We report the longitudinal follow-up of Zika virus (ZIKV) RNA in semen of a traveller who developed ZIKV disease after return to the Netherlands from Barbados, March 2016. Persistence of ZIKV RNA in blood, urine, saliva and semen was followed until the loads reached undetectable levels. RNA levels were higher in semen than in other sample types and declined to undetectable level at day 62 post onset of symptoms.

Case report
In March 2016, a previously healthy man in his 50s, reported to the travel clinic at Erasmus MC, with arthralgia, conjunctivitis, fever (≥ 38.1°C) and rash. The patient had returned eight days earlier from a four day business trip to Barbados. He had been vaccinated according the Dutch guidelines for travellers, including for yellow fever (vaccinated in 2012). He remembered being bitten by mosquitoes during early mornings on the island. Four days after returning to the Netherlands he suffered from staggered joint pain in ankles, elbows and hand joints. Two days after the start of the joint pain he developed fever up to 38.5 °C, a total body macular rash, followed by conjunctivitis. The fever disappeared after three days as well as the joint pain, rash and conjunctivitis. No haematospermia was reported. The patient recovered completely. At the time of his visit in Barbados both ZIKV and chikungunya virus (CHIKV) were circulating on the island.

Laboratory observations
Upon first presentation at the clinic, plasma and urine were collected. CHIKV RNA was not detectable by real-time reverse-transcription polymerase chain reaction (RT-PCR) [1], while ZIKV RNA was detectable for two independent RT-PCR targets in plasma and urine [2], confirming ZIKV disease. The patient agreed to follow-up sampling and ZIKV testing of blood, saliva, urine and semen until the sample type turned negative. Additional urine was collected and found positive for the most sensitive target at 6, 10, 13 and 19 days post onset of illness (dpi) (Figure).

Real-time RT-PCR analysis was validated according to ISO15189:2012 guidelines, briefly total nucleic acids were purified from 200 µL of plasma, saliva, urine or semen sample and eluted in a final volume of 100 µL using MagNaPure LC total nucleic acid isolation kit and the HP200 protocol (Roche Life Science, Almere the Netherlands). Subsequently, 8 µL of eluate in a final volume of 20 µL was used per reaction. Real-time RT-PCR was performed using the primers and probe set 1086/1162c/1107-FAM developed by Lanciotti et al. [2], and which annealed the most sensitive target mentioned above, and Fast Virus 1-Step Master Mix (Life technologies, Nieuwkerk a/d IJssel, the Netherlands) on a LC480-II (Roche), with 5 min 50 °C, 20 s 95 °C, and 45 cycles of 3 s 95 °C and 30 s 60 °C as thermal profile. The real-time RT-PCR was internally controlled for inhibition by addition of phocine distemper virus [3].

Saliva was collected at 6 and 10 dpi and was only positive at day 6. Additional plasma samples were taken at 6 and 10 dpi and ZIKV genome levels turned undetectable at 10 dpi. Semen was collected at 10, 13, 19, 20, 32, 34, 47 and 62 dpi and turned negative at the last sampling point.

Based on the semi-quantitative real-time RT-PCR data for the different sample types, the ZIKV RNA load appeared to be the highest in semen and remained higher in semen than in urine in the follow-up samples. Both urine and semen increased the window of ZIKV detection in clinical samples of this patient, with up to six-fold for semen vs plasma or saliva, and show
Background

ZIKV is a mosquito-borne flavivirus that has emerged in South and Central America and the Caribbean since 2015, affecting 39 countries in the region with an estimated 270,000 suspected and 40,000 confirmed cases as of 19 May 2016 [3]. Barbados reported its first three autochthonous ZIKV cases on 15 January 2016 [4] and has reported 317 suspected and seven confirmed cases since (status 19 May 2016 [3]). The association of ZIKV infection during pregnancy with microcephaly in newborns and other neurological disorders urged the World Health Organization (WHO) to declare the outbreak of microcephaly-associated ZIKV a Public Health Emergency of International Concern on 1 February 2016 [5]. With the outbreak of ZIKV in some European overseas countries and territories the risk of importation is currently high [6]. In the diagnostic unit at Erasmus MC, 49 confirmed ZIKV patients have been diagnosed in the period between 29 November 2015 and 23 May 2016, including two travellers returning from Barbados in March 2016 (A. van der Eijk, personal communication, May 2016).

Discussion

Aedes aegypti mosquitoes are the main vector for ZIKV transmission. However, the increasing import of ZIKV cases in non-endemic countries has put a previously obscure mode of non-mosquito-borne ZIKV transmission in the limelight: sexual transmission through infectious semen [7,8]. An increasing number of cases describing sexual transmission of ZIKV from symptomatic men-to-women or from asymptomatic men-to-men have been reported from areas where Aedes aegypti is not present, including Europe [7,9-11]. Although sexual transmission seems to represent the greatest risk for ZIKV transmission in areas without competent mosquito vectors, little is known about the prevalence of ZIKV in semen of symptomatic and asymptomatic infected men, the kinetics of infectious ZIKV and ZIKV RNA in semen in both of these groups, the possible gonadotropism of ZIKV in men and the minimal infectious dose for semen-borne transmission to men and women. Single time point sampling of semen reported the presence of viral RNA at 14 [7], 27 and 62 dpi [12] and isolation of virus 18 and 24 dpi [10].

An important implication of the teratogenicity of ZIKV and semen as source for ZIKV infection [13], is the necessity for protected sexual intercourse at least during pregnancy for men returning from ZIKV endemic areas or when residing in those areas. The European Centre for Disease prevention and Control (ECDC) recommends that male travellers returning from areas with active transmission should consider using a condom with a pregnant partner until the end of pregnancy, but should consider the use of a condom for at least one month upon return to reduce the potential risk of onward sexual transmission with non-pregnant female or male partners [14]. Some national guidelines [15-17] advise condom use for one to two months probably based on the very limited ZIKV isolation data that has been reported for semen up to 24 dpi [7,10].

In line with a report by Mansuy et al. [7], our data show higher ZIKV RNA loads in semen than in other sample types for our patient. Moreover in our study, the viral RNA loads in semen only slowly declined with time to undetectable levels at 62 dpi. Based on this observation and that of others, whereby another study reports a patient’s semen still positive for ZIKV RNA 62 dpi, a patient’s semen still positive for ZIKV RNA 62 dpi, however, with no end-point determination [12], the advice to use a condom with a non-pregnant partner for one month after return from an endemic area might be reconsidered to at least 90 days upon return (including the 62 days [12] and the subsequent maximum incubation time of 13 days [18]) as long as some of the crucial questions mentioned above have not been answered and a fully evidence-based recommendation on condom use is not possible yet. Another implication is the need for review of inclusion criteria for sperm donation.

The higher ZIKV RNA load in semen than in urine samples taken at the same time points found here, has also been previously observed for two other patients [7,10].

Figure

Semi-quantitative kinetics of Zika virus RNA loads in various types of clinical samples according to time post-disease onset, in a Dutch traveller returning from Barbados, March 2016*
This, together with the longevity of ZIKV RNA in semen indicates that semen should be taken into consideration as a sample of choice for sensitive ZIKV diagnostics with a broad detection window in males. While serology is usually the diagnostic method of choice in convalescent and asymptomatic patients with a suspected arbovirus infection [19], flavivirus serology is highly complex due to co-circulation of cross-reactive other flaviviruses, the existence of cross-reactive flavivirus vaccines and the occurrence of original antigenic sin [18]. Therefore molecular testing on semen might have an added value to serology in males up to 2 months dpi.

**Conclusions**

We describe the longitudinal follow-up of the presence of ZIKV RNA in semen and other sample types in a returning traveller. Semi-quantitative ZIKV RNA levels were consistently higher in semen than in urine in the convalescent phase and slowly declined to an undetectable level at 62 dpi. Testing of semen could have an added value to testing of urine and serology in men up to 2 months dpi. Although ZIKV was not isolated here, the use of condoms and the abstinence of semen donation up to 90 dpi as a precautionary measure to prevent sexual transmission of ZIKV, might need consideration until a fully evidence-based assessment can be made based on data generated within larger patient datasets.

*Erratum*

The Figure was corrected on 16 June 2016.

**Acknowledgements**

We are grateful to the patient for his willingness to provide additional samples for the study. We thank the team of the Clinical Virology unit for excellent technical support.

The patient provided written informed consent to participate in the study and for publication of this case-report.

**Conflict of interest**

None declared.

**Authors’ contributions**

CR data analysis, figure, writing; SP molecular data generation, data analysis; GvK, RM serology; JvK tissue culture; TL, MK co-writing; AvdE, medical coordinator; EvG, co-writing, treating physician. All authors reviewed and commented on the manuscript.

**References**

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