

# OrNV Witch's Brew Experiment 1: A Last Ditch Attempt to Find Virus Pathogenetic for the Guam Coconut Rhinoceros Beetle Genotype

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December 2, 2015

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 April 10, 2015, mortality of the virus treated beetles (78%) was significantly greater than that of the control group (54%). Treatment mortality corrected for experimental control mortality by Abbott's formula was 51%.

## 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 in a large container filled with moist, 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.ipynb'). Significance of differences in mortality were determined using a Fisher's exact test, and final mortality was adjusted using Abbott's formula.

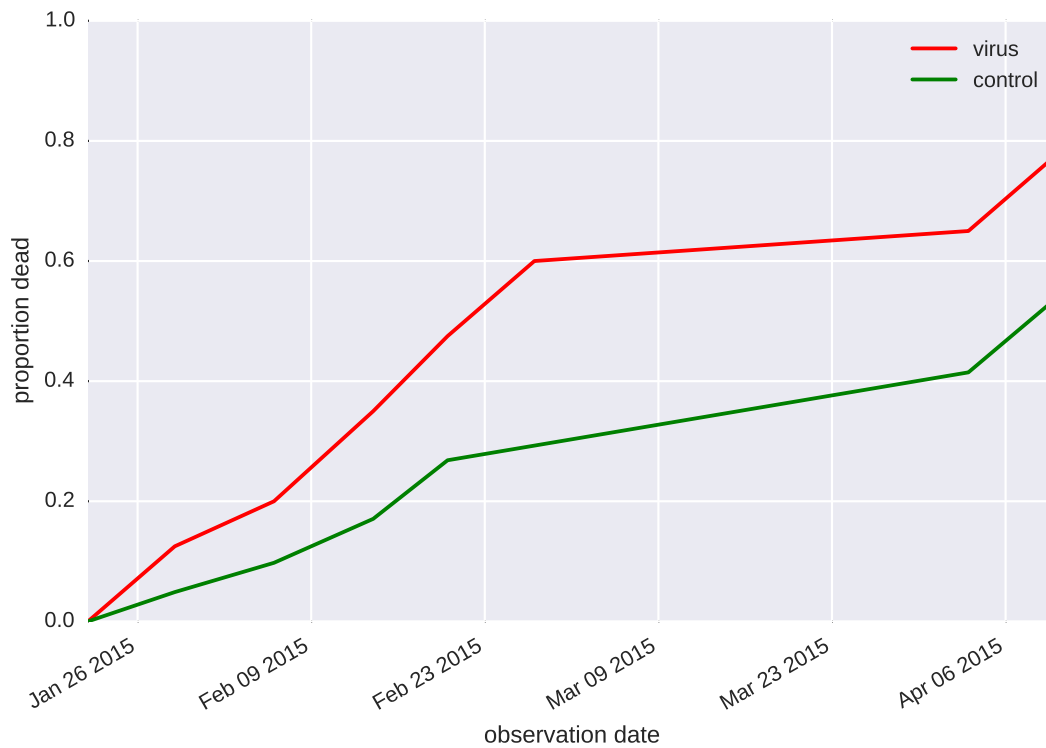


Figure 1: Cumulative mortality.

## Results and Discussion

Cumulative mortality of virus-treated beetles (78%) on April 10 (Fig. 1) was significantly greater than that of control beetles (54%); ( $p = 0.0005$ ; Fisher's exact test). Treatment mortality corrected for experimental control mortality by Abbott's formula was 51%.