

Evaluation of *Aedes aegypti* Densonucleosis (AeDNV) Infection in Adult Mosquito Mortality

Emily M. Plake, Malena Doehling, Stacey Bray, Dr. Jon Carlson, and Dr. Erica Suchman
Department of Microbiology, Immunology, and Pathology, Colorado State University, 80523

Methods and Materials

1. Mosquito Collection and Insectary Environement.
   A Reville-D strain of *Aedes aegypti* mosquitoes, cultured since 1991, was obtained (C. Meredith, Arthropod-Borne Infectious Disease Laboratory, Colorado State University Fort Collins, CO) and used for all experiments. This room is on a 12 hour photoperiod and maintains a constant temperature of 80°F and 80% humidity by daily observation and monitoring. Larvar and Pupae were fed 50 ul of a mixture of mouse and fish food and water on an as needed basis.

2. Virus Production.
   AeDNV was produced by transfecting cell cultures grown in L-15 medium supplemented with 10% fetal bovine serum and streptomycin-penicillin in 75 cm2 polystyrene culture with infectious clones (Afanasev, 1991). Transfection was done using the Qiagen Effectene TRANSFUSION protocol and cells were incubated at 28°C for 4 days following transfection. Post incubation, virus pellets were collected through multiple centrifugations. The pellets collected were then resuspended in 1ml of double distilled water per 30ml of original solution and filtered using a 0.45 μm syringe style filter (Sartorius*, Kenne, NH) (Ledermann et al., 2004).

   Mosquito eggs were batched in approximately 500ml of tap water and 1 ml of Blood, Brain and Heart infusion. One hour post batching 25 larvae were isolated and placed into eight 100ml plastic cups (25 larvae per cup for a total of 200 larvae) containing 5mls of tap water. Each cup was then infected with the appropriate volume of virus to get the desired dose (1x10^7 genome equivalents per ml) and fed 5 ul of food. The cups were allowed to incubate at 80°F and 80% humidity for 24 hours without food. After 24 hours 75 milliliters of water were added to the cup to total eighty milliliters. Dead adults were recorded and removed from existing populations. Removed adults were placed in a 1.2 microfuge tubes for further analysis by Quantitative PCR.

4. Quantitative PCR.
   The primer and probe design: Degenerate primers within the NS1 gene of densonucleosis genome were designed to detect viral genome. The probe has a Fam fluorescent dye and a black hole quencher at the 3' end of the sequence (forward: CAT ACT ACA CAT CTC TCC ACA A, probe: FAMUGG CCA AAG CAG CCG C, rev: CCT GTG GAC TCT TCT GCC TCT T). (Ledermann et al., 2004).

   -Quantitative of Viral Genomes: A plasmid containing the viral NS1 gene was used as a standard to establish a linear regression. The viral genome equivalent (geq/ml) in the samples were then calculated based on the fluorescence values obtained from these standards (Ledermann et al., 2004).

5. Virus (DNA) Isolation
   Qiagen® DNeasy protocol for insects is used to extract these biological samples to be analyzed for virus concentration by Quantitative PCR.

*Figure 1: Graph of Adult Mortality (percentage vs. Day Post Infection). This graph is from the primary studies of adult mortality and shows the highlighted areas of early and late mortality.*

*Figure 2: Viral genome equivalent per ml isolated from mosquitoes that died in the early and late parts of the mortality graph. Note that in both the early and late parts of the mortality graph there were individuals that contained viral titers anywhere from 1 x 10^7 genome equivalents per ml to 1 x 10^12 genome equivalents per ml.*

*Figure 3: Average genome equivalents per ml for both early and late mortality groups. This graph allows for visual comparison between the two.*

Abstract

*Aedes aegypti* densonucleosis virus (AeDNV) has long been considered a likely agent in biologic control against arthropod-borne diseases. Extensive studies have been done to evaluate infection and pathogenesis in *Aedes aegypti* larvae and pupae, but only primary studies have been performed in adults. The primary studies on adult mortality produced a two part mortality curve that showed high levels of early mortality, low levels of intermediate mortality and high levels of late mortality. It was hypothesized that the high levels of early mortality were due to high levels of virus infection, whereas the high levels late mortality were due to age. In this study we analyzed the mosquitoes that died early and late in the mortality assay with quantitative virus titers in individual mosquitoes. We found that there was no significant difference between titers of the first and second mortality groups. This graph allows for visual comparison between the two.

Discussion

• Our hypothesis that early adult mosquito mortality is caused by high virus levels, whereas late mortality is caused by old age is incorrect. In fact, high virus levels were found in individuals that died in both the early and late parts of the mortality curve.

• There is no correlation between the time of death in adult individuals and the virus titer of that individual. Therefore, there is no correlation between death and virus titer. This supports previous data that showed high virus titers in adult mosquitoes that were infected with less virulent strains of densonucleosis viruses (Ledermann et al., 2004).

• No correlation between death and virus titer suggests that the viruses ability to cause mortality is dependent on something other than the amount of virus present in the individual. The viruses ability to cause mortality could be dependent on many other factors such as; the location of infection, the amount of time it takes for the virus to reach that location, host (mosquito) immunity to the virus, and environmental factors related to the viruses ability infect.
Longevity 3: Total Death/Total # Hatched vs. Days Post Hatch

- Day 0 Control
- Day 3 Control
- Day 0 $10^8$
- Day 3 $10^8$
- Day 0 $10^9$
- Day 3 $10^9$
- Day 0 $10^{10}$
- Day 3 $10^{10}$

Days Post Hatch

Total Death/Total Emerged