Experimental Zika Virus Infection in a Neotropical Primate Model

John A. Vanchiere MD PhD1,2, Julio C. Ruiz DVM1, Thomas J. Kuehl PhD3, Scott C. Weaver PhD4, Nikos Vasilikis PhD4, and Christian R. Abee DVM1

1Department of Veterinary Sciences, University of Texas MD Anderson Cancer Center, Bastrop, TX
2Department of Pediatrics, Louisiana State University Health Sciences Center, Shreveport, LA
3Department of Obstetrics and Gynecology, Baylor Scott & White Hospital, Temple, TX
4Department of Pathology and Center for Biodefense and Emerging Infectious Diseases, University of Texas Medical Branch, Galveston, TX

ABSTRACT

Background: The epidemic of Zika virus (ZIKV) infection in South and Central America and its serious complications during pregnancy have highlighted the need for animal models of ZIKV disease for the study of pathological and therapeutic strategies prior to testing in humans. The purpose of this study was to determine whether inoculation of squirrel monkeys (Saimiri species) with Asian-lineage ZIKV results in infection similar to that seen in humans.

Methods: Two Bolivian squirrel monkeys (Saimiri boliviensis boliviensis) and two Guyanese squirrel monkeys (Saimiri sciureus) were inoculated with ZIKV, subcutaneously inoculated in early pregnancy with 10^5 plaque-forming units (PFU) of ZIKV isolated from Mexico during the current epidemic. Blood, urine and saliva samples were obtained for virologic and serologic analyses. Virologic assessments were performed by plaque reduction neutralization test (PRNT) and plaque assay. Concomitant intravenous inoculation was performed. Seropositivity was determined by plaque neutralization assay at 28 days post-inoculation.

Results: All four animals remained clinically well after ZIKV inoculation and there were no abnormalities in the hematologic and chemist profile performed at 5 days post-inoculation. Viremia was detected in two of the four animals inoculated with ZIKV, with peak viremia occurring at 14 days post-inoculation, followed by a rapid decline in detectable ZIKV. Viremia was detected at all 14 time points sampled in the study. Overall, 3 out of 4 inoculated animals had demonstrable neutralizing antibodies at 28 days post-inoculation.

Conclusions: This study provides evidence of the susceptibility of squirrel monkeys to infection with Asian-lineage ZIKV, supporting the hypothesis that these neotropical primates could serve as a reservoir and amplification host if a cyclic model of ZIKV could be established in South America.

MATERIALS AND METHODS

This study was performed at the NIH-funded Squirrel Monkey Breeding and Research Resource (SMBRR) at the University of Texas M.D. Anderson Cancer Center (MDACC) after IACUC approval.

Animals: Animals used in this study were bred in captivity at the SMBRR. All ZIKV-inoculated animals were housed in BSL-2 qualified research rooms at the SMBRR. Caregiver Section (C-vaccination) deliveries were performed 1-2 days prior to natural labor and delivery in order to avoid cannibalization of potentially infective placentas and to allow for the placement of anesthetic for pathology analysis.

Zika Virus: ZIKV (strain Mexico, 1, 44) obtained from the current South/ Central American outbreak was provided by the NIH-funded World Reference Center for Emerging Viruses and Arboviruses within the Center for Biodefense and Emerging Infectious Diseases at the Institute for Human Infections and Immunity at the University of Texas Medical Branch. The virus was produced in Vero cells and diluted to the working concentration of 10^5 plaque-forming units (PFU) per 100μl in normal saline for use and stored at −80°C until used. Higher doses may be necessary to produce more severe neurologic disease in the developing fetus.

Clinical and Laboratory Assessments: Study animals were observed daily by animal care technicians and complete physical examinations were performed every two days for the first two weeks of the study by neotropical primate veterinarians. Subsequently, physical examinations were performed weekly until the conclusion of the study. Complete blood count and comprehensive metabolic panel were performed at 5 days post-inoculation and then clinically indicated. Ultrasound examination was performed weekly beginning prior to ZIKV virus inoculation for evaluation of fetal length, head growth, placental abnormalities and general well-being.

Virologic assessments: Blood, urine and saliva specimens for quantitative qPCR detection of ZIKV were performed using established techniques. The volume of blood, urine and saliva collected for each virologic assessment was 250μl. Blood collected in the protocol was minimized (10μl per week) in order to avoid inadvertent inoculation. ZIKV-neutralizing antibody titers were determined by plaque reduction neutralization test (PRNT) in 96-well plates.

RESULTS AND CONCLUSIONS

Unlike the other endemic and epidemic arboviruses of South and Central America, Zika virus (ZIKV) is a novel pathogen with limited human data. Zika virus infection during pregnancy has been associated with microcephaly and other congenital abnormalities. The purpose of this study was to characterize the dynamics of ZIKV infection during pregnancy of squirrel monkeys (Saimiri species), neotropical primates which may be both a natural host of ZIKV in South America and an appealing nonhuman primate model for studies of human ZIKV infection during pregnancy. This project provides new insights into the pathogenesis of congenital ZIKV infection and the potential for experimental evidence of congenital ZIKV neuroinvasive disease in a primate model, adding to our understanding of the sylvatic cycle of ZIKV in South America and to fully characterize the pathogenesis of ZIKV in the developing embryo.

In this study, we characterized the dynamics of ZIKV infection during pregnancy of squirrel monkeys (Saimiri spp.), a neotropical primate which may be both a natural host of ZIKV in South America and an appealing nonhuman primate model for studies of human ZIKV infection during pregnancy. This project provides new insights into the pathogenesis of congenital ZIKV infection and the potential for experimental evidence of congenital ZIKV neuroinvasive disease in a primate model, adding to our understanding of the sylvatic cycle of ZIKV in South America and to fully characterize the pathogenesis of ZIKV in the developing embryo.

Table 1. Summary of Demographic, Clinical and Virologic Data on Zika Virus-Exposed and Control Animals

<table>
<thead>
<tr>
<th>Animal</th>
<th>NEHP</th>
<th>Species</th>
<th>Age (y)</th>
<th>Sex</th>
<th>Placental Inoculation</th>
<th>Virus Inoculation</th>
<th>Viremia</th>
<th>Viruria</th>
<th>PRNT80</th>
<th>Infant #</th>
<th>Sex</th>
<th>Placental Inoculation</th>
<th>Virus Inoculation</th>
<th>Viremia</th>
<th>Viruria</th>
<th>PRNT80</th>
</tr>
</thead>
<tbody>
<tr>
<td>5834</td>
<td>3y</td>
<td>S. sciureus</td>
<td>3y</td>
<td>NA</td>
<td>NA/144d</td>
<td>NA/144d</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>6391</td>
<td>male</td>
<td>NA/144d</td>
<td>NA/144d</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>5817</td>
<td>3y</td>
<td>S. sciureus</td>
<td>3y</td>
<td>NA</td>
<td>NA/144d</td>
<td>NA/144d</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>6391</td>
<td>female</td>
<td>NA/144d</td>
<td>NA/144d</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
</tbody>
</table>

Figure 1. Timeline of Viremia (A) and Salivary Excretion (B) for Zika Virus-exposed Squirrel Monkeys by RT-qPCR

Figure 2. Time Course of Fetal Femur Length (A) and Biparietal Diameter (B) from Gestational Age Using Ultrasound Measurement for Zika Virus-exposed Squirrel Monkeys Compared to 54 Historical Controls

REFERENCES

(4) Kalika ECS, Gillette MA. Time-Resolving Sharing of Zika Virus Data in an Interdisciplinary World. JAMA Pediatr 2016 Mar 31:

RESULTS AND CONCLUSIONS

1. This study confirmed the susceptibility of Bolivian and Guianan squirrel monkeys to infection with ZIKV with resultant viremia, viruria and salivary excretion for 14 days post-inoculation.
2. Based on this study, we estimate the dose of ZIKV required to obtain infection in 50% (ID50) of squirrel monkeys is ~10^5 PFU. Higher doses may be necessary to produce more severe neurologic disease in the developing fetus.
3. This study demonstrates the sensitivity of fetal ultrasound for monitoring in utero growth and for evaluation of the placentas in a neotropical primate model of ZIKV infection.
4. ZIKV infection of pregnant squirrel monkeys results in fetal abnormalities of the cerebral cortex and placenta consistent with those seen in humans, despite the absence of detectable viruria in some animals.
5. This neotropical primate model of ZIKV infection and congenital ZIKV disease may be useful for evaluation of antiviral agents and candidate ZIKV vaccines for prevention and/or treatment of congenital ZIKV disease of the brain.
6. Further studies of ZIKV in neotropical primates are necessary to help define the role of nonhuman primates in the sylvatic cycle of ZIKV in South America.