Zika Virus and the Safety of Blood Supply in Brazil: A Retrospective Epidemiological Evaluation

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Abstract. The potential for transfusion transmission of dengue virus (DENV), chikungunya virus (CHIKV), and Zika virus (ZIKV) has raised concerns about the safety of the blood supply in endemic areas. In this study, nucleic acid testing (NAT) for ZIKV, DENV, and CHIKV RNA was performed in asymptomatic blood donor samples in the city of Campinas, located in the southeast region of Brazil (1962 in 2015 and 1775 in 2016). The prevalence of reactive NAT was 0.15% in 2015 and 0.62% in 2016 for dengue, 0.05% in 2015 and 0.17% in 2016 for Zika, and 0% in both years for chikungunya. These results demonstrate the weakness of the clinical interview in screening these blood donors. Furthermore, positivity for ZIKV was detected in March 2015, 1 year before the first reported cases in the region. These data attest the feasibility of using donor samples held in library as a tool for retrospective epidemiological evaluation, which is particularly interesting considering emerging pathogens, for which data on their spread and penetrance are initially scarce.

INTRODUCTION

Zika virus (ZIKV) is a mosquito-borne *Flavivirus* and emerging infectious disease which was considered a public health emergency by the World Health Organization (WHO) from February until November 2016.¹ International concern has arisen about its also well-described congenital and sexual transmission.^{2–4} The potential for ZIKV transmission through blood transfusion was demonstrated during the ZIKV outbreak in French Polynesia as researchers found that 3% of asymptomatic blood donors were infected with ZIKV in 2013–2014.⁵ In Brazil, possible cases of viral transmission through blood transfusions were investigated in 2016.^{6,7} However, no recipients had signs or symptoms that could be credibly related to ZIKV infection. The current evidence suggests that transfusion-transmitted ZIKV is likely; however, clinical significance has not been established.⁸ However, this is a subject worth considering because in the summer of 2016, a high incidence of cases of the disease was observed in Brazil.⁹

Epidemiologically, dengue virus (DENV) and chikungunya virus (CHIKV) together with ZIKV are mosquito-borne viruses (arboviruses) transmitted to humans by mosquitoes belonging to the *Aedes* genus. Dengue virus poses only a marginal risk to the blood supply despite of its high prevalence in tropical regions of the world. Dengue has been shown to be transfusion-transmitted and clinical manifestation, although rare, do occur.^{10–12} No cases of chikungunya transmission have been described after blood transfusions; however, there is concern because of the many similarities with DENV, including the same routes of transmission.¹³

Blood donor screening on the basis of a questionnaire, without a laboratory test, is insufficient for identifying ZIKV-infected donors in areas with active mosquito-borne transmission because of high rates of asymptomatic infection.¹⁴ Zika virus diagnosis is primarily based on the detection of viral RNA by reverse transcription polymerase chain reaction (RT-PCR). A

recent study demonstrated an incidence of 0.5% of viremic donors in Puerto Rico, from April to June of 2016.¹⁵ The viremic period has yet to be better established and may be very short in peripheral blood, allowing direct virus detection only a few days after the onset of symptoms. An immunoglobulin M antibody response in ZIKV-infected patients has been reported; however, cross-reaction with other flaviviruses, including DENV, is common.¹⁶

Transmission of the arboviruses ZIKV, DENV, and CHIKV in Brazil can occur during all months of the year because of the tropical weather. The concurrent arbovirus epidemic and overlapping of endemic regions invoke the need of differential diagnosis for Zika, dengue, and chikungunya. Thus, the aim of this study was to evaluate retrospectively, by nucleic acid testing (NAT), the presence of ZIKV, DENV, and/or CHIKV RNA in the blood of asymptomatic donors, comparing the summer periods of 2016 with that of 2015, when no ZIKV epidemic had yet been reported in the southeast region of Brazil.

MATERIAL AND METHODS

Subjects and sample collection. Plasma samples from blood donors were collected in the city of Campinas, located in the state of São Paulo, in the southeast region of Brazil. The population size of Campinas is 952,659. Molecular testing was performed retrospectively in two time points corresponding to the summer period in the region, when there is a significant increase in the cases by the proliferation of the transmitting mosquito. The samples were evaluated as follows: 1,775 donors in 2016, March and 1,962 donors in 2015, March. The samples were collected sequentially from all donor candidates present at the collection unit on four consecutive Fridays of March 2015 and 2016, 52% of samples were from females and 48% were from males. The samples collected in 2016 were processed the day after the collection, and those of 2015 were stored at –30°C in freezers with strict temperature control (approximately 1 year of storage).

According to the standard procedures of blood banks in Brazil, all donors underwent a predonation questionnaire, and blood was collected only from voluntary donors who were asymptomatic at the time of donation. A signed informed

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consent statement was obtained from all participants (the informed consent term for blood donation contemplates the possibility of performing tests for emerging pathogens, even if not mandatory by law).

Molecular testing. One hundred microliters of plasma from each donor was pooled into a pool of six samples; this volume (600 μ L) was submitted to RNA extraction using the MDX equipment (Qiagen, Hilden, Germany), with a final eluate volume of 65 μ L, from which 15 μ L was used for PCR.

A multiplex real-time PCR was performed using the molecular assay Zika, dengue, and chikungunya (ZDC) developed by Bio-Manguinhos/Fiocruz (National Health Surveillance Agency [ANVISA] registration number 80142170032) for the detection of Zika, chikungunya, and dengue on clinical samples, according to the manufacturer's instructions. Briefly, the multiplex real-time PCR setup was performed automatically with JANUS instrument (PerkinElmer, Waltham, MA) and the amplification was carried on the sequence detection system ABI7500 (ThermoFisher Scientific, Waltham, MA). Positive controls for Zika, chikungunya, and dengue were used for all assays. The samples were tested in minipools of six, using 15 μ L of nucleic acid in each reaction. An internal control (IC, patent PI0600715-5; Bio-Manguinhos, Rio de Janeiro, Brazil) was used to control all steps and reactions. The RNA extraction and real-time PCR process were considered to be valid if the IC was positive.

The amplification conditions were 30 minutes at 51°C, 10 minutes at 95°C and, 40 cycles of 30 seconds at 95°C and 1 minute at 60°C. Samples were considered negative if the IC was positive but the signals for Zika, chikungunya, or dengue were negative.

The molecular assay ZDC (Bio-Manguinhos) is a commercial product, registered by the Brazilian regulatory agency ANVISA. Probit analysis reported for its registration revealed a 95% limit of detection (LOD) of 8.99 copies/mL and 50% LOD of 4.42 copies/mL for ZIKV RNA; the 95% LOD to chikungunya was 480 copies/mL and the 95% LOD to Dengue was 100 copies/mL, calculated with sample one from the commercial panel of dengue (Dengue Early Infection AccuSet Performance; Seracare Milford, MA). The LOD assay was performed with individual samples, from the "Collaborative study for the characterization of a chikungunya virus RNA reference reagent for use in nucleic acid testing"¹⁷ and "Harmonization of nucleic acid testing for Zika virus: development of the 1st World Health Organization International Standard."¹⁸ The copy number was established by Droplet Digital PCR (ddPCR; BioRad, Hercules, CA) because by the time of this study, the standards had not yet been established. The analysis of nine batches of the product with 828 samples true negative and 972 samples true positive revealed a specificity of 99.6–100% (confidence interval: 95%).

Reactive minipools were resolved by testing the individual members of the reactive pool; individual sample testing was performed using a new RNA extraction of each individual sample, and the amplification curve and the multicomponent results were evaluated (the positive samples had cycle threshold [Ct] < 30).

RESULTS

Blood donor samples were tested in minipools of six samples. All positive pools resulted in one individual reactive

TABLE 1
Results of molecular testing for dengue virus, Zika virus, and chikungunya virus in blood donors' samples in the city of Campinas, Brazil, in March 2015 and March 2016

	2015 (n = 1,962)	2016 (n = 1,775)
Dengue	3 0.15% (0.05–0.44)	11 0.62% (0.35–1.11)
Zika	1 0.05% (0.01–0.29)	3 0.17% (0.06–0.5)
Chikungunya	0 0% (0–0.2)	0 0% (0–0.22)

Values are presented in absolute number, percentage, and 95% confidence interval.

sample. The positive samples had Ct < 30 (cutoff of the ZDC test is Ct < 38) and the IC was in between the acceptable Ct values. Table 1 presents the results of the multiplex real-time PCR assay.

The Epidemiologic Surveillance Agency of Campinas published official reports with 65,634 confirmed cases of dengue in the period from January 1 to October 31, 2015, and 3,388 from January 1 to October 31, 2016. Regarding ZIKV, in 2016, 500 cases were confirmed (44 confirmed by laboratory testing and 456 confirmed by clinical and epidemiological criteria). In respect of chikungunya, 41 suspected cases were reported until November 2016: 18 were subsequently considered negative, 17 were still under investigation, and the six confirmed cases consisted of imported infection.⁹

DISCUSSION

Despite the short viremia period, arboviruses have become a threat for transfusion safety because of their high incidences in human populations during outbreaks and mainly because a considerable proportion of those infected individuals remain asymptomatic.^{15,19–21} Given the necessity of detecting donors in the preseroconversion viremic phase of infection, NAT-based assays are recommended by the U.S. Food and Drug Administration, the European Center for Disease Prevention and Control, and the WHO for sensitive and specific detection of ZIKV infection, and particularly for donor screening.^{22–24} In Brazil, those recommendations were made by the Ministry of Health and the regulatory agency ANVISA,⁹ and the data presented in this study emphasize the importance of these tests also in the retrospective evaluation of cases of emerging pathogens, constituting an important ally in the epidemiological investigation.

There are few published case reports of transfusion-transmitted dengue, but it is likely that cases are underreported. During a large epidemic of DENV-4 infection in Brazil, Sabino et al.²⁵ have found that > 0.5% of donations were RNA positive. They postulated that during epidemics in dengue-endemic countries, transmission through transfusion is occurring, with more than one-third of components from RNA-positive donations transmitting infection. According to the Epidemiologic Surveillance Agency of Campinas, the number of confirmed cases of dengue in 2015 was much higher than 2016.⁹ Surprisingly, we have found more DENV RNA-positive samples in 2016 than 2015. This fact can possibly indicate that the virus circulating in 2016 may have caused more asymptomatic infections, making it impossible to recognize these infected donors by clinical screening alone. Another explanation could be an underestimated burden of DENV in 2016. It has been shown that dengue surveillance substantially

underestimate disease burden in Brazil, especially in what are considered low-transmission periods.²⁶

Previous studies following ZIKV outbreaks in French Polynesia and Martinique have detected, by NAT, 3% and 2% of contaminated blood donations, respectively.²⁷ These rates are quite higher than those presented at our institution, but we have previously reported the case of a probable transfusion-transmitted ZIKV in a patient submitted to liver transplantation. The blood donor made a voluntary telephone call 3 days after donation informing he started a febrile illness following donation. The patient had been transfused with a platelet concentrate from the infected donor, but medical evaluation excluded any clinical signs or symptoms that could be related to ZIKV.⁷ Although in this case the recipient was not clinically affected by the infection, the prevalence of RNA-positive donations demonstrated in this study highlights the alarm about the potential for morbidity over transfusion recipients, considering the lack of an effective treatment strategy and, subsequently, the possibility of severe illness, especially in cases of immunosuppressed patients.

The autochthonous transmission of ZIKV in the northeastern region of Brazil was confirmed as of April 2015.²⁸ However, the results of our study demonstrate that there was positivity for the nucleic acid of the virus in asymptomatic blood donors' samples in March 2015 in the southeastern region, that is, a month before the time when cases were being reported in a region more than 2,000 km away,^{29,30} and almost a year before the first autochthonous case was identified in the city of Campinas (January 2016), uncovered after investigation of a blood donor who reported symptoms suggestive of infection days after donation.^{7,31} Interestingly, a recent study also demonstrated positive results for Zika virus in up to 70% of the samples tested by RT-PCR in symptomatic individuals in Mexico who were previously tested negative for dengue and chikungunya, evidencing the presence of the virus in individuals of these sites at least since July 2015.³² Interestingly, the virus appears to have been circulating in Haiti since 2014,³³ and phylogenetic analysis has demonstrated a close relationship with the virus circulating in Brazil. These studies thus demonstrate the complex dispersion of the virus between different continents without a yet fully identified pattern.

Therefore, our results provide data on the penetrance of the virus in the southeast region of Brazil in the periods analyzed and demonstrate the possibility of using donor samples in the library as an important tool for retrospective epidemiological evaluation. This is particularly important in the case of emerging pathogens, where knowledge about their population dynamics is still scarce, allowing for projections and institution of measures against their spread.

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Conflicts of Interest: DR, EA, DTG, and PA are Bio-Manguinhos employees.

Disclosures: DR, EA, and DTG designed the experimental procedures and analyzed its results; DR and EA performed the laboratory tests; BDB and MAC analyzed the results in view of the epidemiologic data; BDB, DTG, and PA wrote the manuscript; and MAC coordinated the study.

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