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Experimental Zika Virus Infection in a Neotropical Primate Model

John A. Vanchiere MD PhD[1,2], Julio C. Ruiz DVM[1], Thomas J. Kuehl PhD[3], Scott C. Weaver PhD[4], Nikos Vasilakis PhD[4], and Christian R. Abee DVM[1]

[1]Department of Veterinary Sciences, University of Texas MD Anderson Cancer Center, Bastrop, TX
[2]Department of Pediatrics, Louisiana State University Health Sciences Center, Shreveport, LA
[3]Department of Obstetrics and Gynecology, Baylor-Scott & White Hospital, Temple, TX
[4]Department 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 prophylactic 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 in early pregnancy with 10e5 plaque-forming units (PFU) of ZIKV isolated from Mexico during the current epidemic. Blood, urine and saliva specimens were collected every other day for the first 14 days post-inoculation and then every 14 days thereafter for quantitative PCR assessment of Zika virus replication. Seroconversion was documented 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 hematology and chemistry profiles performed at 5 days post-inoculation. Viremia was detected in two of the four animals inoculated with Zika virus, with peak viremia occurring at 5 and 9 days post-inoculation, followed by a rapid decline in detectable ZIKV. Viruria was detected at 9 and 14 days post-inoculation and salivary excretion was detected as late as 14 days post-inoculation. Three of the four ZIKV-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 should a sylvatic cycle of ZIKV 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 BSL2-qualified research rooms at the SMBRR. Caesarian Section (C-section) delivery was performed ~7 days prior to natural labor and delivery in order to avoid cannibalization of potentially sick infants and to allow recovery of the placenta for anatomic 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 10e5 plaque-forming units (PFU) per 100ul in normal saline for subcutaneous inoculation between the scapulae of two Bolivian and two Guyanese squirrel monkeys.

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 5 days post-inoculation and then as clinically indicated. Ultrasound evaluation was performed weekly beginning prior to Zika virus inoculation for evaluation of femur length, head growth, placental abnormalities and general wellbeing.

Virologic assessments: Virologic assessments (blood, urine and saliva specimens for quantitative PCR detection of ZIKV) were performed using established techniques. The volume of blood, urine and saliva required for each virologic assessment was 200ul. Blood collections in the protocol were minimized (<3cc per week) in order to avoid iatrogenic anemia. ZIKV-neutralizing antibody titers were determined by plaque neutralization. Complete necropsy was performed on the newborn animals within 2 hours of delivery and specimens were collected in appropriate media for virus cultivation *in vitro*, DNA extraction, and routine histologic studies. Routine evaluation of necropsy tissues was performed by the primate pathologists at the SMBRR. Tissue viral loads were determined in fresh-frozen tissues obtained at necropsy using established assays.

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BACKGROUND

Unlike the other endemic and epidemic arboviruses of South and Central America, Zika virus (ZIKV) is now known to be the cause of severe neurologic damage in an estimated 1-10% of infants born to mothers with primary ZIKV infection in early pregnancy.(3) The true magnitude of *in utero* neurologic damage attributable to ZIKV is not yet known, but the 20-fold increase in microcephaly reported in Brazil during 2015 may be the proverbial "tip of the iceberg" since significant neurologic development continues well into the second year of life in humans. As such, the urgency to understand the pathogenesis of congenital ZIKV infection and to develop prophylactic and therapeutic strategies to abrogate the impact of ZIKV in naïve populations is clear. While many important questions will be answered over the next several years by observational studies in South and Central America, animal models to study ZIKV are critically necessary to test both the safety and efficacy of vaccine and antiviral agents prior to the consideration of deploying such strategies in humans, especially during pregnancy.

Until recently, the data on experimental infections of laboratory animals with ZIKV was limited to a small number of manuscripts that were published in the two decades after the discovery of ZIKV. (1,2) Recent and ongoing studies of ZIKV infection in rhesus macaques (4) and rodents (5) provide *in vivo* models for testing of prevention and treatment strategies, but additional models are necessary to broaden 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 environmental maintenance of ZIKV in South America among neotropical primates. Furthermore, it provides experimental evidence of congenital ZIKV neuroinvasive disease in a primate model, adding to the accumulating clinical data that supports a causal association between ZIKV infection in pregnancy and microcephaly. The results of this study will be used to develop additional protocols for testing of antiviral agents and ZIKV vaccine candidates that may be useful to prevent congenital ZIKV infection in humans.

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

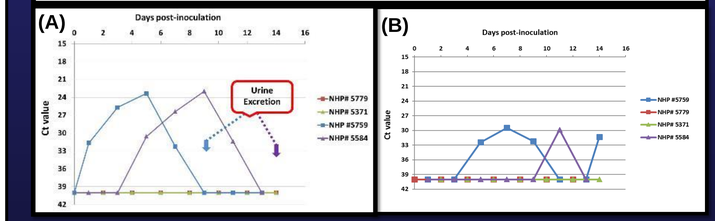

Figure 2. Time Course of Fetal Femur Length (A) and Biparietal Diameter (B) By Gestational Age Using Ultrasound Measurement for Zika Virus-exposed Squirrel Monkeys Compared to 54 Historical Controls

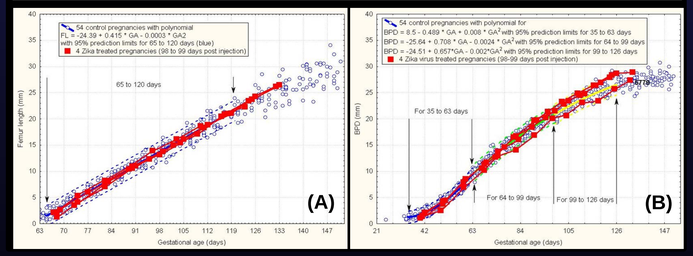

Table 1. Summary of Demographic, Clinical and Virologic Data on Zika Virus-Exposed and Control Animals

Animal	NHP#	Species	Age	EGA at inoculation	DPI/EGA at delivery	Viremia	Viruria	ZIKV PRNT80	Infant #	Sex	Placental anomalies	Placenta Weight	Fetal anomalies	Brain weight
1	5759	S. boliviensis	3y	33d	111d/145d	+	+	1:80	6391	male	Reflections	22.86g	Calcification	16.4g
2	5779	S. boliviensis	3y	34d	111d/144d	-	-	1:80	6392	male	Reflections, infarction	18.19g	↓ BPD	16.0g
3	5371	S. sciureus	5y	25d	119d/145d	-	-	<1:20	6394	male	Villous degeneration	21.47g	gliosis	14.9g
4	5584	S. sciureus	4y	24d	119d/144d	+	+	1:160	6395	male	Zika +	17.49g	-	15.1g
5	5834	S. sciureus	3y	NA	NA/144d	-	-	-	6389	male	-	20.25g	-	15.25g
6	5817	S. sciureus	3y	NA	NA/146d	-	-	-	6393	female	-	16.83g	-	13.9g

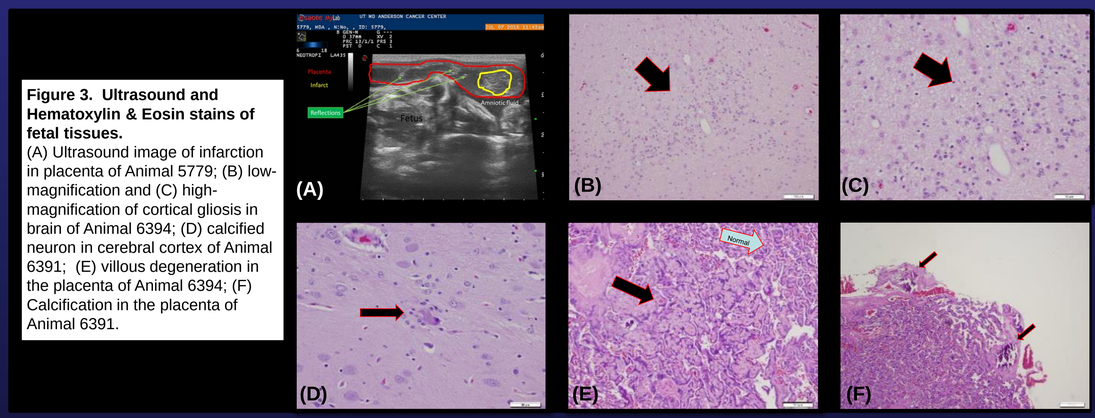

Figure 3. Ultrasound and Hematoxylin & Eosin stains of fetal tissues.
(A) Ultrasound image of infarction in placenta of Animal 5779; (B) low-magnification and (C) high-magnification of cortical gliosis in brain of Animal 6394; (D) calcified neuron in cerebral cortex of Animal 6391; (E) villous degeneration in the placenta of Animal 6394; (F) Calcification in the placenta of Animal 6391.

RESULTS AND CONCLUSIONS

1. **This study confirmed the susceptibility of Bolivian and Guyanese 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% (ID_{50}) of squirrel monkeys is ~10e5 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 placenta 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 viremia 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.**