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SHORT COMMUNICATION

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Chikungunya and Mayaro infective viruses in components of blood

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Abstract

Objective: Evaluate the infectivity of Alphavirus Chikungunya and Mayaro in blood products in plaque forming units (UFP/ml).

Background: Arboviruses are responsible for sporadic diseases or epidemics which cause serious public health issues. Due to the high number of asymptomatic infections and high viremia, blood donors may pass on these viruses by transfusion.

Methods/Materials: This study used blood bags that would be discarded after evaluation and certification of the absence of infections. The blood products obtained by centrifuging a unit of whole blood were called blood components. All blood bags were infected with viable viruses (previously quantified) compatible with Chikungunya and Mayaro viremia.

Results: Blood bags inoculated with both Chikungunya and Mayaro viruses were able to keep infective viruses during the processing of blood products (red blood cell concentrate, platelet concentrate and fresh frozen plasma) and also after the recommended storage for each component, which may infect individuals transfused with those.

Conclusion: The results indicate that in order to prevent infections by Mayaro and Chikungunya viruses in blood products it is necessary to stimulate the development and use of diagnostic tests for these pathogens in donated blood.

KEYWORDS
Alphavirus Chikungunya, *Alphavirus* Mayaro, arbovirus, blood, components, viremia

1 | INTRODUCTION

Blood transfusion is an important lifesaving medical act based on transferring blood from one individual to another in order to compensate patient's blood losses.[1] Thus, there are strict blood donor screening criteria to minimise the risk of transmission and spread of infectious diseases.[2] Many arboviruses circulate in Brazil producing outbreaks or sporadic cases with important social and economic impacts. However, contamination by these pathogens are not taken into consideration by Brazilian blood banks.[3] Chikungunya (CHIKV) and Mayaro (MAYV) may be included among the most important arboviruses circulating in Brazil. Despite not being highly lethal, both viruses cause acute febrile episodes and also painful and disabling joint disorders that may last for months or years.[4,5] CHIKV and MAYV are positive single-stranded RNA Alphavirus (Togaviridae) with a genome of 11.5 kb that contain two genes encoding four non-structural proteins (nsP1 to nsP4) and five structural genes (C, E3, E2, 6K, E1).[6]

CHIKV is known to affect the safety of blood transfusions, especially during epidemics.[7] In the Caribbean Island of Saint Martin, in 2014, of 2149 plasma samples evaluated in a blood bank during a CHIKV outbreak, 4 were positive for CHIKV genome and 2 of the plasma donors manifested febrile syndrome after donation.[8] Estimation rates of asymptomatic people infected with CHIKV range from 3% to 28%[9] and these asymptomatic individuals have a high probability of donating contaminated blood.[7,10–12] For the emerging American

arbovirus MAYV there are no reports of contamination of blood products.

This study shows that MAYV and CHIKV are capable of infect possible blood donors and remain viable in blood products even after their processing and storage, leading to a risk of viral transmission when these products are transfused.

2 | MATERIALS AND METHODS

The Mayaro virus strain BeAr20290 (MAYV) and the Chinkungunya virus strain S27-African (CHIKV) were used to infect African green monkey kidney (VERO) cells (ATCC, CCL81). VERO cells were maintained in DMEM (Vitrocell, Brazil) supplemented with 10% fetal bovine serum (Vitrocell, Brazil), 1% antibiotic and antimycotic solution (100 U/ml penicillin, 1 mg/ml streptomycin, Vitrocell, Brazil). For infection experiments, confluent monolayers of VERO cells in 75 cm^2 vials (Corning, USA) were washed with PBS and then inoculated with the virus. Infected flasks were incubated at 37°C and under gentle shaking for 1 h and had DMEM supplemented with 2% fetal bovine serum added. Infected cells were kept at 37°C and 5% CO_2 until appearance of cytopathic effect. At this point the medium in the infected flasks was harvested, centrifuged (1000 rpm, at 4°C for 20 min), aliquoted and stored at −80°C until the time of use. VERO cell infections with CHIKV and MAYV were confirmed by indirect immunofluorescent test and virus presence in the medium harvested was confirmed by RT-PCR.[13-15]

2.1 | Virus quantification

CHIKV and MAYV in the virus samples and in the blood products were quantified in plaque forming units per ml (PFU/ml).[15] Plates of 24-wells were seeded with 2×10^5 VERO cells per well and incubated for 24 h at 37°C. Following, wells having confluent cell monolayers were washed with PBS and inoculated with 1 ml of each decimal dilution of the viral solution, ranging from 10^1 to 10^{10}. Plates were then incubated for 1 h at 37°C under gentle shaking and 1 ml of an overlay solution including DMEM supplemented with 3% sterile carboxymethylcellulose (Sigma-Aldrich, USA) and 2% fetal bovine serum (Vitrocell, Brazil) was added to the wells. The plates were incubated at 37°C and 5% CO_2 until appearing of lysis plaques when subsequently the overlay solution was discarded and the cells were fixed in each well with a 10% formaldehyde solution in PBS and finally stained with naphthalene black (Sigma-Aldrich, USA). Clearly visible plaques were counted and the viral titre was determined in PFU/ml as previously mentioned.[16]

2.2 | Inoculation of MAYV and CHIKV in blood components

This study was approved by two Research Ethics Committees: the one in Santa Casa of Sao Carlos and the one at the General Hospital of the School of Medicine of Ribeirão Preto of the University of São Paulo (protocol n^0 2019/3.252.505).

Blood bags were obtained from 8 healthy donors under approval in an informed consent form. All experiments were done in duplicate using 2 blood bags for CHIKV, 2 bags for MAYV and 4 bags for negative control. The blood bags with 450 ml each were inoculated with 10^6 PFU/ml for CHIKV or 10^7 PFU/ml for MAYV. After virus inoculation, the bags were kept for 12 h under gentle shaking at room temperature. Twelve hours is the regular time for processing a blood bag and also an exceeding time for viral replication. Negative controls were inoculated with culture medium only.

Twelve hours after virus inoculation, the whole blood bags were centrifuged (2600 g, 3.5 min) in order to obtain red blood cells and plasma rich in platelets. After this process, a second centrifugation of the platelet rich plasma (5000 g, 4 min) was performed in order to separate fresh plasma and platelet concentrate. Each unit of blood product was derived from a single donor.

Samples for quantification of MAYV and CHIKV were collected from freshly prepared blood products and at different times of their storage including a sample collected at maximum storage time.

3 | RESULTS

For all experiments, duplicate results of levels of CHIKV or MAYV were similar and averages of both values are shown in Figure 1. Freshly prepared platelet concentrates showed 10^5 PFU/ml for CHIKV and 10^4 PFU/ml for MAYV. Viruses levels were relatively stable during all 5 days of the storage period at 20°C to 28°C under

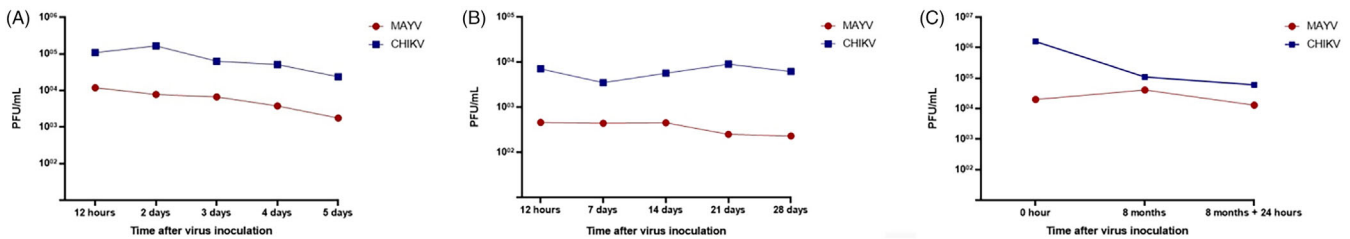

FIGURE 1 Temporal evolution of CHIKV and MAYV infective levels in freshly prepared blood products and after storage. (A) Concentrate of platelets. (B) Concentrate of red blood cells. (C) Fresh frozen plasma

gentle shaking in an incubator. Remaining similar at the fifth day with $10^{4.6}$ PFU/ml for CHIKV and $10^{3.6}$ PFU/ml for MAYV, as shown in Figure 1A. Freshly prepared concentrates of red blood cells showed $10^{3.9}$ PFU/ml for CHIKV and $10^{2.7}$ PFU/ml for MAYV. Viruses levels were stable in samples collected during 28 days of storage at 4°C, remaining $10^{3.7}$ for CHIKV and $10^{2.5}$ for MAYV on the last day, as shown in Figure 1B. Fresh plasma samples showed $10^{6.2}$ PFU/ml for CHIKV and $10^{4.3}$ PFU/ml for MAYV. Viruses levels after a 8 month frozen period at −20°C followed by 24 h at room temperature were $10^{4.9}$ for CHIKV and 10^{4} for MAYV, as shown in Figure 1C.

4 | DISCUSSION

The transmission of some arboviruses in blood transfusions is recognised in some viruses such as dengue, West Nile and Chikungunya, but little is known about this type of transmission.[7,8,17,18]

The protocol currently used for processing donated blood bags aims at reducing the risk of viral transmission by transfusion of blood products and include: screening to obtain clinical and epidemiological information that allows the exclusion of potentially infected donors, detection of viruses by the presence of the virus itself and/or nucleic acid testing (NAT) in bags of donated blood.[19]

In this study, MAYV (10^{7} PFU/ml) and CHIKV (10^{6} PFU/ml) viruses were inoculated in 450 ml of concentrated whole blood. The levels of both viruses were compatible with those observed in asymptomatic infected individuals.[10] These blood bags were processed following the observed protocol in blood banks. Before storage, the platelet concentrates showed 10^{5} PFU/ml for CHIKV and 10^{4} PFU/ml for MAYV; red cell concentrates showed $10^{3.9}$ PFU/ml for CHIKV and fresh plasma showed $10^{4.3}$ PFU/ml for MAYV. Once inoculated and diluted 450-fold in packaged blood, the virus level found for MAYV was expected but when looking at CHIKV levels, it is possible to note a high titre of virus. A high level of CHIKV was observed in fresh plasma, $10^{6.2}$ PFU/ml. The results showed the maintenance of the viruses inoculated in blood components, with a slight increase in titres to blood bags inoculated with CHIKV, indicating the possibility of mild viral replication. Other authors have observed DENV replication in red blood cell concentrates.[20,21]

Although the proportion of symptomatic to asymptomatic cases caused by CHIKV varies in different endemic regions,[10,11] donation from asymptomatic or pre-symptomatic donors can become a source of potential contamination for different blood products.[11] Another study conducted by our research group showed the presence of IgM for CHIKV and MAYV in healthy blood bank donors in the city of Sao Carlos, Brazil, suggesting the maintenance of the virus circulation and active infected donors enabling transmission to recipients from blood bags.[22]

Although the penetration of CHIKV into blood systems has been established by other research groups,[10] it is not known whether this pathogen remains infectious during the storage of certain blood products. For three types of blood cells, CHIKV and MAYV will remain infectious during storage, consistent with reported cases of the transfusion-transmitted disease.

Despite the absence of clinical reports and studies that prove the transmission of these viruses through blood bags, in this study it was possible to observe, through in vitro infection, the maintenance of the infective capacity of Chikungunya and Mayaro viruses in blood bags and blood components. Thus other publications about these viruses by our group, and other studies with Dengue virus,[20] alert for a closer look at the selection of blood donors travelling to endemic areas, especially during outbreaks of these arboviruses. Thus, the implementation of measures to prevent the spread of Chikungunya and Mayaro viruses in blood banks are guidelines to be considered during outbreaks and epidemics. Some institutions, such as the French blood services, have successfully implemented the systematic surveillance of CHIKV RNA for platelet donors.[8,9,11,12] The lack of resources for pre-donation screening, therefore, was responsible for creating a significant challenge to ensure transfusion safety. Other blood bag collection and screening centres carry out differential research for endemic viruses and of local clinical interest. More recently, an internal NAT for blood donations in Caribbean territories, showed a rate of 1:500 viremic units.[23] The West Nile NAT test was applied for the first time in the USA and Canada and was also extended to the regions of Veneto-Friuli-Venice-Giulia and Sardinia, among other areas of Europe in a period of 1 to 2 years of the outbreak.[23,24]

It is of great importance to implement proactive surveillance of emerging viral infections in blood donor populations from different parts of the world, especially in those geographic areas with high rates of endemic regions. After the identification of a new agent, a process for evaluating its transmission routes by blood transfusion and its potential pathogenicity according to the immune status of the recipients should be implemented. Moreover, the risk assessment will help to define the implementation of new preventive strategies to reduce transfusion transmission risk of this emerging agent.

As previously described, genomic surveillance was essential for the implementation of NAT tests in countries with endemic areas, so the development of suitable nucleic acid detection assays for arboviruses presents itself as the ideal scenario and can be valuable in providing a higher level of transfusion safety.[24,25]

5 | CONCLUSION

This study shows the maintenance in vitro of infectious CHIKV and MAYV viruses. In this way, these viruses can remain viable after the processing of blood components, in platelet concentrate, red cell concentrate and plasma, even after the storage recommended by guidelines of blood banks. The maintenance of infective viruses in blood components brings the possibility of accidental transmission of these viruses through blood donation. Further studies on the susceptibility of blood components to infection by these Alphaviruses and on their circulation in asymptomatic blood donors are necessary. Due to the risk of this possible transmission, it may be in the public interest to

screen healthy blood bank donors at the time of outbreaks caused by arboviruses that are neglected in the blood bank context.

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CONFLICT OF INTEREST

The authors have no competing interests.

AUTHOR CONTRIBUTIONS

Ana Beatriz dos Anjos Souza and Luiz Tadeu Moraes Figueiredo contributed to the design of the study. Ana Beatriz dos Anjos Souza collected blood packs in the Blood Bank of São Carlos City. The laboratory work and data analysis was performed by Ana Beatriz dos Anjos Souza, William Marciel de Souza, Victoria Thomazelli Garcia, and Luiz Tadeu Moraes Figueiredo. All the authors critically revised the work and approved the final version.

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