Prolonged Zika Virus RNA Detection in Semen of Immunosuppressed Patient

Christina Petridou, David Bonsall, Aleem Ahmed, Mark Roberts, Carolyn Bell, Mariateresa de Cesare, Rory Bowden, Victoria Graham, Daniel Bailey, Andrew Simpson, Emma Aarons

Author affiliations: Rare and Imported Pathogens Laboratory, Public Health England Porton, Salisbury, UK (C. Petridou, V. Graham, D. Bailey, A. Simpson, E. Aarons); University of Oxford, Oxford, UK (D. Bonsall, M. de Cesare, R. Bowden); Leicester Royal Infirmary, Leicester, UK (A. Ahmed); Worcestershire Royal Hospital, Worcester, UK (M. Roberts); South Warwickshire National Health System Foundation Trust, Warwick, UK (C. Bell)

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Zika virus RNA has been detected in semen samples collected <370 days after symptom onset. We report unusual persistence of Zika virus RNA in semen, confirmed by sequencing at 515 days after symptom onset and detectable for >900 days, in a patient with immunosuppression.

Detection of Zika virus RNA in semen was described previously in an immunocompetent man 370 days after symptom onset; envelope and precursor of M protein gene sequencing indicated high genetic stability in semen 3–4 months after symptom onset (1). We report detection of Zika virus RNA in semen over a longer period in a 43-year-old immunosuppressed man in the United Kingdom.

The patient has multicentric reticulohistiocytosis (MRH), a rare rheumatologic condition, which was diagnosed in 2015. When MRH was diagnosed, the patient had multiple pruritic, firm papules and nodules on his face and neck. He also had lesions with a characteristic coral bead appearance at periungal sites. In addition, he had severe joint pain and stiffness affecting his hands and knees and drenching sweats. His MRH diagnosis was confirmed by testing of a punch biopsy of a lesion. He was HIV negative, and his immunoglobulin levels and immunoglobulin electrophoresis results were normal. He was initially treated with topical steroids and antihistamines, but he only had limited relief. He was prescribed oral steroids and required high doses to control his symptoms. Clinicians added methotrexate and hydroxychloroquine to his medications as steroid-sparing agents and to reduce the chance his MRH would progress to erosive disease.

References


Address for correspondence: David T. Williams, CSIRO, Australian Animal Health Laboratory, 5 Portarlington Rd, Geelong, VIC 3220, Australia; email: d.williams@csiro.au

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In April 2016, seven months after starting his disease-modifying antirheumatic drugs, the patient experienced fever and a new widespread maculopapular rash. He had returned to the United Kingdom from Brazil 7 days before. We detected Zika virus RNA in plasma taken 1 day after symptom onset by using real-time reverse transcription PCR methods described by Pyke et al. (2), with modifications (Appendix, http://wwwnc.cdc.gov/EID/article/25/8/1543-App1.pdf). We did not detect Zika virus nonstructural protein 1–specific antibodies by ELISA (EUROIMMUN, https://www.euroimmun.com) in initial samples, but we noted seroconversion on day 13 (Appendix Figure). After diagnosing Zika virus infection, clinicians stopped the patient’s methotrexate alone to prevent a flare-up of his MRH (Appendix Figure). We attempted viral culture on multiple semen samples, as previously described (3), but were unsuccessful (Table). We constructed sequencing libraries from total seminal plasma-extracted RNA enriched by using a panel of oligonucleotide probes, 120 nt in length, designed to capture all known Asian Zika virus strains, according to previously described methods (4). We prepared libraries for previously collected semen samples from before day 326 and sequenced these using MinION (Oxford Nanopore Technologies, https://nanoporetech.com); MiSeq (Illumina, https://www.illumina.com). We did the same for the day 515 sample and sequenced it using MiSeq (Illumina, https://www.illumina.com). We used double indexing to prevent cross-contamination and index misassignment errors.

We also prepared 90 plasma samples from patients infected with hepatitis C, collected for a separate study, in parallel with the day 515 sample. We did this to exclude the possibility of cross-contamination from our patient’s previous samples, particularly the day 13 sample, which were shipped, prepared, and sequenced 6 months earlier. We did not detect Zika virus in any of the hepatitis C samples. We found no evidence of cross-contamination with Zika virus sequences during processing that could explain the whole-genome genomes detected in the day 515 sample. Consensus sequences were consistent with all samples having come from the same patient with only 2 mutations, 1 synonymous change at codon 2921 and a K3272E substitution, acquired during the 502 days between the first and last samples sequenced (Table). We deposited sequence data in GenBank (accession nos. MH763832–3).

We present the raw text as follows:

In April 2016, seven months after starting his disease-modifying antirheumatic drugs, the patient experienced fever and a new widespread maculopapular rash. He had returned to the United Kingdom from Brazil 7 days before. We detected Zika virus RNA in plasma taken 1 day after symptom onset by using real-time reverse transcription PCR methods described by Pyke et al. (2), with modifications (Appendix, http://wwwnc.cdc.gov/EID/article/25/8/1543-App1.pdf). We did not detect Zika virus nonstructural protein 1–specific antibodies by ELISA (EUROIMMUN, https://www.euroimmun.com) in initial samples, but we noted seroconversion on day 13 (Appendix Figure). After diagnosing Zika virus infection, clinicians stopped the patient’s methotrexate alone to prevent a flare-up of his MRH (Appendix Figure). We attempted viral culture on multiple semen samples, as previously described (3), but were unsuccessful (Table). We constructed sequencing libraries from total seminal plasma-extracted RNA enriched by using a panel of oligonucleotide probes, 120 nt in length, designed to capture all known Asian Zika virus strains, according to previously described methods (4). We prepared libraries for previously collected semen samples from before day 326 and sequenced these using MinION (Oxford Nanopore Technologies, https://nanoporetech.com); MiSeq (Illumina, https://www.illumina.com). We did the same for the day 515 sample and sequenced it using MiSeq (Illumina, https://www.illumina.com). We used double indexing to prevent cross-contamination and index misassignment errors.

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**Table.** Serial Zika virus genome sequence and culture results from semen of a patient with immunosuppression, United Kingdom*

<table>
<thead>
<tr>
<th>Sample no.</th>
<th>Days after symptom onset</th>
<th>RT-PCR C. value†</th>
<th>Sequence coverage, %‡</th>
<th>Average read depth</th>
<th>Sequencing platform§</th>
<th>Mutations detected</th>
<th>Culture result</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>13</td>
<td>19</td>
<td>99.9 (min depth 2), 88.4 (min depth 40)</td>
<td>386.9</td>
<td>MiniON</td>
<td>Reference</td>
<td>Frozen sample: unsuccessful</td>
</tr>
<tr>
<td>2</td>
<td>46</td>
<td>26</td>
<td>ND</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>Fresh sample: unsuccessful</td>
</tr>
<tr>
<td>3</td>
<td>167</td>
<td>No RNA detected¶</td>
<td>ND</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>ND</td>
</tr>
<tr>
<td>4</td>
<td>194</td>
<td>Subthreshold</td>
<td>ND</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>ND</td>
</tr>
<tr>
<td>5</td>
<td>241</td>
<td>31</td>
<td>Unsuccessful</td>
<td>3.3</td>
<td>NA</td>
<td>None</td>
<td>Frozen sample: unsuccessful</td>
</tr>
<tr>
<td>6</td>
<td>257</td>
<td>34</td>
<td>44 (min depth 2), 0 (min depth 40)</td>
<td>NA</td>
<td>MiniON</td>
<td>None</td>
<td>ND</td>
</tr>
<tr>
<td>7</td>
<td>278</td>
<td>No RNA detected¶</td>
<td>ND</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>ND</td>
</tr>
<tr>
<td>8</td>
<td>326</td>
<td>26</td>
<td>76 (min depth 2), 6 (min depth 40)</td>
<td>13.2</td>
<td>MiniON</td>
<td>None</td>
<td>Fresh sample: unsuccessful</td>
</tr>
<tr>
<td>9</td>
<td>396</td>
<td>29</td>
<td>ND</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>ND</td>
</tr>
<tr>
<td>10</td>
<td>515</td>
<td>24</td>
<td>98.1 (min depth 5)</td>
<td>33.3</td>
<td>MiSeq</td>
<td>NA</td>
<td>ND</td>
</tr>
<tr>
<td>11</td>
<td>687</td>
<td>39</td>
<td>ND</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>ND</td>
</tr>
<tr>
<td>12</td>
<td>941</td>
<td>32</td>
<td>ND</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>ND</td>
</tr>
</tbody>
</table>

*Cc. cycle threshold; min, minimum; NA, not applicable; ND, not done; RT-PCR, reverse transcription PCR.
†Before PCR, nucleic acid was extracted from samples 1–7 using the EZ1 Virus Mini Kit (QIAGEN, https://www.qiagen.com). Samples 8–12 were extracted using the MagNA Pure 96 DNA and Viral NA Small Volume Kit (LifeScience-Roche Diagnostics Corporation, https://lifescience.roche.com). Cc values >40 with acceptable amplification curves are interpreted as positive, but results for samples with Cc values >35 are confirmed by reextraction and repeat PCR in triplicate, where possible.
‡Conservative read-depth thresholds were selected for comparative analyses of the day 13 (sample 1) and day 515 (sample 10) consensus genomes.
¶Confirmed on reextraction and repeat PCR testing.
Counotte et al. systematically reviewed all available evidence on the risk for sexual transmission of Zika virus (5). Data from case reports, case series, cohort studies, in vitro work, and animal studies indicate that the infectious period for sexual transmission of Zika virus is considerably shorter than the period during which viral RNA can be detected in semen. As a result, the World Health Organization now recommends male travelers with potential Zika virus exposure delay conception for ≥3 months rather than ≥6 months (6).

In our case, Zika virus RNA might have persisted in semen because of failed immune clearance secondary to the patient’s MRH or his immunosuppressive drug treatment. However, when advising returning male travelers in couples planning pregnancy, clinicians should be aware that Zika virus RNA shedding in semen might be intermittent and persist for longer in patients with immunosuppression.

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About the Author
Dr. Petridou is an infectious diseases registrar working at the Rare and Imported Pathogens Laboratory at Porton Down, Salisbury, United Kingdom. Her research interest is in Zika virus infection and she previously worked as the Zika virus registrar at Porton Down.

References

No Evidence for Role of Cutavirus in Malignant Melanoma
Ulrike Wieland, Steffi Silling, Martin Hufbauer, Cornelia Mauch, Paola Zigrino, Frank Oellig, Alexander Kreuter, Baki Akgül

Author affiliations: University of Cologne, Cologne, Germany (U. Wieland, S. Silling, M. Hufbauer, C. Mauch, P. Zigrino, B. Akgül); Institute of Pathology, Mülheim an der Ruhr, Germany (F. Oellig); Helios St. Elisabeth Hospital Oberhausen, University Witten/Herdecke, Witten, Germany (A. Kreuter)

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Cutavirus was previously found in cutaneous melanoma. We detected cutavirus DNA in only 2/185 melanoma biopsies and in 0/52 melanoma metastases from patients in Germany. Viral DNA was localized in the upper epidermal layers. Swab specimens from healthy skin were cutavirus positive for 3.8% (9/237) of immunocompetent and 17.1% (35/205) of HIV-positive men.

Cutavirus, a novel human protoparvovirus with linear single-stranded DNA, has been detected in fecal samples from children with diarrhea and in cutaneous T-cell lymphomas (CTCL) (1,2). Recently, Mollerup et al. reported the identification of cutavirus in 1 of 10 cutaneous malignant melanomas using viral enrichment methods with high-throughput sequencing and real-time PCR (3). This discovery raised questions concerning tropism and pathogenicity of cutavirus in human skin. We performed a retrospective study to determine cutavirus DNA prevalence and viral load in a large collection of formalin-fixed paraffin-embedded tissue biopsy specimens of malignant melanomas and in forehead swabs of healthy skin of immunocompetent and HIV-positive persons in Germany.

We used 185 cutaneous malignant melanoma biopsy specimens from 179 patients and 52 melanoma metastases from 42 patients from Germany for analyses with cutavirus real-time PCR (Appendix, http://wwwnc.cdc.gov/EID/article/25/8/19-0096-App1.pdf). We detected cutavirus DNA only in 2 nodular malignant melanomas, located on the abdomen of a 64-year-old man (MM-A) and on the cheek of an 85-year-old woman (MM-B). Viral DNA loads in these biopsies were 0.3 (MM-A) and 2.8 (MM-B) cutavirus DNA copies per μg DNA. Cutavirus was not detected in other malignant melanomas using viral enrichment methods and other flaviviruses: a living systematic review. PLoS Med. 2018;15:e1002611. http://dx.doi.org/10.1371/journal.pmed.1002611

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