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Detection of Zika virus RNA in semen of asymptomatic blood donors

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Abstract

Objectives—Zika virus (ZIKV) transmission through semen donation has never been reported but the risk is supported by the detection of ZIKV in semen and the demonstration of ZIKV sexual transmission. The potential impact of ZIKV on assisted reproductive procedures should be evaluated.

Methods—We tested longitudinally collected semen samples provided by asymptomatic blood donors who tested positive for ZIKV RNA in plasma during ZIKV outbreaks in Puerto Rico and Florida in 2016.

Results—Five of the 14 (35.7%) asymptomatic blood donors provided semen samples that tested positive for ZIKV RNA, with ZIKV RNA loads ranging from 8.03×10^3 to 2.55×10^6 copies/mL. Plasma collected at the same time as the semen tested negative for ZIKV RNA for most ZIKV RNA-positive semen collections; all corresponding plasma samples tested positive or equivocal for anti-ZIKV IgG antibodies and all except one tested positive for ZIKV IgM antibodies. The rate of detection of ZIKV RNA in semen in asymptomatic donors is not significantly different from the rate previously reported for symptomatic patients.

Conclusions—Our results that show a high percentage of detection of ZIKV RNA in the semen of asymptomatic men confirm that ZIKV is a new threat for reproductive medicine and should have important implications for assisted reproductive technology. We recommend that semen donations from men at risk for ZIKV infection should be tested for ZIKV RNA, regardless of symptoms of ZIKV infection.

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Transparency declaration

The authors have declared that they have no conflicts of interest.

Keywords

Blood-borne infections; Emerging diseases; Sexually transmitted; Vector-borne infections; Zika virus

Introduction

Zika virus (ZIKV) is mainly transmitted through mosquitoes but sexual transmission has also been reported, mostly from men, with only one case of suspected female-to-male transmission [1–3]. ZIKV transmission through medical procedures involving reproductive cells, tissues and organs has not been reported, although there is a high level of concern over a potential risk, particularly for semen donations [4]. The risk of ZIKV transmission by donated semen used in assisted reproductive technology (ART) is supported by the detection of ZIKV in cultures of human semen, and the demonstration of ZIKV sexual transmission in humans and mouse models [1,2,5]. There are several gaps in the knowledge of ZIKV transmission by semen: what is the percentage of ZIKV RNA-positive and culture-positive semen samples from men who had asymptomatic infections; what is the duration of detection of ZIKV RNA and of infectivity in semen; are ZIKV RNA-positive semen samples infectious by sexual transmission even if *in vitro* cellular cultures of semen are negative; is testing for ZIKV RNA or antibodies warranted for ART semen donations from men potentially exposed to ZIKV; and what is the impact of ZIKV infection on male fertility?

To answer some of these questions, we tested longitudinally collected semen samples provided by asymptomatic blood donors who tested positive for ZIKV RNA during ZIKV outbreaks in Puerto Rico and Florida in 2016. Although the study is ongoing, we are reporting interim results because they could significantly impact guidelines for the prevention of sexual transmission of ZIKV and for policies for testing ART donations for ZIKV.

Materials and methods

Nucleic acid testing (NAT) of blood donations was implemented in Puerto Rico in April 2016 and in Florida beginning in August 2016 [6,7]. Routine NAT by RT-PCR was performed on plasma samples using the Roche (Basel, Switzerland) cobas™ Zika test under a US Food and Drug Administration approved investigational protocol. Detection of IgM and IgG antibodies against ZIKV was performed as previously reported [7].

Donors that tested positive for ZIKV RNA in plasma were contacted and asked to provide additional research samples (whole blood, urine, semen and saliva) collected at different intervals (6–181 days) after the ZIKV NAT-positive index donations. Specimens were shipped overnight at ambient temperature to the Blood System Research Institute, San Francisco, CA, USA, where they were processed into frozen aliquots within 24 h of collection. Aliquots of 0.3 to 0.5 mL of semen were sent under code from the Blood System Research Institute on dry ice to the Institut Louis Malarde (Papeete, Tahiti, French Polynesia). Semen ZIKV RT-PCR, including RNA viral load determination, and cultures on

Vero cells with immunofluorescent staining to detect potential replicating virus, were performed as previously reported [8].

The percentage of asymptomatic blood donors with ZIKV RNA-positive semen was compared using chi-squared test with the percentage obtained in symptomatic Puerto Rican patients (31/55 or 56.4%) reported in a previous study [3].

The study was approved by the ethics committee of the University of California San Francisco and supported by the Recipient Epidemiology and Donor Evaluation Study (REDS-III) Central Laboratory (NHLBI Contract No. HHSN268201100001I). Written consent was obtained from all participants before sample collection.

Results

Twenty-nine semen samples were available from 14 blood donors who tested positive for ZIKV RNA on plasma collected at the time of asymptomatic blood donations (Table 1). Among the 14 donors who submitted semen samples, five (35.7%) tested positive for ZIKV RNA (three from Puerto Rico and two from Florida). ZIKV RNA was detected in semen collected from 7 to 54 days after the index donations.

Zika virus RNA loads in semen ranged from 8.03×10^3 to 2.55×10^6 copies/mL. All samples were negative in culture even after two successive passages.

Plasma collected at the same time as the positive semen tested negative for ZIKV RNA for six of eight ZIKV RNA-positive semen collections.

All available plasma from ZIKV RNA-positive time-points tested positive or equivocal for IgM and IgG antibodies against ZIKV, except for one sample that tested negative for IgM.

The percentage of ZIKV RNA-positive semen samples was not significantly different between asymptomatic blood donors in the current study (35.7%) and symptomatic men reported in a previous study (56.4%) (p 0.17) [3].

Discussion

Zika virus is a new challenge for blood transfusion safety and leading organizations, such as the World Health Organization, US Food and Drug Administration, AABB (formerly the American Association for Blood Banks) and the European Centre for Disease prevention and Control, have issued guidelines to prevent ZIKV transfusion–transmission infections that now include NAT screening of blood donations [4,9–11]. With the confirmation of ZIKV transmission by semen, ZIKV has also become an important challenge for ART, with deferral of semen donations in some countries during outbreaks.

To date, only two ZIKV sexual transmissions have been reported from asymptomatic men [12,13] and ZIKV RNA was detected in the semen of an asymptomatic traveller returning from Thailand, which was collected because he underwent aggressive chemotherapy [14].

The rate of detection of ZIKV RNA in follow-up semen samples from asymptomatic blood donors in our study was not significantly different from the rate previously found in symptomatic ZIKV-infected patients in Puerto Rico [3], confirming that the potential for ZIKV transmission by semen should be considered for all exposed men regardless of the presence of symptoms, as acknowledged by current guidance on avoidance of sexual transmission. However, the rates of detection in semen in symptomatic and asymptomatic men should be confirmed and compared in larger studies.

As our cohort included follow-up samples from blood donors who previously tested positive for ZIKV RNA in plasma, it was not surprising that all of them, expect one, tested positive or equivocal for anti-ZIKV IgM and IgG at the time of semen donations. ZIKV-specific IgG antibodies are detectable for at least several years after infection and IgM persists for 3–6 months, whereas ZIKV RNA and infectious ZIKV in culture have been detected up to 188 and 69 days postinfection, respectively [1]. Consequently ZIKV IgM or IgG screening in regions that have experienced epidemics would lead to rejection of numerous donors with ZIKV-positive antibody results who would probably be safe semen donors.

Infectious particles of ZIKV were not detected in culture from the ZIKV RNA-positive semen samples in the current study. A possible deterioration of ZIKV particles during storage due to the low pH of semen [15] or a possible neutralization of ZIKV by the adaptive immune response [5] may be explanations for negative cultures in RNA-positive semen samples. In symptomatic patients from Puerto Rico [3], infectious ZIKV in culture was detected in three of 20 (15%) semen samples but the sample cycle thresholds of the RT-PCR assay were lower (19 to 27) and the input volume of semen used for culture was higher (500 μL compared with 100–200 μL) than in the current study. As the limit of detection of ZIKV infectious particles in our culture model has not yet been determined, we cannot interpret the lack of detection of ZIKV infectivity in *in vitro* cultures as indicating lack of risk for transmission following sexual or ART exposure.

Assessing the risk of ZIKV transmission through semen donation is challenging due to the scarcity of data. Results reported in this interim analysis show a high percentage of detection of ZIKV RNA in semen from asymptomatic blood donors, which has clear implications for ART programmes and confirms that ZIKV is a new threat for reproductive medicine. There is an urgent need for licensed molecular tests to detect ZIKV RNA in semen, and systematic serological screening of semen donors should also be evaluated.

References

1. Moreira J, Peixoto TM, Machado de Siqueira A, Lamas CC. Sexually acquired Zika virus: a systematic review. Clin Microbiol Infect. 2017; 23:296–305. [PubMed: 28062314]
2. Atkinson B, Thorburn F, Petridou C, Bailey D, Hewson R, Simpson AJH, et al. Presence and persistence of Zika virus RNA in semen, United Kingdom, 2016. Emerg Infect Dis. 2017; 23:611–5. [PubMed: 27997333]
3. Paz-Bailey G, Rosenberg ES, Doyle K, Munoz-Jordan J, Santiago GA, Klein L, et al. Persistence of Zika virus in body fluidsdpreliminary report. N Engl J Med. 2017 epub ahead of print.
4. European Centre for Disease Prevention and Control. Zika virus and safety of substances of human origin. A guide for preparedness activities in Europe. Jul, 2016. Available at: http://ecdc.europa.eu/en/publications/Publications/Zika-virus-safety-of-substances-of-human-origin.pdf.

5. Duggal NK, Ritter JM, Pestorius SE, Zaki SR, Davis BS, Chang G-JJ, et al. Frequent Zika virus sexual transmission and prolonged viral RNA shedding in an immunodeficient mouse model. Cell Rep. 2017; 18:1751–60. [PubMed: 28199846]

6. Kuehnert MJ, Basavaraju SV, Moseley RR, Pate LL, Galel SA, Williamson PC, et al. Screening of blood donations for Zika virus infectiondPuerto Rico, April 3–June 11, 2016. MMWR Morb Mortal Wkly Rep. 2016; 65:627–8. [PubMed: 27337368]

7. Galel SA, Williamson PC, Busch MP, Stanek D, Bakkour S, Stone M, et al. First Zika-positive donations in the continental United States. Transfusion. 2017; 57:762–9. [PubMed: 28164310]

8. Musso D, Roche C, Robin E, Nhan T, Teissier A, Cao-Lormeau VM. Potential sexual transmission of Zika virus. Emerg Infect Dis. 2015; 21:359–61. [PubMed: 25625872]

9. World Health Organization. Maintaining a safe and adequate blood supply during Zika virus outbreaks. 2016. Available at: http://www.who.int/csr/resources/publications/zika/safe-blood/en/.

10. Food and Drug Administration. Revised recommendations for reducing the risk of Zika virus transmission by blood and blood components. Guidance for industry. 2016. Available at: http://www.fda.gov/BiologicsBloodVaccines/Guidance.

11. American Association for Blood Banks. Association bulletin # 16-07 Updated recommendations for Zika, dengue, and chikungunya viruses. 2016. Available at: http://www.aabb.org/advocacy/regulatorygovernment/.

12. Brooks RB, Carlos MP, Myers RA, White MG, Bobo-Lenoci T, Aplan D, et al. Likely sexual transmission of Zika virus from a man with no symptoms of infectiondMaryland, 2016. MMWR Morb Mortal Wkly Rep. 2016; 65:915–6. [PubMed: 27585037]

13. Freour T, Mirallie S, Hubert B, Splingart C, Barriere P, Maquart M, et al. Sexual transmission of Zika virus in an entirely asymptomatic couple returning from a Zika epidemic area, France, April 2016. Euro Surveill. 2016; 21(23)

14. Mansuy JM, Pasquier C, Daudin M, Chapuy-Regaud S, Moinard N, Chevreau C, et al. Zika virus in semen of a patient returning from a non-epidemic area. Lancet Infect Dis. 2016; 16:894–5.

15. Froeschl G, Huber K, von Sonnenburg F, Nothdurft H-D, Bretzel G, Hoelscher M, et al. Long-term kinetics of Zika virus RNA and antibodies in body fluids of a vasectomized traveller returning from Martinique: a case report. BMC Infect Dis. 2017; 17:55. [PubMed: 28068904]

Table 1

Results of plasma IgM/IgG and nucleic acid testing, semen nucleic acid testing (cycle threshold) and Zika virus RNA loads in semen

Participant	Day since index donation	Origin of sample	Plasma IgM/IgG	Plasma NAT	Semen NAT (Ct)	ZIKV RNA load in semen (copies/mL)
1	47	PR	P/P	N	N	—
2	27	PR	P/P	N	P (37.15)	8.03×10^3
3	6	PR	P/P	N	N	—
	26	PR	P/P	N	N	—
	181	PR	Eq/Eq	N	N	—
4	7	PR	P/P	N	N	—
	15	PR	P/P	N	N	—
	104	PR	Eq/P	N	N	—
	181	PR	Eq/P	N	N	—
5	10	PR	P/P	N	N	—
6	7	PR	P/P	N	N	—
	21	PR	P/P	N	N	—
	46	PR	Eq/P	N	N	—
	90	PR	N/P	N	N	—
7	7	PR	P/P	Eq	P (27.77)	1.55×10^6
	18	PR	P/P	N	P (30.96)	2.59×10^5
	54	PR	P/P	N	P (31.37)	2.05×10^5
	108	PR	P/P	N	N	—
8	10	PR	P/P	N	N	—
9	8	PR	P/P	N	N	—
	21	PR	P/P	N	N	—
	42	PR	P/P	N	N	—
10	25	US	P/P	N	P (26.88)	2.55×10^6
	38	US	P/P	N	P (29.97)	4.49×10^5
11	9	PR	P/P	P	P (33.27)	7.06×10^4
12	24	US	NA/NA	N	N	—
13	21	PR	P/P	N	N	—
	43	PR	NA/NA	N	N	—

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Participant	Day since index donation	Origin of sample	Plasma IgM/IgG	Plasma NAT	Semen NAT (Ct)	ZIKV RNA load in semen (copies/mL)
14	22	US	P/Eq	N	P (34.44)	3.67×10^4

Abbreviations: Ct, cycle threshold; PR, Puerto Rico; US, USA (Florida); P, positive; Eq, equivocal; N, negative; NA, not available; ZIKV, Zika virus.