

# **OrNV Witch's Brew Experiment 4: A Last Ditch Attempt to Find Virus Pathogenetic for the Guam Coconut Rhinoceros Beetle Genotype**

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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 the Witch's Brew 3 bioassay. Sixty-two adult beetles were forced to swim in the slurry for 30 minutes on June 3, 2016. A control group of 62 beetles were forced to swim in water. Beetles were checked weekly.

By July 1, 2016, mortality of the virus treated beetles (89%) was significantly greater than that of the control group (31%). Treatment mortality corrected for experimental control mortality by Abbott's formula was 84%.

## **Methods**

Frozen, dead beetles from previous bioassays were added to one liter of water and made into an aqueous slurry using a blender. The slurry was poured into a small pail and 62 were made to swim in this for thirty minutes. A control group of 62 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 = 'witchesBrew4.ipynb'). Significance of differences in mortality were determined using a Fisher's exact test, and final mortality was adjusted using Abbott's formula.

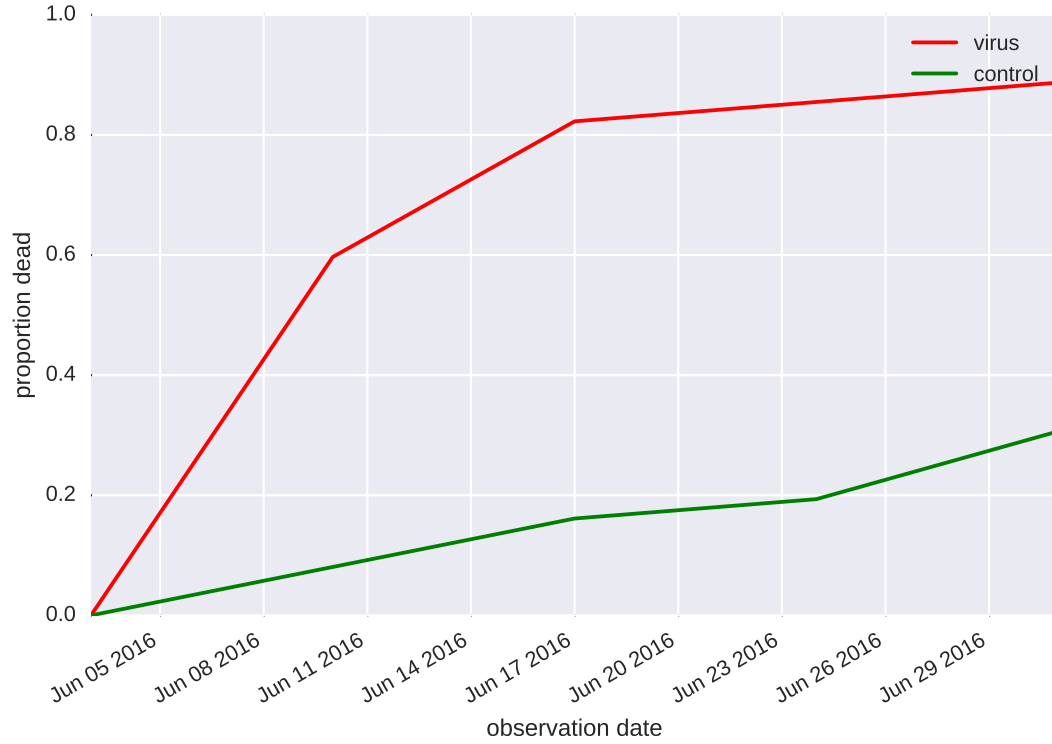


Figure 1: Cumulative mortality.

## Results and Discussion

Cumulative mortality of virus-treated beetles (89%) on July 1, 2016 (Fig. ??) was significantly greater than that of control beetles (31%); ( $p = 0.0000$ ; Fisher's exact test). Treatment mortality corrected for experimental control mortality by Abbott's formula was 84%.