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Research Article

The Frequency of Zika Virus in a Corneal Donor Population

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Abstract

Purpose: To examine the frequency of Zika virus in the corneal donor population.

Methods: This was a prospective, masked, cross-sectional study May and November 2016 and July and November 2016. Serum and vitreous samples were obtained from cadaveric donors consented with research permission at one of three eye banks: New York, New York, San Juan, Puerto Rico, and Dallas, Texas. Samples were obtained post mortem and shipped to Transplant Services Center, University of Texas Southwestern Medical Center, Dallas, Texas. Serum samples were tested for the presence of IgM and IgG seropositivity using a non-FDA approved immunoassay. A subset of serum samples and all vitreous samples were tested for presence of viral RNA using real time RT-PCR.

Results: Serum antibody testing was performed on a total of 309 samples. Four were positive for IgM, 14 positives for IgG, and 2 positives for both IgG and IgM. The remaining 289 samples were seronegative. The frequency of seropositivity across geographical regions was 3% for New York, 41% for Puerto Rico, and 1% in Texas. All serum samples were negative for viral RNA. Of the 36 vitreous samples tested, only one sample from an IgG positive donor was positive for Zika RNA.

Conclusions: This study is the first to estimate the frequency of Zika virus in the cornea donor population in varying United States cities. In areas endemic for Zika virus, there is a high likelihood of donor seropositivity. It is unknown at this time whether the presence of Zika RNA in the vitreous represents active virus or persistent RNA but does suggest the potential reservoir of virus in the eye. Further studies are needed to determine potential tissue transmissibility.

Keywords: *Cornea; Donor; Transplantation; Virus; Zika; Rna*

Abbreviations

IgM: Immunoglobulin M; IgG: Immunoglobulin G; FDA: Food and Drug Administration; RNA: Ribonucleic Acid; RT-PCR: Reverse Transcriptase Polymerase Chain Reaction; STAT2: Signal Transducer and Activator of Transcription 2; cDNA: Complementary Deoxyribonucleic Acid; CDC: Centers for Disease Control and Prevention

Introduction

Zika virus is a flavivirus and belongs to the same family of viruses that include West Nile virus, Dengue virus, and Yellow Fever virus. The primary mode of transmission of Zika Virus is through the bite of an infected Aedes aegypti mosquito. Unlike other flaviviruses how-

ever, Zika virus is also transmitted via sexual contact and undergoes vertical transmission from mother to fetus in utero [2-6]. In the case of pregnancy, the placenta, amniotic fluid and fetus are all affected, with the greatest effects occurring if exposure takes place during the first trimester [7,8]. Once infected, the virus quickly attacks neural progenitor cells and can then spread to the developing eye, and in some cases, can also cause sensorineural hearing loss [9-14]. Mature cortical neurons and astrocytes in adults are also highly susceptible to infection by Zika virus [1]. Zika virus infects neural cells by antagonizing the signal transducer and activator of transcription [2]. (STAT2), preventing the induction of interferon and an antiviral response [15]. This allows the virus to replicate in stealth mode within the infected neuron.

Zika virus has been shown to be present in human ocular fluids [16-18]. The first human case report of Zika virus RNA present in aqueous humor was accompanied by uveitis and a non-mucopurulent conjunctivitis [16].

Viral transmission has been shown to occur although rarely, by corneal transplantation when tissue is recovered from an infected donor and grafted to an uninfected recipient [19-22]. These finding raise the question of the ability of Zika virus in ocular tissue and/or ocular fluids to transmit from donor to recipient during corneal transplantation. Given the severity of complications arising from Zika viral disease, particularly the high risk for severe birth defects, and the long residence time of Zika virus in body fluids after viremia has cleared, the ability of Zika virus to persist in ocular fluids is of high concern to transplantation biology. In this study, we sought to determine the frequency of Zika virus in the corneal donor population by testing donor serum for the presence of immunoglobulins IgG and IgM. Serum and vitreous were then tested for the presence of viral RNA.

Materials and Methods

This is a prospective, cross-sectional study to determine the frequency of Zika Virus in the corneal donor population. Serum samples were recovered from consecutive cadaveric donor's post mortem between May and November 2016 from three participating eye banks. Donor tissue could only be recovered when research permission was obtained as part of the consent for donation. Vitreous samples were collected between July and November 2016. This included Transplant Services Center at UT Southwestern Medical Center (UTSWMC) in Dallas, Texas, the Eye Bank for Sight Restoration, Inc., New York City, New York, and the Lions Eye Bank of Puerto Rico, San Juan, Puerto Rico. Consent for tissue donation from cadaveric donors was obtained according to standard Food and Drug Administration (FDA) regulations at all three eye banks. All donors underwent a complete screening history per the March 2016 guidance by the FDA. This includes four questions pertaining to travel to Zika endemic areas, in addition to history of a previous diagnosis or symptoms of Zika virus. Since Puerto Rico is classified as an endemic region, only the previous diagnosis or symptoms were exclusionary not the travel questions. Serum and vitreous samples were obtained post mortem and were shipped on ice to Transplant Services Center at UTSWMC stored at 80°C. All samples were numerically coded and the laboratory technicians were masked to location of sample origination.

Zika virus immunoassay

The presence of Zika virus immunoglobulins IgG and IgM were tested using a non-Food and Drug Administration approved test kit from Artron Laboratories Inc. Test kit (Burnaby, British Columbia, Canada). According to the manufacturer, the test kit has a sensitivity of 95% and a specificity of 93%. Testing was performed according to manufacturer instructions. Briefly, 5 µLs of serum and 80 µLs of diluent buffer were inserted into the testing cassette and allowed to develop for 10 minutes at room temperature. The formation of a control band at the top of the cassette confirmed activity of the assay. The formation of separate bands within the cassette window allowed for the positive identification of IgG and/or IgM.

Real Time Reverse Transcription Polymerase Chain Reaction (RT-PCR)

To determine the level of Zika virus RNA in serum and vitreous samples, real time RT-PCR was performed using the protocol from the Centers for Disease Control and Prevention (CDC). Vitreous samples were tested in as masked grouping from which one or more serum antibody test positive samples had been found. RNA was extracted using the QIAmp viral RNA kit (Qiagen, Valencia, CA) according to man-

ufacturer instructions. RNA was measured using a Qubit 3.0 Fluorometer (Fisher Scientific, Houston, TX). RNA was reverse transcribed, and cDNA amplified using the QuantiTect Probe RT-PCR kit (Qiagen, Valencia, CA). Primers and probes were ordered from Integrated DNA Technologies (Coralville, IA). Water only, no template controls, were used as negative controls in all reactions. A positive control that originated at the Communicable Disease Center at Centers for Disease Control and Prevention CDC, was obtained through Dallas Health and Human Services, Dallas, TX. The final 50 µL reaction underwent 1 cycle at 50°C for 30 minutes (RT reaction) followed by 1 cycle at 95°C for 15 minutes to allow for enzyme activation. The cDNA was then amplified for 45 cycles at 95°C for 15 secs followed by 60°C for 1 minute. Primer and probe sequences are detailed in table 1. Test interpretation was performed according to the CDC algorithm as follows: a cycle threshold (Ct) value of < 38 in duplicate wells is read as positive, a Ct value of < 38 in one of two wells is equivocal, and a Ct value of > 38 in duplicate wells is negative. All samples were performed in duplicate. For positive samples, real time RT-PCR was performed again on a subsequent day to ensure reproducibility of the results.

All genotypes	Sequence
Zika1087	CCGCTGCCCAACACAAG
Zika1163c	CCACTAACGTTCTTTTGCAGACAT
Zika1108FAM	AGCCTACCTTGACAAGCAGTCAGACACTCAA
Asian genotypes	
Zika4481	CTGTGGCATGAACCCAATAG
Zika4552c	ATCCCATAGAGCACCACTCC
Zika4507cFAM	CCACGCTCCAGCTGCAAAGG

Table 1: *Primers and Probes for the Asian and all genotype strains.*

Statistical Analysis

All data are expressed as mean ± standard deviation. To determine a difference in the rate of seropositivity across regions, a chi-square test was used. Statistical significance was set at $P < 0.05$.

Results

A total of 309 donors were examined in this study (Figure 1). For serum immunoglobulin testing, 189 samples originated from New York City, 32 samples from Puerto Rico, and 88 samples from Transplant Services at UTSWMC in Dallas, TX (Figure 2). Six serum samples from New York City were positive. Four were IgM positive, indicating that they had just seroconverted, and the other two were IgG positive. Eleven samples from Puerto Rico were IgG positive and 2 samples were both IgM and IgG positive. The single positive sample from Dallas was IgG positive. The calculated frequency of Zika Virus immunoglobulin positivity was 3.2% for New York City, 40.6% for Puerto Rico, and 1.1% for Texas. The increased rate of seropositivity in Puerto Rico, an endemic region, was statistically significant ($P < 0.001$, chi-square). None of the serum samples tested were positive for Zika virus RNA, indicating that none of the donors were viremic at the time of death.

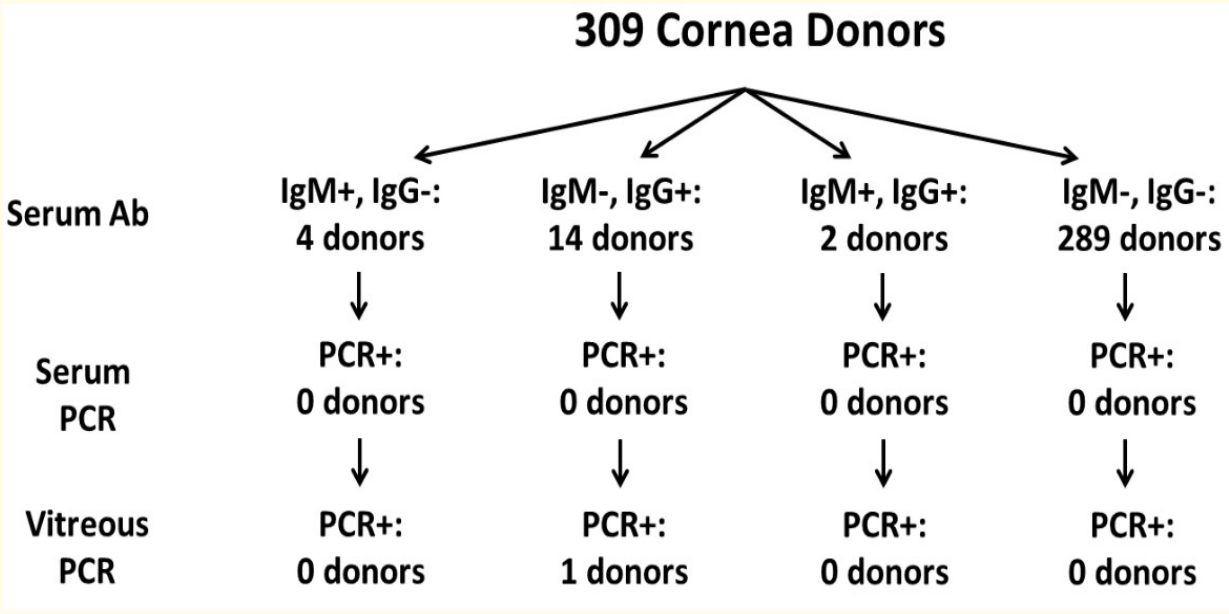

Figure 1: *Experimental workflow. Serum from 309 donors was tested by immunoassay for presence of IgG or IgM. Of those, 92 patients had serum tested for viral RNA using RT-PCR. Additionally, all 36 vitreous samples were also tested for viral RNA using RT-PCR.*

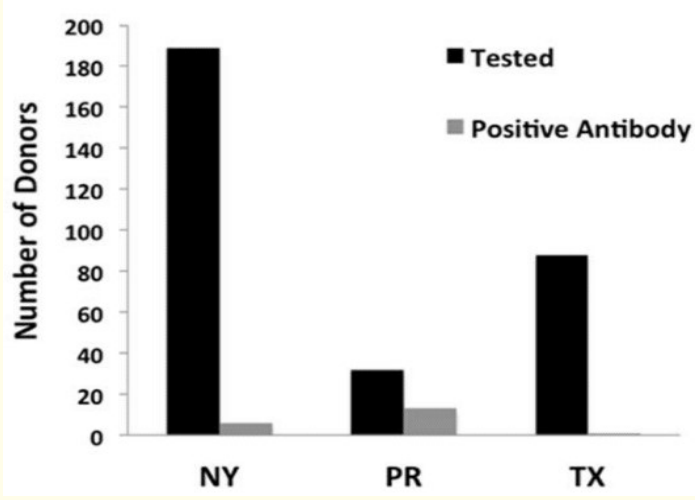

Figure 2: *Distribution of seropositive samples across geographic regions. Puerto Rico, which was an area endemic for Zika virus, had approximately 41% of donors test positive by serology for Zika viral immunoglobulins.*

Real time RT-PCR testing for the 36 vitreous samples, which included all donors that tested positive for immunoglobulin IgG or IgM in serum, resulted in one positive vitreous sample for both the primer/probe sets for the Asian genotype that is circulating within the western hemisphere and for the primer/probe sets recognizing all genotypes. Mean cycle thresholds were 34.29 ± 0.08 and 32.36 ± 0.14 for the Asian genotype and all genotypes, respectively. Subsequent repeat testing was performed to confirm this finding, with cycle thresholds of 32.55 ± 0.20 and 36.32 ± 0.27. As we have previously reported, this patient was from a 56-year-old man in Puerto Rico who expired from multiple gunshot wounds [18]. The patient had no history suggestive of Zika virus infection but was IgG positive at the time of death. There were no signs of active Zika infection in the eye at the time of collection.

Discussion

This is the first study to establish the prevalence of Zika Virus in the eye bank donor population. While none of the donors in this study were viremic at the time of infection, persistence of active virus has been shown to remain in non-ocular body fluids in the post-viremic phase. This includes saliva, urine, semen, cervical mucus, aqueous humor, and in our report, vitreous [1,4,16,18,23] In fact, during the French Polynesia outbreak, saliva was used for testing of Zika virus in infected individuals due to the rapid clearance of the virus from blood within the first 7 - 10 days. [24] Since all corneal donor tissue was transplanted in the study, we were unable to acquire donor corneas for tissue testing. Importantly, other studies have shown that Chikungunya virus, a related arbovirus, has been reported to be present in corneas of non-viremic donors [25]. In that study however, all patients were IgM positive indicating recent conversion to a seropositive state. In contrast, our finding of positive viral RNA in the vitreous was in a donor with serum positive for IgG and negative for IgM. It is not known whether the viral RNA we detected was infectious or just viral remnants that persisted in the ocular immune privileged environment; however, since no recipient complication was reported viral load or viability may have been insufficient for recipient transmission. Thus, further studies are necessary to examine the significance of these findings.

Of high relevance to the cornea donor population is the finding that 41% of cornea donors in an endemic area such as Puerto Rico were positive for IgG and/or IgM. This finding strengthens the need for adoption of a rapid FDA approved test to screen for Zika virus in cornea donors in endemic areas until risk of transmission can be ascertained. In non-endemic areas, we found a frequency of 3% in New York donors and 1% in Texas donors. Further, of the 36 vitreous samples analyzed, 1 sample representing approximately 3% of the vitreous samples were positive for viral RNA. These values are concerning as they are much higher than the those reported for Hepatitis B and Rabies, both viruses that have been shown to undergo transmission from donor to recipient during corneal transplantation [20-22] When considering these findings, it is important to consider the possibility of cross-reactivity associated with immunoglobulin testing. Zika virus antibodies have been shown to cross react with both Dengue and Chikungunya; however, at the time of this study no outbreaks of either Dengue or Chikungunya were reported in the sampling areas.

The ability of Zika virus to undergo vertical transmission to the fetus and infect neural progenitor cells in the eye is of great concern to the transplantation community. Likewise, the long-term neurological consequences of Zika virus infection in adults are not yet established. It is unknown the course that Zika virus will take in the coming years; however, it is possible that Zika virus may potentially become endemic in other areas where the Aedes aegypti are present. This includes a large portion of the United States [26]. Further, it is also unknown whether mutations in the Zika viral genome over time will impact virulence or confer a greater ability of different host strains of mosquitos to carry the virus, as reported with Chikungunya [27].

It is important to note that in this study, all donors died of unrelated natural causes or trauma. All donors underwent a thorough screening for high-risk behaviors and infectious disease in accordance with the FDA regulations for human cells and tissues. Currently, the FDA mandate for Zika virus states that cells or tissues from a donor with known Zika virus infection within 6 months of death are not suitable for transplantation [28]. At the present time, it is unknown whether the corneas that originated from the donor with the positive vitreous sample resulted in Zika virus transmission in either transplant recipient, but no report of complications has occurred. However, since only 20% of Zika-positive patients are symptomatic, it is not possible to determine transmission in the absence of serological testing. Attempts to recover serum from corneal recipients have been unsuccessful.

Conclusion

The finding of Zika virus RNA in the vitreous of a cornea donor in an endemic population heightens concern for the potential for transmission during corneal transplantation. Further studies are needed to determine whether detectable RNA in the vitreous is infectious or represents residual non-infectious RNA that is persisting in an immune privileged site. This finding further emphasizes the combination of a thorough medical and social history during screening and serological testing for optimal assurance of recipient patient safety. Continued efforts to develop and make widely available testing modalities, which can be readily implemented, for donor screening for Zika virus is a critical priority for public health and safety.

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Bibliography

1. Miner JJ and Diamond MS. "Zika Virus Pathogenesis and Tissue Tropism". *Cell Host and Microbe* 21.2 (2017): 134-142.

2. Chouin-Carneiro T., *et al*. "Differential Susceptibilities of Aedes aegypti and Aedes albopictus from the Americas to Zika Virus". *PLoS Neglected Tropical Diseases* 10.3 (2016): e0004543.

3. Foy BD., *et al*. "Probable non-vector-borne transmission of Zika virus, Colorado, USA". *Emerging Infectious Diseases* 17.5 (2011): 880-882.

4. Barzon L., *et al*. "Infection dynamics in a traveller with persistent shedding of Zika virus RNA in semen for six months after returning from Haiti to Italy". *Euro Surveillance* 21.32 (2016): 21.

5. Mansuy JM., *et al*. "Zika virus: high infectious viral load in semen, a new sexually transmitted pathogen?". *The Lancet Infectious Diseases* 16.4 (2016): 405.

6. D'Ortenzio E., *et al*. "Evidence of Sexual Transmission of Zika Virus". *New England Journal of Medicine* 374 (2016): 2195-2198.

7. Cauchemez S., *et al*. "Association between Zika virus and microcephaly in French Polynesia, 2013-15: a retrospective study". *Lancet* 387.10033 (2016): 2125-2132.

8. Reynolds MR., *et al*. "Vital Signs: Update on Zika Virus-Associated Birth Defects and Evaluation of All U.S. Infants with Congenital Zika Virus Exposure - U.S. Zika Pregnancy Registry, 2016". *MMWR Morbidity and Mortality Weekly Report* 66.13 (2017): 366-373.

9. Roach T and Alcendor DJ. "Zika virus infection of cellular components of the blood-retinal barriers: implications for viral associated congenital ocular disease". *Journal of Neuroinflammation* 14.1 (2017): 43.

10. Russo FB., *et al*. "Zika infection and the development of neurological defects". *Cell Microbiology* 19.6 (2017).

11. Tang H., *et al.* "Zika Virus Infects Human Cortical Neural Progenitors and Attenuates Their Growth". *Cell Stem Cell* 18.5 (2016): 587-590.

12. Leal MC., *et al*. "Sensorineural hearing loss in a case of congenital Zika virus". *Brazilian Journal of Otorhinolaryngology* 16 (2016): 30127-30126.

13. Leal MC., *et al*. "Hearing Loss in Infants with Microcephaly and Evidence of Congenital Zika Virus Infection - Brazil, November 2015-May 2016". *MMWR Morbidity and Mortality Weekly Report* 65.34 (2016): 917-919.

14. de Paula Freitas B., *et al*. "Ocular Findings in Infants with Microcephaly Associated With Presumed Zika Virus Congenital Infection in Salvador, Brazil". *JAMA Ophthalmology* (2016).

15. Grant A., *et al*. "Zika Virus Targets Human STAT2 to Inhibit Type I Interferon Signaling". *Cell Host Microbe* 19.6 (2016): 882-890.

16. Furtado JM., *et al*. "Uveitis Associated with Zika Virus Infection". *New England Journal of Medicine* 375 (2016): 394-396.

17. Miner JJ., *et al*. "Zika Virus Infection in Mice Causes Panuveitis with Shedding of Virus in Tears". *Cell Reports* 16.12 (2016): 3208-3218.

18. Heck E., *et al*. "Zika virus RNA in an asymptomatic donor's vitreous: risk of transmission?". *American Journal of Transplantation* 17.8 (2017): 2227-2228.

19. Dubord PJ., *et al*. "Eye banking and corneal transplantation communicable adverse incidents: current status and project NOTIFY". *Cornea* 32.8 (2013): 1155-1166.

20. Srinivasan A., *et al*. "Transmission of rabies virus from an organ donor to four transplant recipients". *The New England Journal of Medicine* 352.11 (2005): 1103-1111.

21. Vetter JM., *et al*. "Survival after transplantation of corneas from a rabies-infected donor". *Cornea* 30.2 (2011): 241-244.

22. Hoft RH., *et al*. "Clinical evidence for hepatitis B transmission resulting from corneal transplantation". *Cornea* 16.2 (1997): 132-137.

23. Barzon L., *et al*. "Isolation of infectious Zika virus from saliva and prolonged viral RNA shedding in a traveller returning from the Dominican Republic to Italy, January 2016". *Euro Surveillance* 21.10 (2016): 30159.

24. Bonaldo MC., *et al*. "Isolation of Infective Zika Virus from Urine and Saliva of Patients in Brazil". *PLOS Neglected Tropical Diseases* 10 (2016): e0004816.

25. Musso D., *et al*. "Detection of Zika virus in saliva". *Journal of Clinical Virology* 68 (2015): 53-55.

26. Couderc T., *et al*. "Chikungunya virus infection of corneal grafts". *The Journal of Infectious Diseases* 206.6 (2012): 851-859.

27. "Estimated range of Aedes albopictus and Aedes aegypti in the United States". [Centers for Disease Control and Prevention web site] (2017).

28. Tsetsarkin KA., *et al*. "A single mutation in Chikungunya virus affects vector specificity and epidemic potential". *PLOS Pathogens* 3 (2007): e201.

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