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ID CASE

Occupational Exposure to the Ugandan Research Strain (MR766) of Zika Virus

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A laboratory worker suffered an accidental needle-stick resulting in an exposure to the Ugandan strain (MR766) of Zika virus, which has rarely been studied in humans. We report the clinical presentation and outcomes, molecular and serological diagnostic results, and antibody response.

Keywords. antibody responses; occupational exposure; viral persistence; ZIKV.

Zika virus (ZIKV) was identified in Uganda in 1947 from a sentinel rhesus macaque during the course of surveillance for yellow fever virus (YFV) [1]. Since then, there have been 3 major ZIKV human outbreaks: the Pacific Island of Yap in 2007 [2], the French Polynesian islands in 2013 [3], and the Americas from 2014 to 2016 [4]. Miami (Florida), became ground zero for the first outbreak of ZIKV in the continental United States. The most common mode of ZIKV acquisition is vector-borne through infected mosquitoes, but vertical and sexual transmission have also been described [5]. Occupational exposure to ZIKV has seldom been reported.

Approximately 20% of ZIKV infections are symptomatic, and symptoms include a mild febrile illness and rash that resolves within 1 week [5]. Low-grade fever, itchy maculopapular rash, arthritis or arthralgias, headache, retro-orbital pain, and nonpurulent conjunctivitis have also been reported [5]. In rare cases, ZIKV infection is associated with neurological complications, including Guillain-Barré syndrome and meningoencephalitis [5].

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The Occupational Safety and Health Administration (OSHA) and the National Institute for Occupational Safety and Health (NIOSH) within the Centers for Disease Control and Prevention received reports of 3 ZIKV occupational exposures during the most recent outbreak. The first case, reported in Pennsylvania in 2016, was a result of a needle-stick injury with biological samples containing ZIKV during a vaccine trial. The second occurred in New York State from the bite of an infected mouse. The third involved an occupational exposure occurred when a laceration was inflicted by an infected chicken in Pennsylvania in 2017. From the 3 cases reported, only the first case developed symptoms. Details of the clinical presentation, outcome, and any virology or immunology related to the case have not been reported [6].

Diagnosis of ZIKV is based on molecular assays and serological assays. ZIKV isolates have been clustered into 2 major lineages, African and Asian, and because these lineages share approximately 97% sequence homology, molecular detection can be used to detect both lineages. Although molecular testing is the gold standard to diagnose acute infection, serological assays are the standard for diagnosis of postacute infection. However, cross-reactive immune responses to other flaviviruses, especially in flavivirus-endemic areas, make serological diagnosis challenging [7].

Here, we describe a unique ZIKV infection with the Ugandan ZIKV-MR766 laboratory strain in a researcher in Miami, Florida. We report the clinical presentation, molecular and serological diagnostic results, and antibody response.

CLINICAL CASE

In July of 2018, a 34-year-old female laboratory researcher presented at the Employee Health Office with malaise, skin rash, and joint pain for the past 4 days. Ten days before development of symptoms, she had an accidental needle-stick with minimal visible blood on her left middle finger. The injury occurred immediately after inoculation of a mouse with ZIKV-MR766, a common research strain from Uganda, and the syringe contained virus at a concentration of 1×10^7 plaque forming units (PFU)/mL. The patient was healthy and had not traveled out of Florida in the previous 2 months. The patient was born and raised in Brazil, where other flaviviruses, including dengue virus (DENV) and YFV, are known to circulate. She reported receiving the YFV vaccine in 2004 and did not report any previous history of DENV infection, blood transfusions, or sexual contact with individuals who had traveled to ZIKV-endemic areas during the time of the outbreak. Right after the needle-stick accident, the researcher removed her gloves and washed her hands with water and soap. She reported the incident to her supervisor, who instructed her to go to the employee health office if any ZIKV-like symptoms developed.

Ten days (D10) postexposure (PE), she developed generalized malaise and a pruritic rash on her cheeks and upper chest and reported to the employee health office, where she was referred to the Infectious Diseases clinic. These symptoms lasted 5 days. D14 PE, she developed muscle pain, bilateral arthralgias, and joint effusions in her hands and ankles, which resolved by D17 PE. She did not report fever, headache, or conjunctivitis.

On D14 PE, her physical examination revealed a faint maculopapular rash with an erythematous base on both cheeks, bilateral joint tenderness upon palpation, nonerythematous effusions at the metacarpal and proximal interphalangeal joints of the hands, and nonerythematous effusions at the talocrural articulations or ankle joints. Recommendations were made for the prevention of mosquito bites with insect repellant and prevention of sexual contact in order to limit potential transmission. At D23 PE, there was complete resolution of symptoms and she had a normal physical examination.

The animal involved in the needle-stick incident was a 12-week-old, C57BL/6J male mouse housed and cared for at Institutional Animal Care and Use Committee (IACUC)-approved University of Miami animal facilities. Immediately before the needle-stick of the patient, the mouse was challenged with a dose of 1×10^7 PFU of ZIKV-MR766. ZIKV infection was confirmed 48 hours postchallenge by reverse transcription polymerase chain reaction (RT-PCR) of plasma (6.8×10^4 ZIKV-MR766 copies/mL) and splenic tissue (2.1×10^5 ZIKV-MR766 copies/10^6 cells). There was no detectable infection in the brain tissue.

METHODS

On D14 PE, the patient consented to enroll in multiple IRB-approved studies for additional testing of ZIKV persistence in bodily fluids and assessment of the immunological response against ZIKV. EDTA blood, urine, saliva, and a vaginal swab were collected from D14 PE to D104 PE, per study protocol and participant availability. Physical examination was conducted on PE D14, D17, and D23. Samples were collected on PE D14, D20, D23, D37, and D104.

On D14 PE, serum and urine were collected as part of routine clinical care. Serological testing was performed on the serum sample using the ZIKV IgM enzyme-linked immunosorbent assay (ELISA) test from the Laboratory Corporation of America (Lab Corp) and a confirmatory plaque reduction neutralization test (PRNT), in accordance with the Centers for Disease Control and Prevention (CDC) testing guidelines. A nucleic acid amplification test (NAAT) was performed in both serum and urine (Lab Corp).

A novel, antibody competition-based ZIKV diagnostic test was also performed by Z-Quick Diagnostics, LLC, as a laboratory-developed test [8]. The Z-Quick ZIKV antibody competition-based assay (ACA) is rapid and highly specific to ZIKV IgG antibodies, utilizing a ZIKV-specific monoclonal antibody that recognizes an immunodominant ZIKV-specific epitope [8].

Plasma was isolated from whole blood using Ficoll and centrifugation. Vaginal swab samples were soaked in phosphate-buffered saline with gentle agitation before RNA isolation. RNA was isolated from whole blood, plasma, saliva, urine, and the vaginal swab soak and then tested for the presence of ZIKV-MR766 genomic RNA using strain-specific reagents and an RT-PCR kit. As described previously, patient plasma from multiple time points was screened for IgM and IgG virus-specific binding, and focus reduction neutralization tests (FRNTs) were also performed for DENV and ZIKV [9].

RESULTS

Diagnostic Tests

Complete blood counts and a comprehensive metabolic panel were all within normal values, including platelets, liver, and kidney function tests. The ZIKV IgM ELISA was presumptively positive. The PRNT test for ZIKV at the CDC was negative. ZIKV was qualitatively detected in urine; however, no virus was detected in serum. The Z-Quick ZIKV ACA was negative for ZIKV exposure at D14 PE but positive at all other time points.

Viral Persistence

Additional noncommercial laboratory testing detected ZIKV RNA in whole blood, saliva, urine, and the vaginal swab at D14 PE (Figure 1A). The highest value recorded was in the saliva, at 875 copies/mL (Figure 1A). At D20 PE, virus was only detectable in whole blood at a value of <37 copies per mL, and at D23 PE there was no detectable virus in any sample.

Antibody Response

Analysis of the antibody response (FRNT) in the patient revealed a broad IgM response that bound to both ZIKV-Paraiba (a strain isolated from a symptomatic patient in Brazil during the 2015 outbreak) and ZIKV-MR766 during the acute phase of the infection; however, there was little to no cross-reactivity against DENV1-4 and YFV (Figure 1B). The IgM response against ZIKV-MR766 was higher than the response against ZIKV-Paraiba. An IgG binding response was detected against both ZIKV-MR766 and ZIKV-Paraiba and continued to increase until the last study visit at D104 PE (Figure 1C). IgG against DENV2/4 was detected; however, no IgG binding of DENV1/3 or YFV was seen. The highest neutralizing antibody titers were directed against ZIKV-MR766, and lower and less persistent neutralizing responses were observed against ZIKV-Paraiba. There was no increased neutralization during the course of infection against DENV2/4, but there were elevated levels of binding IgG (Figure 1D). These neutralization results at D14 PE were positive for ZIKV, different than the results reported by the CDC PRNT laboratory test performed at

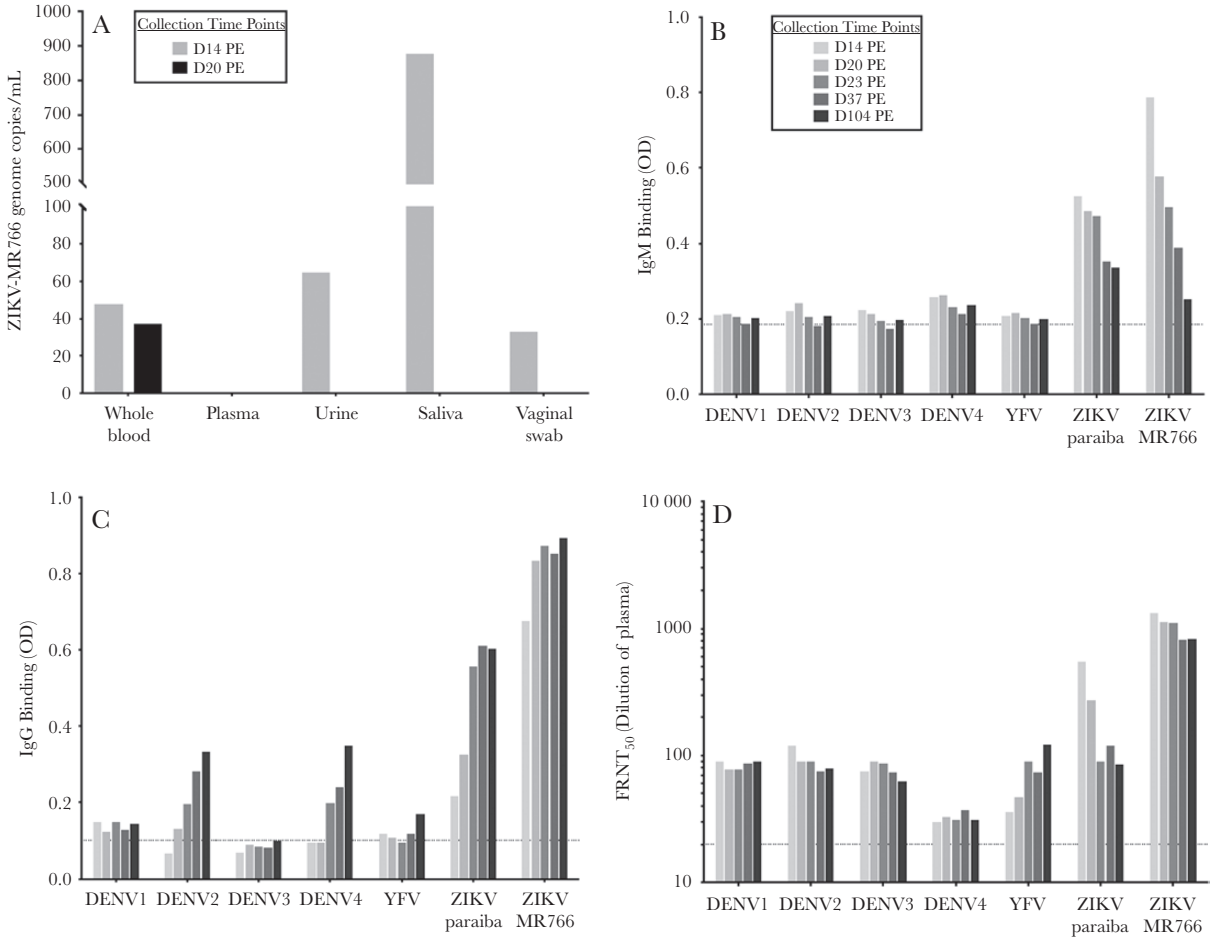

Figure 1. Virus persistence in fluids and antibody response. A, Quantification of ZIKV-MR766 in patient samples. B, IgM binding to whole intact flaviviruses over time. C, IgG binding to whole intact flaviviruses over time. D, Fifty percent neutralization points to flaviviruses over time by focus reduction neutralization test. B, C, Patient plasma diluted 1:100 with phosphate-buffered saline and equal amounts of virus were used among the different types. Dotted lines in (B–D) represent background levels with pooled flavivirus-naïve plasma. Abbreviations: DENV, dengue virus; OD, optical density; PE, postexposure; YFV, yellow fever virus; ZIKV, Zika virus.

this early time point. This is likely due to the CDC using a variety of ZIKV strains for confirmatory PRNTs.

DISCUSSION

This report describes a rare human ZIKV infection with the Ugandan ZIKV-MR766 strain that occurred accidentally after challenging an animal for research purposes. The infection was symptomatic, self-limited, did not result in neurological complications, and was confirmed by both molecular and serological testing in the patient and molecular testing in the animal.

Occupational infection with ZIKV by needle-stick has seldom been reported in the literature, and among the reported cases, details of clinical presentation, outcomes, and detection of the virus in body fluids have not been reported [6, 10, 11]. Workers in biomedical research, health care, and clinical laboratories can be at high risk of contracting ZIKV. It is important that employers implement proper infection prevention and control practices in laboratories and other biomedical research facilities manipulating ZIKV. Furthermore, it is essential to educate workers and supervisors on the potential risks of exposure, reporting regulations, and adverse health effects. Effective engineering controls, including sharps engineered to prevent injuries, combined with administrative controls like proper personal protective equipment and adequate training, help protect workers in laboratories and high-risk workplaces.

Detection of ZIKV RNA in whole blood, urine, saliva, genital secretions, and plasma by RT-PCR can be highly sensitive and specific. In this case, the highest level was detected in saliva, and as previously reported, the longer period of detection was in whole blood [12]. These results underline the importance of testing several different body fluids and highlight that cell-cultured virus may still replicate to high levels in humans, despite being passaged in nonhuman cell lines.

As has previously been reported, analysis of the antibody response shows that there was the generation of flavivirus cross-reactive antibodies [13]. The development of broad ZIKV IgG binding antibodies were not surprising given the IgM response;

however, IgG binding antibodies developing against DENV2/4 and not against DENV1/3 was somewhat unusual. This could be explained by phylogenetic analyses of the flaviviruses that cluster the consensus sequences of DENV2/4 closer to ZIKV than DENV1/3 [14]. Neutralization of ZIKV was sustained and specific to the Ugandan strain, whereas it declined to background levels for the Brazilian ZIKV-Paraiba strain. This could be due to the limited sequence differences in the E protein between the African and South American strains of ZIKV [15].

Furthermore, the negative neutralization results from the CDC's PRNT test differ from the research laboratory-performed FRNT tests. One reason for this difference could be a sensitivity issue of PRNTs compared with FRNTs. In a FRNT assay, infected cells have not yet lysed as they would in a PRNT assay. As not all infected cells will lyse and make plaques, an FRNT assay would allow for a greater number of infectious foci to be recognized by the person performing the analysis and a greater difference between the number of foci in the virus-only control wells than the the number of foci neutralized in wells with the patient's plasma and virus. This may lead to a slightly greater sensitivity in FRNT assays.

This case highlights the potential for ZIKV occupational exposure. Findings may also be used for the development diagnostic tests against ZIKV and to reinforce the need for good infection prevention practices in research laboratories and other biomedical facilities working with ZIKV.

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