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NOVEL ID CASES

Laboratory Acquired Zika Virus Infection Through Mouse Bite: A Case Report

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Zika virus is an arthropod-borne flavivirus mainly transmitted by the bite of infected mosquitoes. However, alternative transmission routes can occur. In this study, we show the accidental transmission of virus from an infected mouse to a human during the experimental manipulation. This study describes the patient clinical manifestations and virus genome identification.

Keywords. mouse bites; transmission; Zika.

Zika virus (ZIKV) is an enveloped single-stranded ribonucleic acid (RNA) flavivirus that was first identified in Uganda (1947) and recently spread throughout Asia, the Western Pacific, and the Americas. In Brazil, ZIKV was first identified in 2015, and the highest number of reported ZIKV cases worldwide reached 3676 cases in December 2018 [1].

The majority of ZIKV infections are asymptomatic. Clinical manifestations include fever, cutaneous rash, and conjunctivitis. However, in rare events, ZIKV infection is associated with neurological disorders including Guillain-Barré syndrome, meningoencephalitis, and acute myelitis [2]. During vertical transmission events, ZIKV infections can be related to congenital fetal malformations during pregnancy, including a severe neurological impairment called congenital Zika syndrome [3–5].

Zika virus is an arthropod-borne virus (arbovirus) that is mainly transmitted through *Aedes* mosquitoes bites. However, transmission can also occur during sexual intercourse, vertical mother-to-fetus transmission, and blood transfusions [6]. After infection, ZIKV RNA can be found in different human body fluids such as saliva, amniotic fluid, urine, cerebrospinal fluid, blood, semen, and tears [7]. Zika virus particles have been isolated and cultured from serum, semen, and saliva [8–10]. Despite the virus being found in the saliva, the transmission potential has not been elucidated yet. In this study, we describe the first case of accidental ZIKV transmission from saliva from an experimentally infected mouse to human with clinical development of Zika fever and virus recovery from plasma during acute phase. Although this transmission route had been well established for other viruses, these results describe a novel route of ZIKV transmission.

THE STUDY

On June 29, 2017, a 30-year-old PhD student arrived at General Clementino Fraga Filho hospital complaining of symptoms that included low-grade fever, cutaneous rash, itching, and headache that started 1 day earlier (Figure 1). These symptoms persisted for 6 days. After that, the patient recovered without medical interventions.

She reported no other family members had been sick or reported any similar symptoms. However, 12 days before symptoms onset, she was accidentally bitten by a ZIKV-infected mouse during animal manipulation involved in her PhD thesis. The experiment she was conducting at the time involved an adult interferon (IFN) $\alpha/\beta/\gamma R^{-/-}$ (AG129) mouse, previously inoculated with 10^6 plaque-forming units of the Brazilian strain ZIKV (GenBank accession number MF352141). The AG129 mouse is a very useful animal model for ZIKV experiments, because it is deficient in IFN-α, -β, and -γ, which are components of innate immunity that play a significant role in preventing viral replication [11]. The accident occurred 6 days post mouse infection. The mouse bite was in student's right hand finger, perforated the glove, and produced bleeding.

The Institute Animal House has the facilities and procedures to perform experiments, and animal models are approved by the Institutional Research Committee of the Federal University of Rio de Janeiro, Brazil, under the protocol number 01200.001568/2013-87/023/16. All users completed all necessary training as required by the Institution to handle animals and perform experimental virus infection. We obtained written informed consent from the patient, who is also an author of the manuscript.

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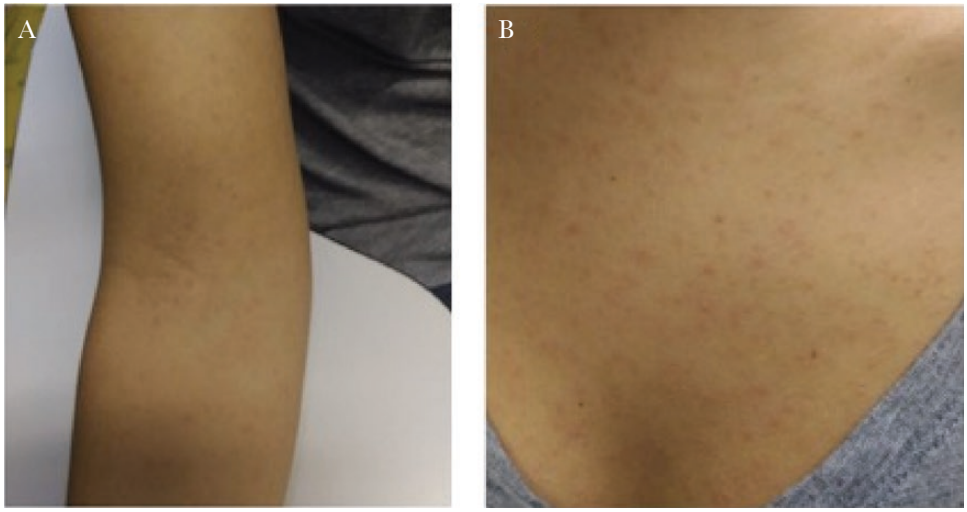

Figure 1. Human clinical symptoms. Cutaneous rash in arms (A) and chest (B) from human case 12 days after the accident.

During the course of the investigation, the patient's blood samples were taken 1, 6, 9, 15, 28, and 49 days after the symptoms onset. At the start of the patient's symptoms, mouse-infected blood samples were also collected from 2 infected animals in the cage.

Viral RNA detection was performed from human and mouse blood samples with specific primers and probe to NS5 gene. Viral RNA was extracted from plasma samples using QIAamp Viral RNA Mini Kit (QIAGEN), following the manufacturer's recommendations. Viral RNA was amplified using One-Step TaqMan RT-PCR (Thermo Fisher Scientific) on a 7500 Real-Time PCR System (Applied Biosystems) as previously described [12]. Zika virus genome was detected on the first day after symptom onset, with 30 cycle threshold (CT) value (Table 1). Zika virus RNA was also detected in the blood of 2 mice confined in the same cage, both with 23 CT values.

Serological findings supported the molecular diagnosis showing anti-ZIKV immunoglobulin (Ig)M antibodies on the patient's blood 6 days postsymptoms onset and remained positive until day 49, when the last sample was collected. Anti-ZIKV IgG antibodies were first detected 9 days after symptom onset (Euroimmun IgM/IgG enzyme-linked immunosorbent assay [ELISA]; Euroimmun AG, Lübeck, Germany). Dengue IgG antibody was negative on Panbio Dengue IgG indirect ELISA (Standard Diagnostic, Inc, Gyeonggi-do, Korea). Finally, ZIKV neutralization antibodies were detected by plaque reduction neutralization test ($PRNT_{90}$), with titers equal to or higher than 1:640 from days 9 to 49, since symptom onset (Table 1).

Although the Zika diagnosis had been well documented, the question is whether the infection was acquired or attributed to the laboratory accident. To answer that, virus whole-genome sequencing was performed using short amplicons (approximately 500 base pairs) covering the whole genome and sequenced using MIseq Plataform (Illumina) [13]. The short-amplicon approach was performed to recover the whole ZIKV genome even in low viral load samples. Negative controls without any read matching the reference ZIKV genome were used to exclude sample cross-contamination. Phylogenetic reconstruction showed that both mouse inoculated ZIKV strain (GenBank accession numbers MT078739 and MT078740) and patient (GenBank accession number MT078742) recovered sequences were grouped at the same branch in a single clade, belonging to Asian genotype, with high SH-aLRT and bootstrap supports (Figure 2). Six viral

Table 1. Patient Laboratorial Findings

Blood Sample	Day After Symptom Onset	RT-PCR CT	$PRNT_{90}$	IgM ZIKV	IgG ZIKV
1	1	30.16	<1:10	Nonreactive	Nonreactive
2	6	Not detectable	Not done	Reactive	Nonreactive
3	9	Not detectable	1:640	Reactive	Reactive
4	15	Not detectable	1:2560	Reactive	Reactive
5	28	Not detectable	1:2560	Reactive	Reactive
6	49	Not detectable	1:1280	Reactive	Reactive

Abbreviations: CT, cycle threshold; Ig, immunoglobulin; PRNT, plaque reduction neutralization test; RT-PCR, reverse-transcriptase polymerase chain reaction; ZIKV, Zika virus.

[a]PRNT titers <1:10 are considered negative.

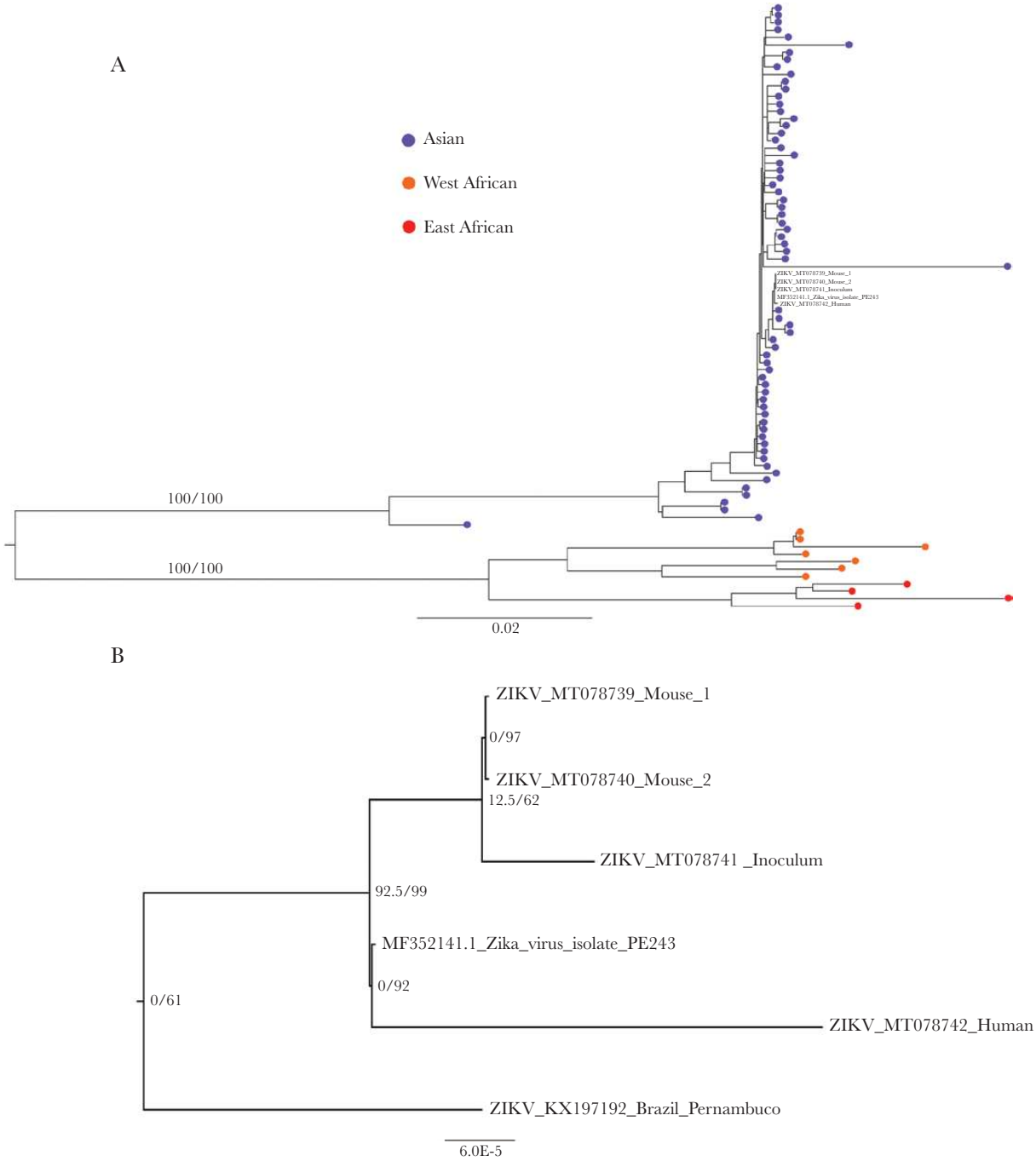

Figure 2. Maximum likelihood phylogenetic reconstruction of mouse-to-human Zika virus (ZIKV) transmission. (A) Zika virus whole-genome sequences dataset from different genotypes across the world, including sequences from ZIKV Asian genotype represented in purple, West African genotype in orange, and East African genotype in red. The study samples including virus isolate inoculum used to infect mice (GenBank accession number MT078741), mouse 1 and 2 (GenBank accession numbers MT078739 and MT078740), and human isolate sample (GenBank accession number MT078742) were identified on the 3. (B) Zoom of patient sequence clade, with mice sequences and inoculum ZIKV strain at the same branch without any genetic distance. Phylogenetic reconstruction was performed using Iqtree software. Support values represent SH-aLRT branch test and bootstrap, respectively.

genetic signatures (1 exclusively from inoculum and 5 exclusively from human isolates) were observed between the samples, showing closely related sequences. Patient and mouse ZIKV sequences do not group at the same branch with worldwide sequences including Rio de Janeiro (Table 2), excluding the possibility of vector transmission and strongly favoring the mouse-to-human transmission.

The detection of ZIKV in human saliva fluid has been reported [8], and although the presence of infectious virus has not been addressed, it opens the possibility of ZIKV transmission

Table 2. GenBank Accession Number and Description of Sequence Dataset Used in Phylogenetic Analyses

GenBank Accession Number	Description
KY014308	Brazil (Rio de Janeiro)
KY559032	Brazil (Rio de Janeiro)
KY785437	Brazil (Rio de Janeiro)
KU926309	Brazil (Rio de Janeiro)
KY272991	Brazil (Rio de Janeiro)
KY559025	Brazil (Rio de Janeiro)
KY785410	Brazil (Rio de Janeiro)
KY785467	Brazil (Rio de Janeiro)
KY785485	Brazil (Rio de Janeiro)
KY785450	Brazil (Rio de Janeiro)
KY559028	Brazil (Rio de Janeiro)
KY559029	Brazil (Rio de Janeiro)
KY817930	Brazil (Rio de Janeiro)
KY559026	Brazil (Rio de Janeiro)
KY785426	Brazil (Rio de Janeiro)
KY014307	Brazil (Rio de Janeiro)
KY559027	Brazil (Rio de Janeiro)
KY559030	Brazil (Rio de Janeiro)
KY785480	Brazil (Rio de Janeiro)
KY785455	Brazil (Rio de Janeiro)
KY785429	Brazil (Rio de Janeiro)
KY785446	Brazil (Rio de Janeiro)
KY014320	Brazil (Rio de Janeiro)
KY014296	Brazil (Rio de Janeiro)
KY014301	Brazil (Rio de Janeiro)
KY014317	Brazil (Rio de Janeiro)
KY559031	Brazil (Rio de Janeiro)
KY014313	Brazil (Rio de Janeiro)
KX197192	Brazil (Pernambuco)
KY558990	Brazil (Pernambuco)
KY558993 MF352141	Brazil (Pernambuco) PE 243
KU509998	Haiti
KX051563	Haiti
KX447518	French Polynesia
KX447510	French Polynesia
KX447519	French Polynesia
KX447513	French Polynesia
KX447512	French Polynesia
KX369547	French Polynesia
KX447511	French Polynesia
KX447520	French Polynesia
KJ776791	French Polynesia
KJ776791	French Polynesia
KX447509	French Polynesia
KX447521	French Polynesia
KX447514	French Polynesia
KX447516	French Polynesia
KX447515	French Polynesia
KX447517	French Polynesia
KU681081	Thailand
KF993678	Canada
JN860885	Cambodia
JN860885	Cambodia
EU545988	Micronesia
EU545988	Micronesia
KU681082	Philippines
HQ234499	Malaysia
KF383119	Senegal
KF383121	Senegal
KF383118	Senegal
AY632535	Uganda
KF268950	Central Africa
KF268949	Central Africa
KF383115	Central Africa
HQ234501	Senegal
KF383116	Senegal
HQ234500	Nigeria
KF383117	Senegal

by this route. Our case involving mouse saliva supports the possibility of transmission by a bite accident. However, it remains to be determined whether the presence of viable and infectious virus particles were in the saliva itself or associated to blood cells. Nevertheless, this case reinforces the importance of biosafety practices in the management and manipulation of ZIKV-infected animals.

CONCLUSIONS

In this case, the timing of symptoms development is compatible with the ZIKV incubation period, after the laboratory accident. In addition, the timing of appearance and duration of diagnostic infectious markers (RNA, IgM, and IgG) agrees with the mouse transmission hypothesis. More importantly, the phylogenetic analysis showed that closer proximity of ZIKV sequences recovered from human- and mouse-infected samples supports this transmission route.

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Potential conflicts of interest. All authors: No reported conflicts of interest. All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest.

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