Direct Delivery of VP19 Double-Stranded RNA into *Litopenaeus vannamei* by Reverse Gavage Induces Protection against White Spot Syndrome Virus Disease

A.S. Leaflet R2753

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Summary and Implications

Double stranded RNA was synthesized *in vitro* and was delivered by reverse gavage (RG) compared to traditional intramuscular injection (IM) 3 days prior to challenge with a lethal dose of WSSV in both groups.

Introduction

Double-stranded RNA (dsRNA) can serve as a potent trigger for an antiviral RNA interference (RNAi) response and has been shown to protect a variety of penaeid shrimp species from infection with viruses including White Spot Syndrome Virus (WSSV). WSSV is one of the most devastating viral agents in the shrimp culture industry. There are no available treatments for WSSV but biosecurity systems have been successful. The aim of this study was to test the efficacy of dsRNA against the WSSV gene, VP19, to protect *Litopenaeus vannamei* from disease when delivered directly to the digestive tract. This study was conducted by delivering VP19 dsRNA through reverse gavage (RG) compared to traditional intramuscular injection (IM).

Materials and Methods

Double-stranded RNA targeting VP19 was generated by *in vitro* transcription.VP19 dsRNA was mixed with red food color to facilitate the observation of dsRNA introduction into the gut.

Specific Pathogen Free (SPF) 3- 5 gram juvenile *L. vannamei* were acquired from Shrimp Improvement Systems (SIS) and divided into 3 groups of 10 shrimp with 3 replicates in each group (n = 90) except 1 replicate in negative control group (n=10). Animals were fasted for 24 h prior to treatment. Treatment groups were received VP19 dsRNA 2 ug/ shrimp either by RG or IM. At 3 days post-VP19dsRNA injection, shrimp in each treatment group and positive control animals were challenged with WSSV. Mortality was observed for 17 days post WSSV challenge. Dead and moribund shrimp were tested for the presence of WSSV by quantitative PCR (qPCR).

Results and Discussion

Animals in the VP19 dsRNA RG, VP19 dsRNA IM, and positive control groups showed survival rates of 100%, 96.5% and 3.45%, respectively (Fig. 1). qPCR revealed that WSSV copies in RG and IM vaccinated group were significantly different ($p<0.05$) from the positive control group with $2.36x10^2$, $1.96x10^2$ and $2.56x10^7$copies, respectively (Fig. 2)

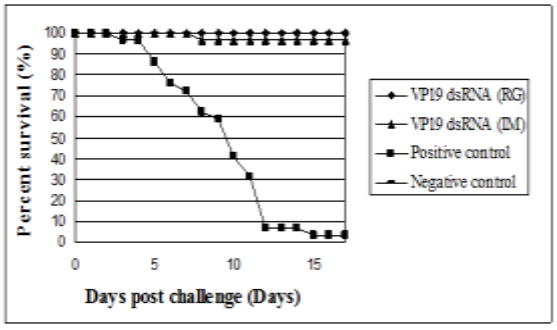

Fig 1. Survival following vaccination via IM or RG.

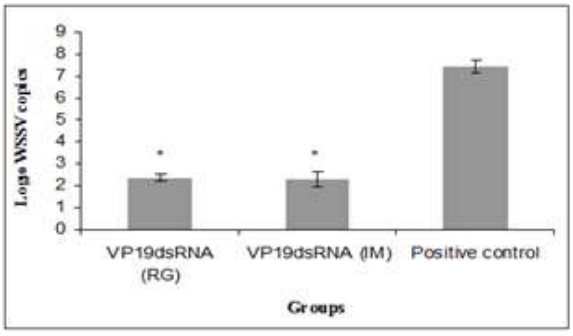

Fig 2. Confirmation of differential expression of WSSV by qPCR.