not detected even once, and none tested positive for *Plasmodium* DNA in the PCR assay. The number of blood donors is estimated to be less than 1% of the total national population. Donor deferral will further reduce repeat donations and universal serological screening is impossible. In this study, follow-up investigations were not conducted, and none of the donors was deferred. Hence, the deferral of malaria-risk donors still relies on the deferral guidelines, and, for a long time, this has been the only method to prevent TTM in China. Donors may give inaccurate information intentionally or unintentionally because they misunderstand the questions or are unaware or have forgotten that they have previously had contact with malaria. Thus, it would not be sufficient to prevent TTM by questionnaires[17,18]. Considering the limitations and cost of screening reagents and methods, setting up appropriate deferral strategies can reduce the risk of TTM to a minimum, providing that they could be properly applied. This can also be achieved by improving the quality of the questionnaire and the interview

techniques, and may require carefully explaining the risk of malaria infection to the donors. Further investigations are of importance to provide elaborate data that might help the authorities to make better-informed decisions regarding donor deferrals.

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AUTHORS CONTRIBUTIONS

HL, SWZ and JS conceived, designed, and supervised the study. HL wrote the draft of the manuscript. SJZ and SWZ performed the serological screening and LS performed the PCR test. LS and NZ contributed to data analysis. CYH and JS revised the manuscript. All authors read and approved the final manuscript.

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