Platform for *Plasmodium* detection in Blood Donors from Endemic and Non-Endemic Brazilian areas: Processing of Pooled Samples using Molecular and Serological Markers

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INTRODUCTION

Malaria transmitted by blood transfusion remains one of the most important infections for hemotherapy services. In Brazil the incidence of malaria by blood transfusion is unknown, and this may contribute to the spread of the disease through cases of failure in the clinical and epidemiological screening or due to asymptomatic donors. Transfusion cases reported in Brazil revealed that donors that caused transfusion malaria had very low parasitaemia, with an estimated rate of 1 to 10 parasites per unit of blood. This requires sensitive methods for diagnosis and prevention. To estimate the frequency of Brazilian blood donors harboring Plasmodium, we used molecular tools and a Rapid Diagnostic Test (RDT) for antibody detection.

MATERIAL AND METHODS

On this retrospective study, that included 147 Brazilian public or private blood banks located in endemic and non-endemic areas for malaria, 13,383 blood donors approved for donation by local screening methods were analyzed for detection of Plasmodium (Fig. 1). Samples were grouped into pools of 10 specimens each (Fig 2) and were processed for genuss detection by three different real-time PCR: Lima qPCR performed in 1,299 pools, PET-qPCR in 1,212 pools and RealAMP performed in positive pools and in 10 negative pools by the other techniques, respectively. Nested PCR was performed for species identification of the positives. Positive pools were disassociated and individual assays were performed to detect positive donors. The RDT SD Bioline Pf/Pv (Standard Diagnostics, Inc.), for antibody detection using recombinant MSP and CSP for *P. vivax* and *P. falciparum*, was applied to pools with samples from endemic areas and pools with samples from non-endemic areas that were obtained randomly among the samples collected.

RESULTS

- 43,299 pools presented amplification of Plasmodium by Lima qPCR, showing a positivity of 3.31% (CI 95%; 2.47-4.43), with positivity of 4.72% (CI 95%; 2.03-10.57) for the Amazon Region and 3.19% (CI 95%; 2.34-4.35) for the extra-Amazon Region (Fig. 3). Table 1 shows the results of all molecular techniques in the pools. Positivity rate for endemic and non-endemic regions are presented in Table 2.
- Nested PCR was able to identify species in 7 pools, all related samples were from the extra-Amazon Region: *P. malariae* (Santa Catarina), *P. falciparum* (Mato Grosso do Sul, 1 Minas Gerais), *P. vivax* (1 São Paulo, 1 Rio Grande do Sul, 1 Paraná, 1 São Paulo/Paraná).
- 25,360 individual samples presented amplification of Plasmodium by Lima qPCR, a positivity rate of 6.94% (CI 95%; 4.75-10.05); the Amazon Region presented 2.50% (CI 95%; 0.44-12.8), while the extra-Amazon Region showed 7.50% (CI 95%; 5.09-10.92) positivity (Fig. 3). Table 1 shows the results of all molecular techniques in the individual blood donors samples.
- Nested PCR identified 8 donor from Santa Catarina and 8 in one donor from Mato Grosso do Sul.
- The RDT SD Bioline Pf/Pv detected antibodies against Pv MSP and CSP recombinant antigens in 3/2 µL pools from the endemic region (1-Bondão, 2-Tocantins), showing 9.38% (CI 95%; 3.24-24.22) positivity; and 13/166 pools from non-endemic region (2-Goiás, 1-Alagoas, 1-Pará, 1-Paraíba, 1-Santa Catarina, 4-São Paulo), showing 7.83% (CI 95%; 4.63-13.54) of positivity (Fig. 4). All pools showed negative results for Pf.

DISCUSSION

This is the first nationwide study that assayed thousands of donors from endemic and non-endemic areas for malaria that were tested for specific markers and for prevalence of Plasmodium infection in donors accepted for donation. Limitations of the study are due to the discontinuation of SD Bioline Pf/Pv in Brazil that interfered with the individual analysis of positive pools. Storage conditions and no availability of individual red blood cells samples, prevented individual molecular analysis of some positive pools. The prevalence rate for malaria in blood donors revealed by this study can assist strategies for blood screening. Blood donors infected with Plasmodium are often asymptomatic, presenting low parasitemias; moreover, Plasmodium can survive in red blood cells stored between 2 and 6°C for up to three weeks, increasing the risk of transmission. This study revealed an alarming positivity in donors from the non-endemic area in agreement with the areas where autochthonous cases are described, showing that this region is vulnerable to the transmission of malaria and should be considered for control measures. Clinical and epidemiological screening using questionnaires with specific questions about displacement or residence in endemic areas, despite the level of transmission, combined with sensitive molecular tests, may be a safer alternative for blood therapy services, avoiding selection of asymptomatic and unnecessary rejection of donors. Lima qPCR that showed the best performance and was able to identify Plasmodium in pools of 10 samples, reducing time and cost of processing, followed by nested PCR for identification of species in the positive pools and individual positivities would be suitable for a platform for malaria screening in blood donors from Brazil.