

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 encephalitis 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 this 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 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 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 immuno-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. Determining the timing of the movement of the virus outside of the midgut and the ability of the virus to be transovarially passed between mosquito generations have important implications for control methods.

Results:

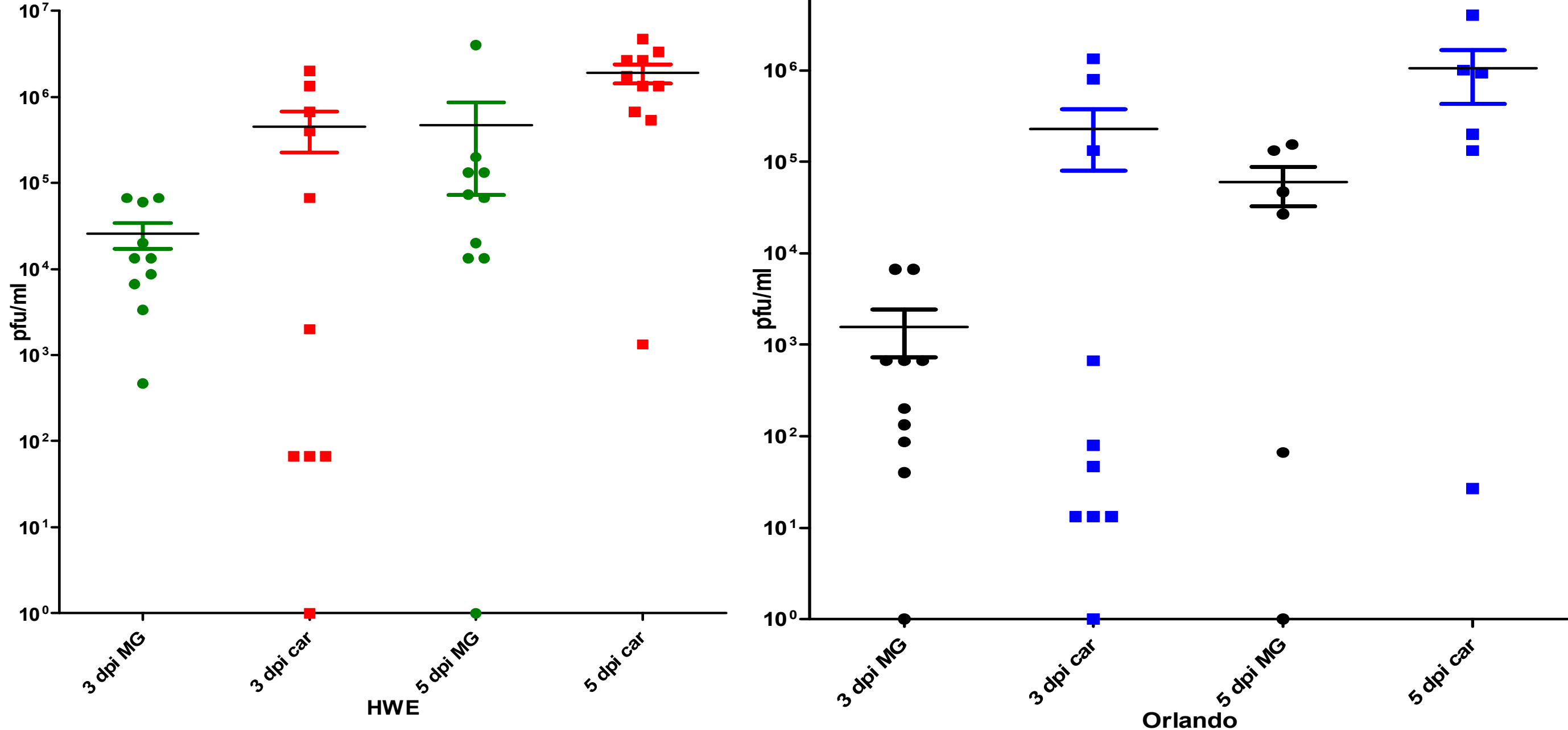


Figure 1: CHIKV 37997 titers in midguts and carcasses of HWE and Orlando *Aedes aegypti* mosquitoes at three and five days post infectious blood meal as determined by plaque assay using Vero cells. The carcass virus titers were significantly higher than the midgut virus titers at each time point. CHIKV titers were also higher in HWE mosquitoes compared to Orlando mosquitoes at each time point.

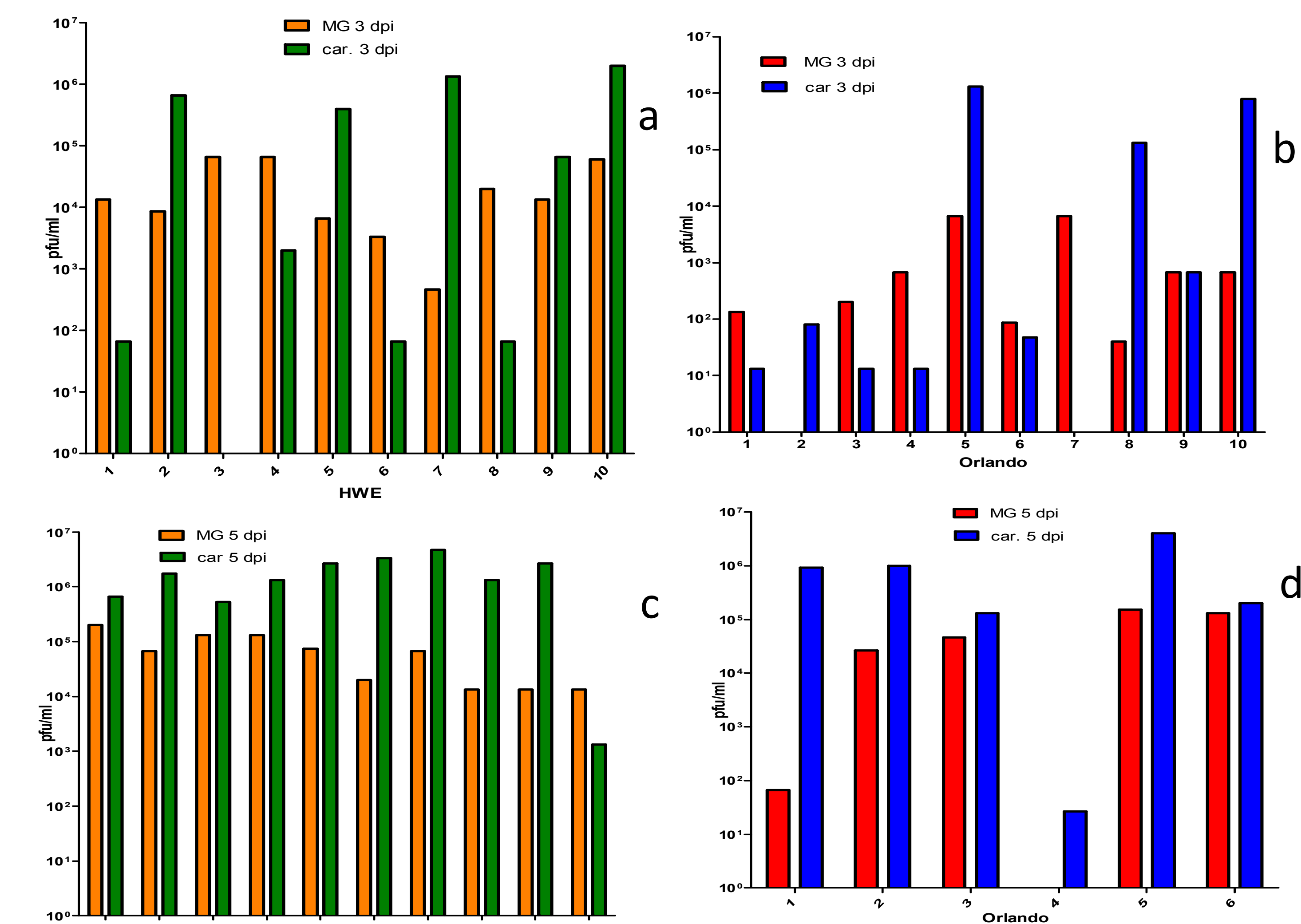


Figure 2: CHIKV titers in midguts and carcasses of individual HWE and Orlando Mosquitoes determined with plaque assays using Vero cells

2a: CHIKV titers in HWE at 3 days post infectious blood meal (dpi)

2b: CHIKV titers in Orlando at 3 dpi

2c: CHIKV titers in HWE at 5 dpi

2d: CHIKV titers in Orlando at 5dpi

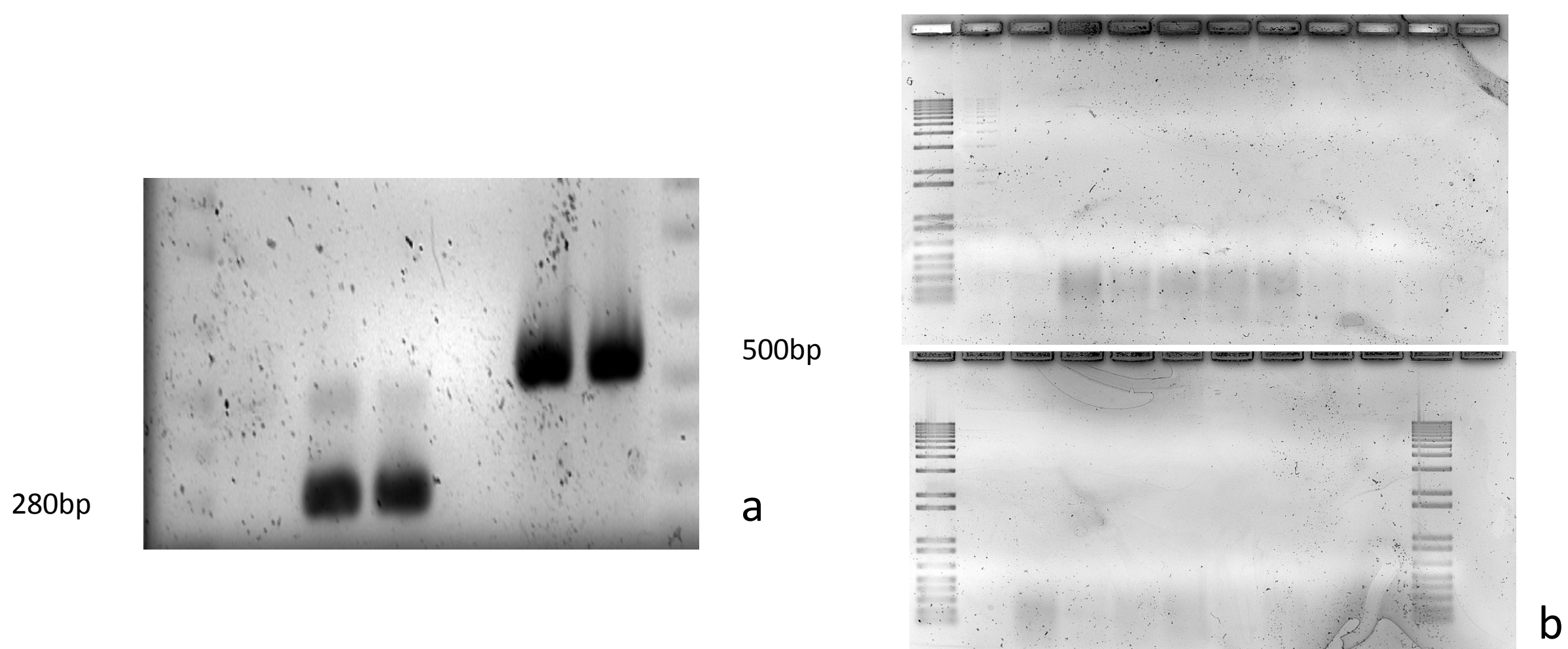


Figure 4: RT-PCR results using total RNA from CHIKV stock and infected mosquito offspring

4a: RT-PCR results from CHIKV stocks using 2 different primer sets

4b: RT-PCR results from mosquito larvae hatched from eggs laid 5 dpi

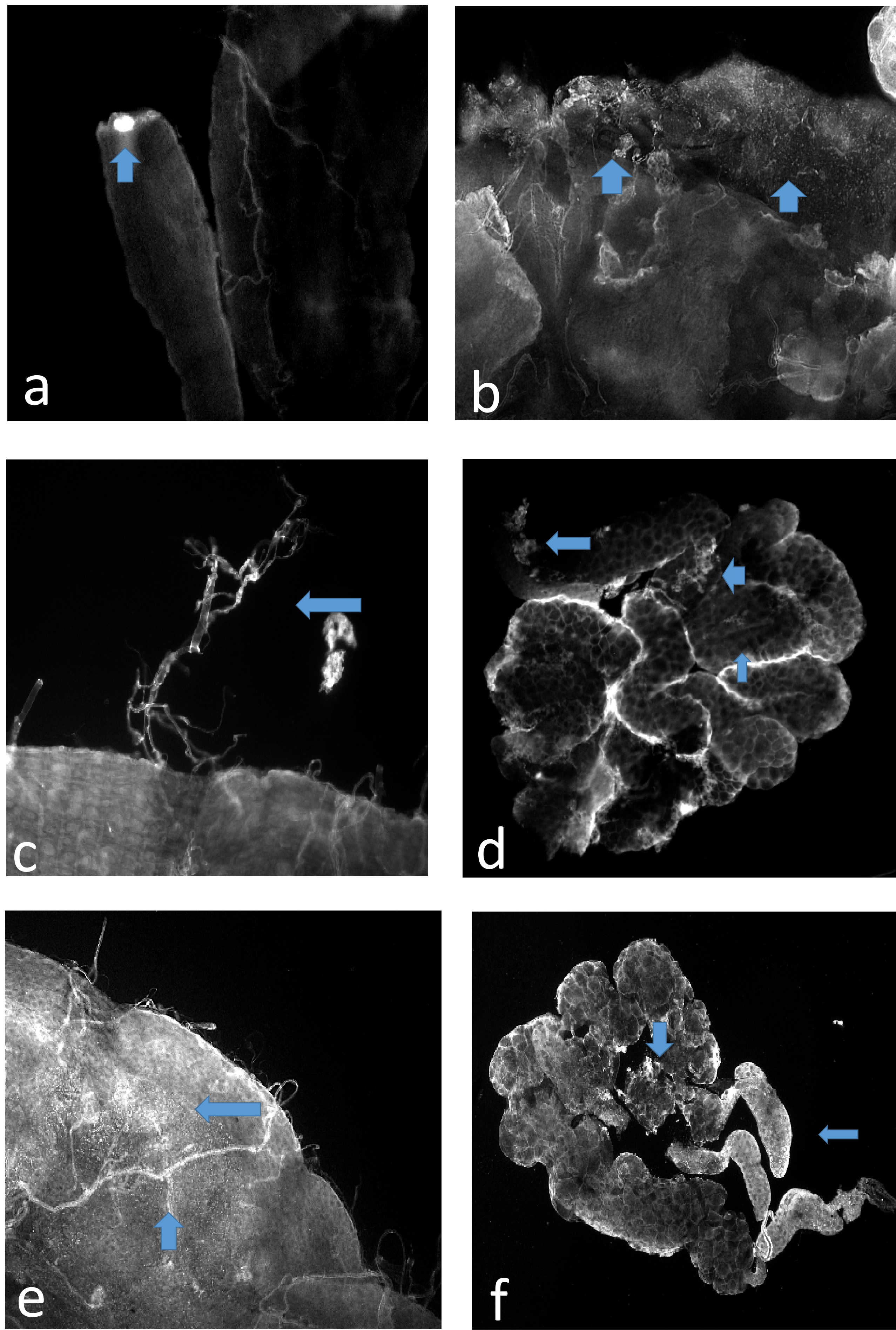


FIGURE 3: IFA samples were incubated with the primary, mouse monoclonal anti-CHIKV antibody followed by an incubation with secondary antibody, biotin-labeled goat anti-mouse IgG antibody and counterstain (Evans Blue). A final incubation with PBS containing streptavidin-fluorescein [FITC] and viewed under a fluorescent microscope, equipped with GFP filter sets

Figure 3a: CHIKV antigen in cardia of mosquito (*Ae aegypti* Orlando) midgut 3dpi.

Figure 3b: CHIKV antigen in midgut epithelium of mosquito (*Ae aegypti* Orlando) 3dpi.

Figure 3c: CHIKV antigen in the trachea of a mosquito (*Ae. aegypti* Orlando) 3dpi.

Figure 3d: CHIKV antigen in the salivary glands of a mosquito (*Ae. aegypti* HWE) 3dpi

Figure 3e: CHIKV antigen in the midgut epithelium and trachea of a mosquito (*Ae. Aegypti* Orlando) 5dpi

Figure 3f: CHIKV antigen in the salivary glands of a mosquito (*Ae. aegypti* Orlando) 5dpi.

Discussion/Conclusions:

- Presence of the virus outside of the midgut at three days after an 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 trachea of the mosquitoes support previous studies that show that arboviruses escape the mid-gut of the mosquito by passing into the trachea where the basal lamina is thinner(1).
- 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 and overall maintained a higher titer compared to the Orlando mosquitoes.
- There is little to no transovarial transmission of CHIKV, indicating that in nature the virus is predominately maintained by horizontal transmission.

References:

- (1)Rosomer, W.S. et al. "Evidence for Arbovirus Dissemination Conducts from the Mosquito (Diptera: Culicidae) Midguts". *Journal of Medical Entomology*. 2004. 41(3):467-475.
- (2) Hardy, J.L. Houk, E.J. Kramer, L.D. and Reeves, W. C. "Intrinsic factors affecting vector competence of mosquitoes for arboviruses". *Annual Reviews of Entomology*. 1983. 28: 229-262.
- (3) Foy, B.D. and Olsen, K.E. "Alphavirus Transducing Systems." *Transgenesis and the Management of Vector-borne Disease*. 2008. 19-24.