Identification of viral reservoirs in Zika virus infected macaques to inform policies for human cells, tissues, and cellular and tissue-based products

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Introduction

Mosquito-borne Zika virus (ZIKV, family *Flaviviridae*, genus *Flavivirus*) was declared a 'public health emergency' by the World Health Organization in February 2016. The rapid and explosive spread of ZIKV in more than 60 new countries and territories in the Americas and Asia since June 2015 has caused millions of infections worldwide, including more than 5,000 and 36,000 symptomatic cases in the continental U.S. and U.S. territories, respectively, in 2016.

As part of its mission to protect public health, FDA creates policies to minimize the risk of transmission of infectious diseases through human cells, tissues, and cellular and tissue-based products (HCT/Ps). This effort includes methods designed to ensure that donors are not infected with certain infectious diseases that could be transmitted through an HCT/P resulting in harm to the recipient. ZIKV presents potential new policy considerations due to lack of knowledge regarding tissue tropism. For example, prolonged persistence of ZIKV has been observed in tissues such as placenta and semen. However, the presence and persistence of ZIKV, and therefore its potential for transmission, in other tissues are largely unknown. In an effort to better understand this risk, FDA partnered with the California National Primate Center at the University of California, Davis to perform studies to investigate ZIKV tissue tropism using a nonhuman primate model.

Methods

- 4 pregnant rhesus macaques were inoculated intravenously and intraamniotically with a 2015 Brazilian strain of ZIKV during the 1st or 2nd trimester and euthanized near the end of gestation, except in 1 animal whose fetus died 7 days post-inoculation (dpi)
- Up to 38 different tissues were collected from each primate and tested for presence of ZIKV RNA by quantitative reverse transcription polymerase chain reaction (qRT-PCR) and/or qualitative transcription-mediated amplification (TMA)
- Tissues with ≥ 3 $\log_{10}$ copies/gram tissue were further tested for presence of infectious ZIKV by plaque assay on Vero cells
- Histopathological analyses by *in situ* hybridization (ISH) was performed on most RNA positive tissues using two sets of probes designed to hybridize to the positive sense ZIKV RNA genome

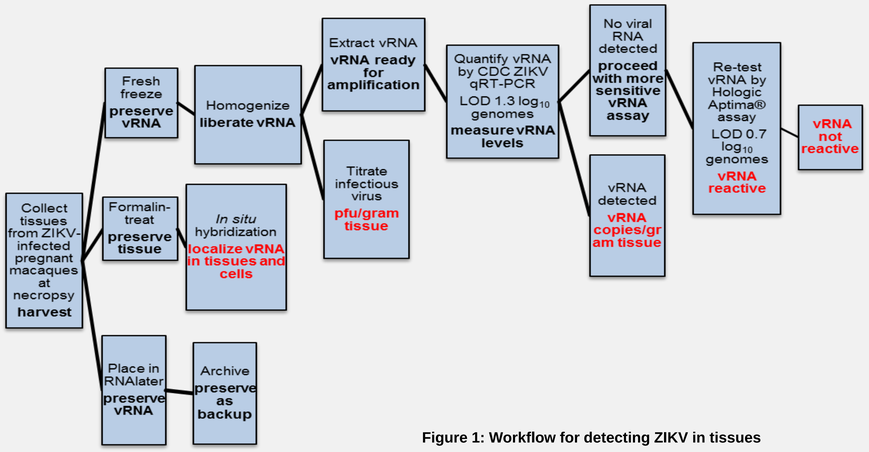

Figure 1: Workflow for detecting ZIKV in tissues

Results

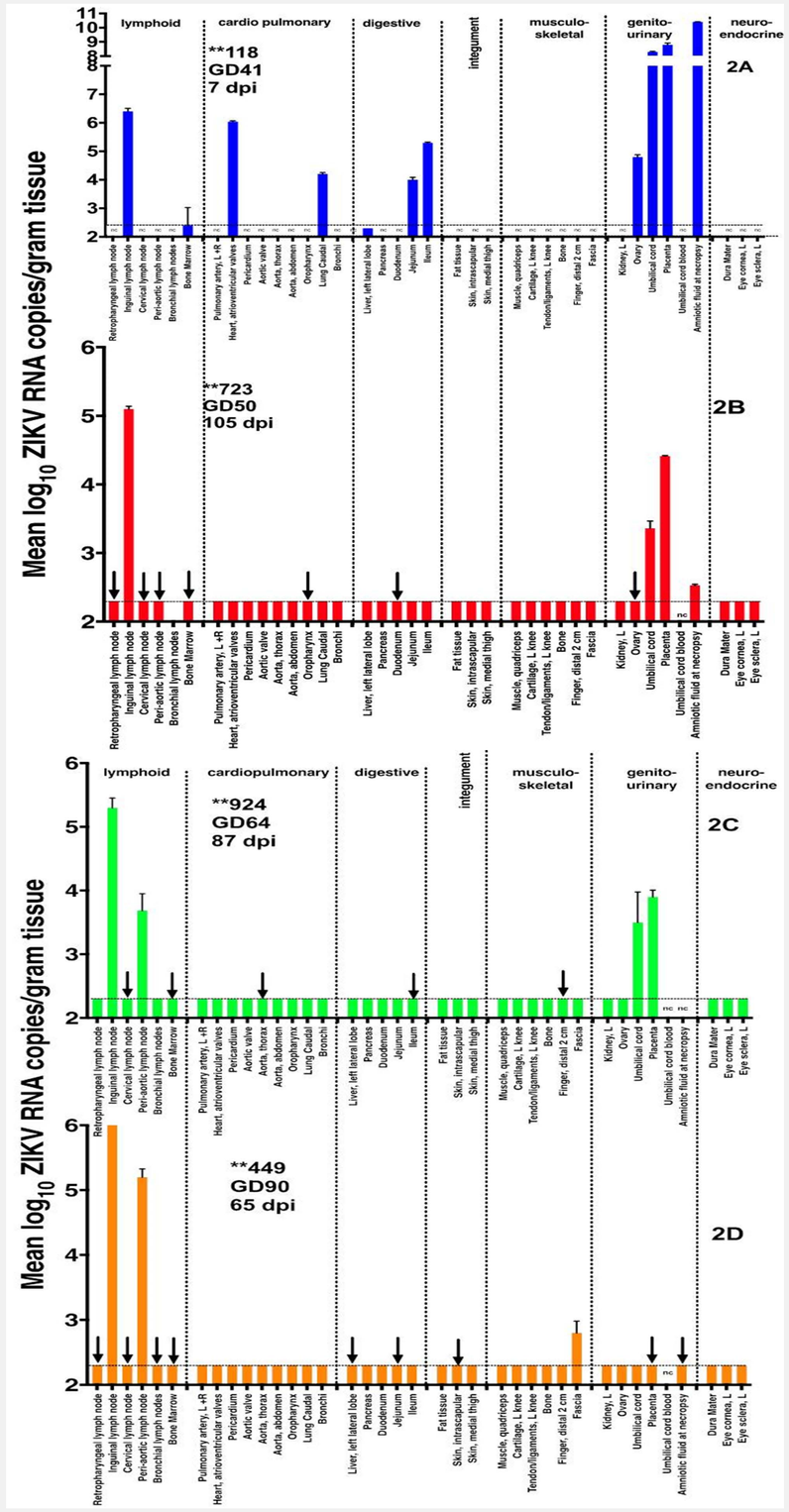

Figure 2: ZIKV RNA copies in tissues of pregnant rhesus macaques inoculated and harvested at (A) GD41 and 7 dpi, (B) GD50 and 105 dpi, (C) GD64 and 87 dpi, and (D) GD60 and 65 dpi. Black arrows show tissues that tested reactive by TMA.

ZIKV RNA and infection rates in tissues from experimentally infected pregnant rhesus macaques

Macaque identifier	**118	**723	**924	**449
Gestation day at inoculation	41	50	64	90
Gestation day at necropsy	48	155	151	155
Days post-inoculation at time of necropsy	7	105	87	65
Tissues containing ZIKV RNA/# tissues tested (%)	11/12 (92%)	11/36 (31%)	9/35 (25%)	12/36 (33%)
# tissues containing infectious ZIKV/# ZIKV RNA positive tissues (%)	3/11 (27%)	1/11 (9%)	1/9 (11%)	0/12 (0%)
# ISH positive tissues/# tissues tested by ISH (%)	3/8 (38%)	5/9 (56%)	7/7 (100%)	6/7 (86%)

Summary

- Detectable ZIKV RNA persists in selected tissues from pregnant rhesus macaques intravenously and intraamniotically inoculated with a 2015 strain of ZIKV from Brazil
- Zika virus RNA was detected in tissues from all 4 animals in this study
- Persistence of ZIKV RNA was detected for up to 105 days, the latest time tissues were sampled post-inoculation
- The dam sampled 7 dpi had the greatest proportion of ZIKV RNA positive tissues, the highest ZIKV RNA levels, and tissues were more likely to contain infectious ZIKV compared to the other 3 dams in the study
- In all 4 animals, ZIKV RNA was detected in lymphatic and genitourinary tissues
- Some animals also contained detectable ZIKV RNA in cardiopulmonary, digestive, and musculoskeletal tissues
- Infectious ZIKV was detected in placental samples from 3 of the dams, the umbilical cord (1 animal), and amniotic fluid (1 animal)
- The peak infectious titer of 6.9 log10 PFU/ml was detected in the umbilical cord of the animal inoculated at GD41 and collected 7 dpi

Collaboration and Response to Public Health Needs

The FDA and University of California, Davis partnered to investigate which tissues Zika virus targets in non-human primates. Information from these studies increase understanding of the risk of Zika virus transmission through infected tissue products and may ultimately improve product safety.

As a result of this study, FDA has gained understanding regarding how the Zika virus impacts a variety of tissues in non-human primate models. This information, together with additional ongoing studies, will inform FDA with regard to the risk of transmission of ZIKV through HCT/Ps and the development of appropriate policies to minimize risk.