Zika Virus MB16-23 in Mosquitoes, Miami-Dade County, Florida, USA, 2016

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We isolated a strain of Zika virus, MB16-23, from *Aedes aegypti* mosquitoes collected in Miami Beach, Florida, USA, on September 2, 2016. Phylogenetic analysis suggests that MB16-23 most likely originated from the Caribbean region.

In 2016, outbreaks of locally transmitted Zika virus occurred in Miami (Wynwood neighborhood) and Miami Beach, in Miami-Dade County, Florida, USA (1). During these outbreaks, a Centers for Disease Control and Prevention (CDC) emergency response team was deployed to assist Miami-Dade County disease surveillance and control efforts. CDC entomologists within the CDC emergency response team worked with Miami-Dade County Mosquito Control and sampled mosquito populations using BG-Sentinel type-2 traps (Biogents AG, Regensburg, Germany) to determine basic entomological parameters. Routinely, mosquitoes were collected, identified to species on the basis of the morphologic characteristics described by Darsie and Ward (2), and shipped inactivated and preserved in RNAlater (Ambion Inc., Austin, TX, USA) to the Bronson Animal Disease Diagnostic Laboratory (Kissimmee, FL, USA) for Zika virus testing.

In addition to the routine outbreak protocol, 2 BG-Sentinel type-2 traps were placed at a construction site near the intersection of James Avenue and Lincoln Road (25°47′25.68″N, 80°07′50.24″W) in Miami Beach on September 1, 2016. This site was selected because it was adjacent to a site where Zika cases had been detected. On September 2, 2016, the mosquitoes captured were frozen and shipped on dry ice to the CDC laboratory in Fort Collins, Colorado, USA. In the laboratory, the mosquitoes were identified to species on chill tables; female *Aedes aegypti* mosquitoes were separated into pools of 50 mosquitoes or less. A total of 293 female *Ae. aegypti* mosquitoes were collected (146.5/trap/day), grouped into 7 pools, and processed for presence of arboviral agents by cytopathic effect (CPE) assay.

We triturated pools of mosquitoes in 500 µL of Dulbecco’s modified Eagle medium complete with penicillin...
(100 U/mL), streptomycin (100 mg/mL), 20% fetal bovine serum, and 50 µg/mL amphotericin B. We used the clarified supernatants from triturated mosquito pools to inoculate Vero (mammalian) cells in 24-well plates. We observed the inoculated cells daily and harvested them upon the appearance of CPE. Of the mosquito pools processed, only 1 pool of 50 female Ae. aegypti mosquitoes caused CPE. Final titration of the Vero passage was 7.02 log₁₀ PFU/mL. We reinoculated the harvested supernatant onto Ae. albopictus C6/36 cells; these cell cultures were maintained at 28°C with complete Dulbecco’s modified Eagle medium supplemented with 2% fetal bovine serum and penicillin/streptomycin. We extracted viral RNA from 140 µL of the supernatant harvested from the C6/36 cell cultures using a QIAamp RNA Mini Kit (QIAGEN, Valencia, CA, USA). We performed reverse transcription PCR confirmation on the extracted RNA using flavivirus-specific primers, as described previously (3).

We performed all procedures using commercial products according to the manufacturer’s protocols unless otherwise noted. We generated cDNA from extracted RNA using the NuGEN Ovation RNA-seq system V2 (NuGEN Technologies, San Carlos, CA, USA). Libraries were constructed using the Ion Xpress Plus gDNA Fragment Library preparation kit (Life Technologies, Carlsbad, CA, USA) by fragmenting cDNA for 1.5 min, generating fragments of
Identification of Wild Boar–Habitat Epidemiologic Cycle in African Swine Fever Epizootic

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The African swine fever epizootic in central and eastern European Union member states has a newly identified component involving virus transmission by wild boar and virus...