doi:10.1017/S1431927617007334
Microsc. Microanal. 23 (Suppl 1), 2017

Zika Virus, a Newly Emergent Flavivirus

Cynthia S. Goldsmith[1], D. Brett Rabeneck[1], Roosecelis B. Martines[1], Julu Bhatnagar[1], Dominique C. Rollin[1] and Sherif R. Zaki[1]

[1.] Infectious Diseases Pathology Branch, Division of High-Consequence Pathogens and Pathology, Centers for Disease Control and Prevention, Atlanta, GA

Zika virus (ZIKV) is a single-stranded, positive-sense RNA virus in the family *Flaviviridae* that was first isolated in 1947 from a rhesus macaque in the Zika forest in Uganda. A large outbreak of ZIKV infection in humans was confirmed in April 2015 in Brazil. Infection early in pregnancy has been associated with congenital microcephaly and other adverse outcomes, including pregnancy loss [1]. ZIKV is the first infectious agent that has been found to cause congenital defects in more than 50 years, and the first mosquito-borne illness that is associated with congenital defects. Understanding the disease biology of ZIKV is crucial for effective intervention. To this end, correlating clinical manifestations with underlying tissue pathology can provide critical insights into disease pathogenesis. The symptoms of ZIKV infection in adults are generally mild, and include fever, rash, joint pain, conjunctivitis (red eyes), muscle pain, and headache, but approximately 80 percent of ZIKV patients are asymptomatic. ZIKV illness may lead to cases of Guillain-Barré syndrome, an illness where the immune system attacks peripheral nerves, which can result in (mostly) temporary partial or total paralysis. ZIKV can be transmitted by mosquitoes, sex and blood transfusions. Other illnesses with similar clinical symptoms which are circulating in the same regions include Chikungunya and dengue.

Electron microscopy of Zika virus-infected LLC-MK2 culture cells showed morphological changes consistent with a flavivirus. Virions, approximately 40 nm in diameter and composed of an inner dense core and an outer envelope, and smooth membrane vesicles, known to be the replication complexes for this virus, were found within the rough endoplasmic reticulum (Fig 1). Additionally, in the brain of a 22 week old fetus, electron microscopy identified virus particles and replicative complexes in neurons, and virions in neuronal processes and extracellularly were also found (Figs 2, 3, 4, 5). Immunohistochemistry (IHC) and in situ hybridization (ISH) assays detected ZIKV antigens, and genomic and replicative RNA, respectively, in the cerebral cortex of fetuses in neurons and degenerating glial cells (Figs 6, 7, 8) [2, 3]. In placentas from Zika virus-infected mothers, viral antigens and RNA and replicative RNA were observed in the chorionic villi, mostly in Hofbauer cells, which are placental macrophages (Figures 9, 10, 11) [2, 3]. Moreover, ZIKV RNA was also identified by RT-PCR and sequencing in formalin-fixed paraffin-embedded brain and placental tissues.

IHC and ISH studies found evidence of viral proteins, virus particles and replicative RNA in the brain in neurons and glial cells. The electron microscopic findings in the fetus provide direct proof that ZIKV is replicating in neurons, as evidenced by the presence of ZIKV replication complexes and virions. Viral particles in dendrites and axons is indicative of virus transport through these neuronal processes.

References:

[1] SA Rasmussen *et al*, NEJM **374** (2016) p. 1981
[2] RB Martines *et al*, Lancet **388** (2016) p. 898
[3] J Bhatnagar *et al*, EID **23** (2017) *in press*

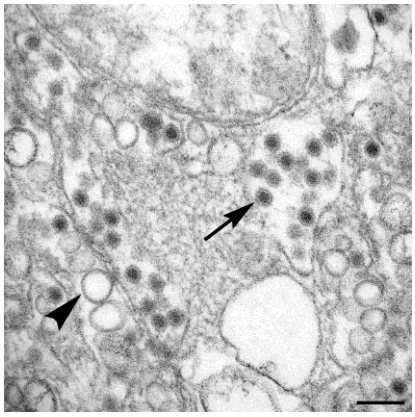

Figure 1. Zika virus grown in cell culture. Arrow, virus particles. Arrowhead, smooth membrane vesicles. Bar, 100 nm

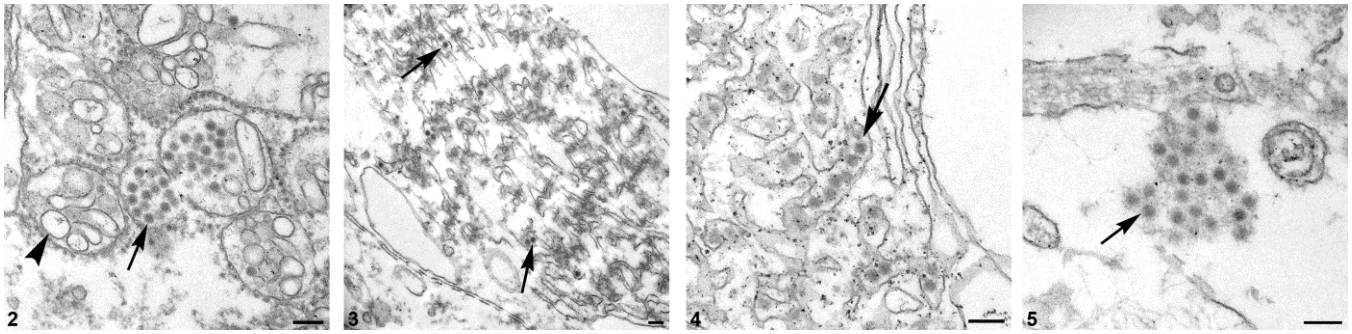

Figure 2. Mature flavivirus particles and replication complexes (Fig 2) were seen within the rough endoplasmic reticulum of neurons. Virions were observed in convoluted membranes in dendrites (Fig 3) and axons (Fig 4). Extracellular particles (Fig 5), surrounded by an amorphous material, were found in clusters. Arrows, virus particles. Arrowhead, smooth membrane vesicles. Bars, 100 nm.

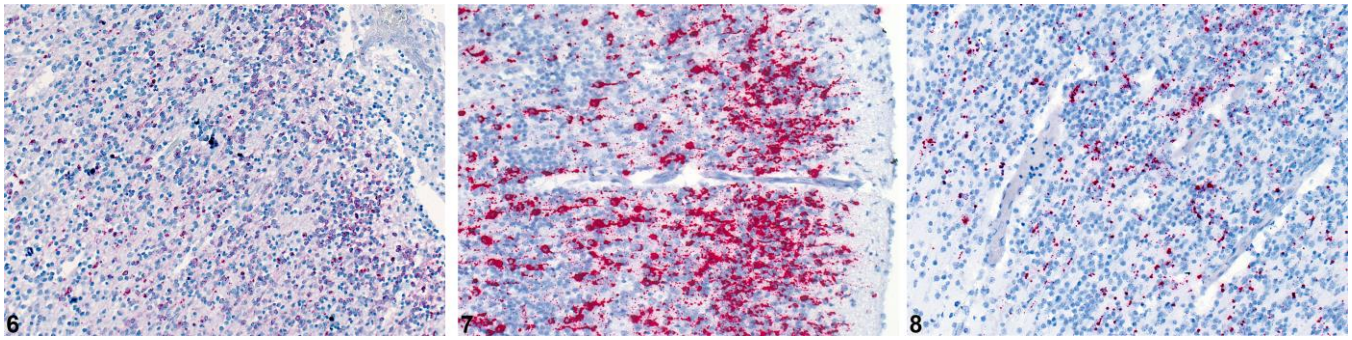

Figure 3. Fetal brain. Fig 6) IHC, which detects viral proteins. Fig 7) ISH using anti-sense probe, which detects viral RNA. Fig 8) ISH using sense probe, which detects replicative RNA. Note the heavy staining of neural cells. Original magnifications, 20x.

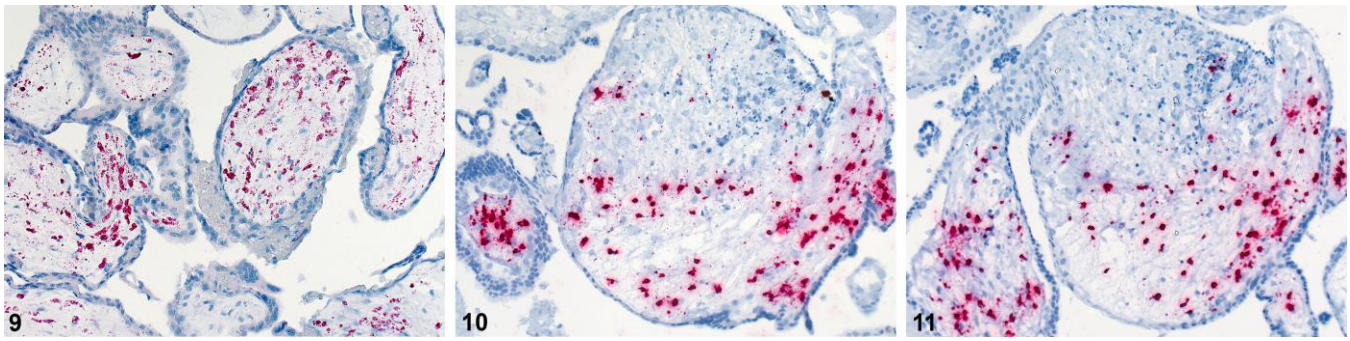

Figure 4. Placenta. Fig 9) IHC, which detects viral proteins. Fig 10) ISH using anti-sense probe, which detects viral RNA. Fig 11) ISH using sense probe, which detects replicative RNA. Most of the staining is within Hofbauer cells. Original magnifications, 20x.

https://doi.org/10.1017/S1431927617007334 Published online by Cambridge University Press