OrNV Witches Brew Experiment: A Last Ditch Attempt to Find Virus Pathogenetic for the Guam Cocoonut Rhinoceros Beetle Genotype

Aubrey Moore, Ian Iriarte and Roland Quitugua

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Bioassays of several isolates of Oryctes nudivirus provided by AgriResearch New Zealand failed to result in significant pathogenicity for the Guam CRB genotype. In a 'last ditch' attempt we made a 'witches brew' slurry containing all frozen dead beetles from previous bioassays plus frozen virus samples in vials. Forty adult beetles were forced to swim in the slurry for 30 minutes on January 22, 2015. A control group of 41 beetles were forced to swim in water. Beetles were checked weekly.

By May 10, 2015, mortality of the virus treated beetles (78%) was significantly greater than that of the control group (54%).

Methods

Frozen, dead beetles from previous bioassays were added to one liter of water and made into an aqueous slurry using a blender. Vials containing remnants of virus samples from AgResearch New Zealand were agitated in 500 ml of water, and this suspension was added to the blender. The slurry was poured into a small pail and forty beetles were made to swim in this for thirty minutes. A control group of beetles was made to swim in water for thirty minutes.

Beetles were kept individually in Mason jars filled with commercially blended steer manure and soil. All beetles were checked weekly. Dead beetles were recorded and frozen.

Analysis

Data were analyzed using an IPython notebook (file name = 'OrNV'). Significance of differences in mortality were determined using a Fisher's exact test.

Results and Discussion

Cumulative mortality of virus-treated beetles (57%) on April 10 (Fig. 1) was significantly greater than that of control beetles (44%); ($p = 0.0005$; Fisher's exact test).

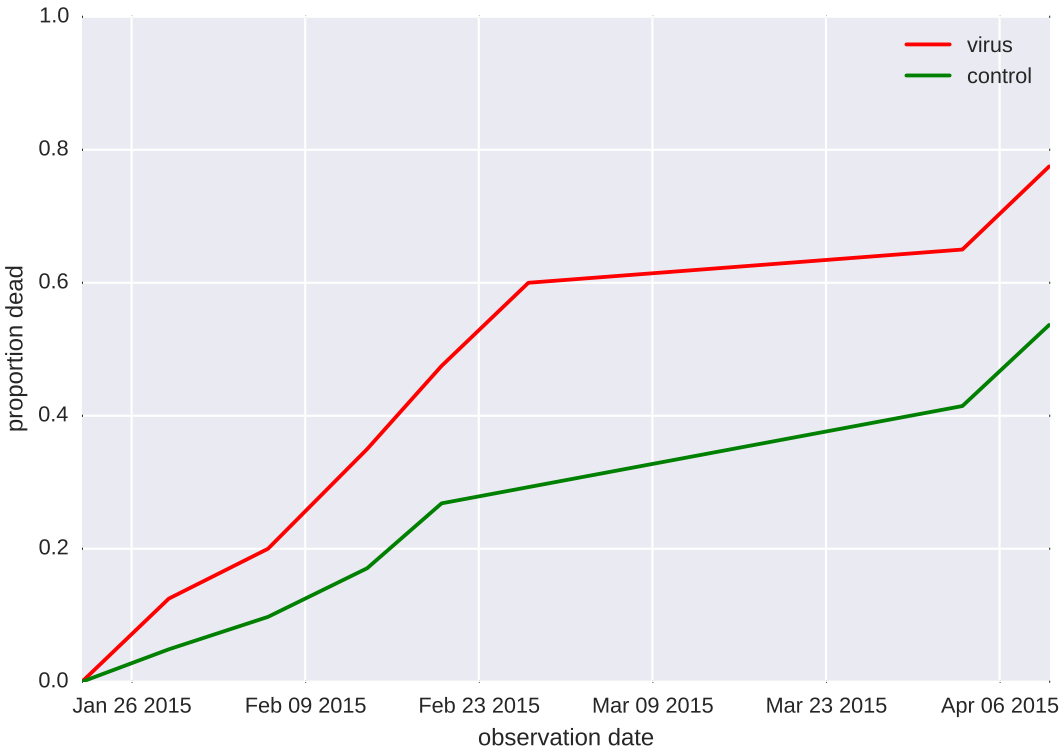

Figure 1: Cumulative mortality.

It is unlikely that the beetles which died during the first two weeks of the experiment resulted from exposure to virus, so we removed these from the experimental data and repeated the significance test. This adjustment did not alter the outcome: Mortality of virus-treated beetles (57%) was significantly greater than that of control beetles (44%); ($p = 0.0005$; Fisher's exact test).

This experiment is incomplete. A postmortem will be done on the dead beetles and the 'witches brew' process will be repeated to see if this also results in significant mortality.