

In Vivo Titration and Development of a Challenge Model for White Spot Syndrome Virus (WSSV) in Pacific White Shrimp (*Litopeneus vannamei*)

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Summary and Implications

A challenge model was developed using a controlled bioassay system for estimation of lethal infective doses (LD 50) of White Spot Syndrome Virus (WSSV), as a model system to be used in further WSSV studies.

Introduction

WSSV is an enveloped double-stranded DNA virus and it has been grouped into the family *Whispoviridae*. WSSV can cause 100% mortality within 7-10 days, resulting in huge economic losses to in the shrimp farming industry. WSSV causes mortality not only in shrimp, but in other crustacean species as well. Therefore, it is important to evaluate the LD50 to shrimp of WSSV in laboratory experiments.

Materials and Methods

Challenge model: Healthy *Litopenaeus vannamei* (*L. vannamei*) shrimp were obtained as postlarvae from Shrimp Improvement Systems, Plantatan Key, FL and

maintained in a specific pathogen free colony at Iowa State University.

Tanks were stocked with 10 SPF shrimp (3- 5 grams body weight each) in 8 x 50 L tanks and allowed to acclimate for 24 hours. Each tank contained artificial seawater with oyster shell biofilters. WSSV dilutions of virus stock were prepared at 1×10^{-3} , 1×10^{-4} and 1×10^{-5} with 2% NaCl. Shrimp were injected with WSSV 100 ul/ shrimp using the different dilutions. Feeding was done daily at 10% biomass, and 10% water exchange were performed daily to remove molts, excess food and fecal material.

Mortality was observed for 14 days, and dead or moribund animals were removed for PCR testing.

WSSV stock: The virus isolate used in this study originated from infected *L. vannamei* shrimp imported from Thailand in 1996. Virus stocks were purified from macerated shrimp tissue, clarified by centrifugation, and filtered at .2 microns. Virus samples were stored in aliquots at -80°C .

Results and Discussion

This study demonstrated the lethal dose 50 (LD 50) of WSSV stock was 1×10^{-4} prepared by using WSSV stock diluted with 2% NaCl as shown (Figure 1). This stock dilution will be utilized as a challenge model for further experiment.

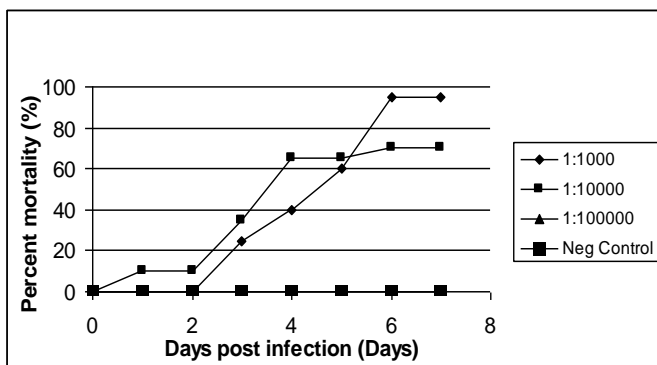


Figure 1. The lethal dose 50 (LD 50) of WSSV.