Early Detection of Chikungunya Virus in *Aedes aegypti* Mosquitoes

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**Introduction:**
Chikungunya virus is a member of the Alphavirus genus (family: Togaviridae), a single stranded RNA virus transmitted by mosquitoes such as *Aedes aegypti* and *Ae. albopictus*. Within its genus, two distinct patterns of disease are seen: New-world viruses, those that cause arthropathies like Eastern equine encephalitis virus and Old-world viruses, those that cause arthralgia like chikungunya virus. Chikungunya virus is endemic in Africa and India and has recently been discovered in the Caribbean with concern for potential spread to the United States. As of July 15th 2014, there have been 357 cases in the United States, including two that were spread from within Florida itself. Of the mosquitoes that transmit Chikungunya virus, *Ae. aegypti* can be regularly found in southern states such as Texas and Florida, Louisiana, etc.. However, the mosquito species is not able to survive any frost periods. *Ae. albopictus* can be found in several northern/mid-western states of the US and the species is adapted to typical winter climates in these regions. Thus, in the near future, there is a high likelihood for CHIKV transmission to occur in various regions of the country. With the epidemic emerging, robust viral detection techniques are needed both to identify new cases and help prevent the spread of the disease.

**Vectorial Capacity** is an epidemiological term that describes the overall ability of a vector in a given location at a specific time to transmit a pathogen. It can be also be looked at as the number of infectious bites a host might receive at a daily basis. Thus, an important component of Vectorial Capacity is the time period the pathogen such as CHIKV spends in the mosquito vector before it can be transmitted by the insect (=extrinsic incubation period) [2]. In this study, we investigated the infection pattern of the virus in two *Ae. aegypti* strains. Most previous studies have focused on detecting the virus in mosquitoes at 7 to 10 days following ingestion of the infectious blood meal by the vector. In this study, virus detection in mosquitoes was performed at 3, 5 and 7 days post infectious blood meal to reveal a minimal length of the extrinsic incubation period of the virus in the vector. For this experiment, we used two detection methods, plaque assays and immune-fluorescence assays (IFA). Plaque assays were used to determine titers of infectious virus in the midguts and carcasses of mosquitoes. IFA looked for the presence of viral antigen in the midgut as well as in salivary glands. Eggs were collected from mosquitoes fed an infectious blood meal and the larvae were tested for the presence of viral RNA. Detection outside of the midgut, especially the presence of viral particles in salivary glands, indicate the potential to transmit the virus to new hosts.

**Discussion/Conclusions:**
- Presence of CHIKV in the midgut of mosquitoes at three days post-infectious blood meal indicates that dissemination of the virus occurs at a rate similarly seen with other Alphaviruses [3].
- The presence of CHIKV antigen in the tracheae of the mosquitoes supports previous studies that show that arboviruses escape the midgut of the mosquito by passing into the trachea where the basal lamina is thinner [3].
- The ability of the virus to escape through the cardia region of the midgut has also been suggested to contribute to fast dissemination rate [3].
- *HWE* mosquitoes had a higher CHIKV titer at three days post-infectious blood meal compared to Orlando mosquitoes.

**Figure 1:** CHIKV 379K77 titers in midguts and carcasses of HWE and Orlando *Aedes aegypti* mosquitoes at three and five days post-infectious blood meal as determined by plaque assay using Vero cells. The carcass virus titers were significantly higher than the midgut virus titers at each time point. CHIKV titers were also higher in HWE mosquitoes compared to Orlando mosquitoes at each time point.

**Figure 2:** CHIKV titers in midguts and carcasses of individual HWE and Orlando Mosquitoes determined with plaque assays using Vero cells.

**Figure 3:** IFA samples were incubated with the primary, mouse monoclonal anti-CHIKV antibody followed by an incubation with secondary antibody, biotin-labeled goat anti-mouse IgG antibody and counterstain (Evans Blue). A final incubation with PBS containing streptavidin-fluorescein (FITC) and viewed under a fluorescent microscope, equipped with GFF filter sets. The antigen was localized to the tracheae and salivary glands of infected mosquitoes.

**Figure 4:** RT-PCR results using total RNA from CHIKV stock and infected mosquito offspring.

**References:**