Susceptibility of *Litopenaeus vannamei, Farfantepenaeus duorarum,* to White Spot Syndrome Virus (WSSV) and Infection of *Menippe adina* with WSSV

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ABSTRACT

White spot syndrome virus (WSSV) can cause 100 % cumulative mortality to farmed shrimp, and there is increasing concern over the possible introduction of this virus into wild shrimp and crab populations in the Gulf of Mexico. In this contribution, we compare the mortality rate of WSSV infected *Farfantepenaeus duorarum* to *Litopenaeus vannamei*. In addition, we demonstrate that the stone crab (*Menippe adina*) is susceptible to WSSV infection.

We used an experimental procedure that is based on a mathematical epidemiology model to compare the survival of *F. duorarum* to *L. vannamei* from exposures to WSSV. The experimental procedure involved exposing 12 uninfected susceptible shrimp to a single infected shrimp cadaver for a specified period of time and then isolating the exposed shrimp individually to determine the number of deaths. *Menippe adina* were challenged by injection of a homogenate containing WSSV and exposed *per os* to WSSV infected tissue. The *L. vannamei* used in the experiment were obtained from the United States Marine Shrimp Farming Program, and *F. duorarum* and *M. adina* were obtained from the wild.

The mean mortality rate from a WSSV exposure was 0.81 for *L. vannamei*, and 0.75 for *F. duorarum*. A statistical difference was not detected in final mean mortality rates between *L. vannamei* and *F. duorarum*. From the *M. adina* challenge, two of the four crabs injected with WSSV died, and both of those were found to be histologically positive for WSSV associated lesions. In addition to the WSSV inclusions, basophilic, intranuclear inclusions were found in hypertrophied nuclei of hepatopancreatic cells which may be caused by another pathogen. Our results suggest *F. duorarum* is as susceptible to mortality from WSSV as *L. vannamei,,* and that *M. adina* is susceptible to infections by WSSV.

KEY WORDS: Crustaceans, Gulf of Mexico, viruses

INTRODUCTION

White spot syndrome virus (WSSV) is a recently described shrimp pathogen that is devastating the shrimp farming industry. WSSV can cause 100 % cumulative mortality to farmed shrimp. Because WSSV is known to have a broad host range, there is concern over the possible introduction of this virus into economically

valuable shrimp and crab populations in the Gulf of Mexico.

Two of the known shrimp hosts of WSSV are *Litopenaeus vannamei*, white-legged shrimp and *Farfantepenaeus duorarum*, pink shrimp (Lightner 1998). *Litopenaeus vannamei* is the most commonly cultured species in the Americas and is one of the most commercially important species comprising the wild shrimp fishery along the Pacific coast of the Americas. *Farfantepenaeus duorarum* is one of three species of shrimp comprising the wild shrimp fishery in the Gulf of Mexico. *Litopenaeus vannamei* is known to be highly susceptible to WSSV, however there is some confusion as to the relative susceptibility of *F. duorarum* to WSSV. Results from two studies have demonstrated that larval *F. duorarum* are as susceptible to WSSV as larval *L. vannamei* and that juvenile *F. duorarum* are less susceptible to WSSV infections than juvenile *L. vannamei* (Lightner et al. 1998 and Wang et al. 1999). However, in the study by Wang et al. (1999) they mention that in a preliminary experiment, juvenile *F. duorarum* were found to be as susceptible to WSSV as juvenile *L. vannamei* (Wang et al. 1999). In addition, preliminary studies performed in our lab have found little difference in mortality rates between juvenile *F. duorarum* and *L. vannamei*.

Menippe adina is a commercially valuable species of stone crab in the Gulf of Mexico (Stuck and Perry 1992). Various crab species have been found to be susceptible to WSSV, but it is not known if *M. adina* is susceptible to WSSV.

In this contribution, we compare the mortality rate of *F. duorarum* to *L. vannamei*, and demonstrate that *M. adina* is susceptible to WSSV infection. In addition, we describe an inclusion in hepatopancreatic cells of *M. adina* that may be caused by a different naturally occurring virus.

MATERIALS AND METHODS

Test Animals and Viral Stock

The *L. vannamei* used in these experiments were obtained from the Oceanic Institute, Hawaii. These shrimp are from the original unselected population of shrimp (Kona stock) that have been maintained by the United States Marine Shrimp Farming Program (Lotz 1997). *Farfantepeneaus duorarum* and *M. adina* used for the experiments were captured in Mississippi Sound, Mississippi, USA. All shrimp used weighed between 2 and 3 grams, and *M. adina* weighed between 0.5 to 25.0 g. The isolate of WSSV used was obtained from mainland China in 1996 and has been maintained in *L. vannamei*.

Experiment 1: Estimating survival rates for *L. vannamei* vs. *F. duorarum*

We used an experimental procedure that is based on a mathematical epidemiology model to compare the survival rates of *F. duorarum* to *L. vannamei* from exposures to WSSV (Soto and Lotz 2000).

In epidemiology models with the Reed-Frost approach to pathogen transmission, transmission from infected host to susceptible individuals is represented by the following equation:

$$S_1 = S_0 - S_0(1-\beta)^{I_0},$$

where S_0 and I_0 are the number of susceptible and infected hosts, respectively at the beginning of some time period, S_1 is the number of susceptible hosts after that time period, and β is the transmission coefficient which is the probability that a contact between a susceptible (S_0) and an infected (I_0) host will result in a transmission. By solving for β, an equation for estimating the transmission rate is obtained:

$$\beta = 1 - \exp\left(\ln\left(\frac{\frac{S_1}{S_0}}{I_0}\right)\right).$$

The transmission rate can be estimated from knowledge of the initial numbers of susceptible (S_0) and infected (I_0) shrimp and the number of susceptible shrimp (S_1) at the end of the time period of interest.

The experimental procedure involved exposing 12 uninfected susceptible shrimp to a single infected shrimp cadaver for a specified period of time and then isolating the exposed shrimp individually to determine the number of successful transmissions. The procedure for obtaining the transmission rate estimates is divided into three phases: preparation of I_0 (initially infected shrimp), exposure, and isolation.

I_0 Preparation

To prepare I_0 (the initial infected shrimp), we exposed two groups of 20 *L. vannamei* to WSSV. The shrimp were exposed in 115 L rectangular aquaria. Each aquarium was filled to a depth of 10 cm of chlorine disinfected seawater. Shrimp were exposed *per os* and received approximately 15 % body weight of frozen, minced cephalothoraces of shrimp known to have died of WSSV.

Exposure

In the exposure phase, susceptible shrimp (S_0) are exposed to infected shrimp (I_0). Twelve susceptible shrimp and one infected shrimp were placed in a cylindrical tank (1 m^2 bottom surface area by 0.6 m height). Each tank was filled to a depth of 10 cm of chlorine treated seawater. The susceptible shrimp were exposed to the infected shrimp for 16 hours. At the end of the exposure, the proportion of the dead infected shrimp consumed by the susceptible shrimp was noted. Water temperature in the exposure tanks was maintained at 27 ± 3 °C.

Isolation

To ensure no secondary transmission from newly infected dying shrimp, the exposed susceptible shrimp were isolated after the initial exposure period. After the 16-h exposure, all shrimp were placed in 1L jars. All jars were placed in a water bath, and each jar was supplied with air. Water temperature in the isolation jars was maintained at 26 ± 2 °C. The time of death of isolated shrimp was recorded. Shrimp were kept in these isolation jars for five days at which time the number of surviving specimens was recorded.

When a single infected shrimp is used, the transmission rate (β) is the proportion of susceptible shrimp getting infected. In a previous study using *L. vannamei*, we found that 100% of shrimp dying during the isolation phase, were WSSV positive, and 98.1% all shrimp that lived through the isolation phase were histologically negative for WSSV (Soto and Lotz 2000). Therefore, the mortality rate of WSSV is a good measure of transmission. We will report mortality rate, the proportion of susceptible shrimp dying, in this study.

Experiment 2: *Menippe adina* Challenge of WSSV

Each *M. adina* was exposed individually in 1 L jars. Each jar was placed in a water bath and supplied with air. Water temperature was maintained at 26 ± 1 °C. Four crabs were challenged by injection with a cell-free shrimp homogenate containing WSSV. The homogenate consisted of a 1:10 dilution of tissue of shrimp known to have died of WSSV in water. Each crab was injected with 0.02 ml per gram of crab into the infrabrachial sinus of the fifth pereiopod. Three crabs were injected with a virus free homogenate. For the per os exposures, 15 crabs received a piece of WSSV infected tissue weighing approximately 5 % body weight, and four crabs received a piece of virus-free shrimp tissue.

Crabs dying were fixed in Davidson's solution following procedures outlined by Lightner (1996). Crabs were kept in these jars for five days at which time all surviving specimens were fixed in Davidson's solution. Each crab was examined histologically after staining with Hematoxylin and Eosin stains for the presence of WSSV intranuclear inclusions. For each crab, two non-serial sagittal sections were analyzed by routine histology. In addition, *in situ* hybridization was performed on a corresponding parallel section. *In situ* hybridization was performed with kits available from DiagXotics, Inc. *L. vannamei* were used as positive and negative controls.

RESULTS

Experiment 1: *L. vannamei* vs. *F. duorarum*

Susceptible *L. vannamei* and *F. duorarum* (S_0) in each tank completely consumed the infected material (I_0). The mean mortality rate from a WSSV exposure was 0.81 (95% CL 0.34 - 0.91) for *L. vannamei*, and 0.75 (95 % CL 0.41-0.87) for *F. duorarum*. A statistical difference was not detected in final mean

mortality rates between *L. vannamei* and *F. duorarum* (chi-square test, P = 0.45). Most animals died between 24 and 60 hours post-exposure for both *L. vannamei* and *F. duorarum* (Figure 1). No animals from either negative control group died during the experiment.

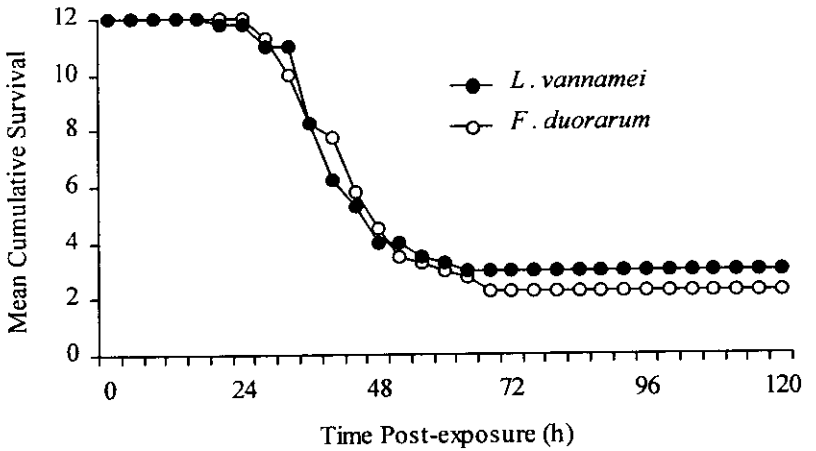

Figure 1. Mean cumulative survival in four-hour increments of *Litopenaeus vannamei* and *Farfantepenaeus duorarum* exposed to WSSV. No difference in final mean survival was detected (chi-square test, P = 0.45).

Experiment 2: *Menippe adina* Challenge of WSSV

From the *M. adina* challenge, two of the four crabs injected with WSSV died, and both of those were found to be histologically positive for WSSV associated lesions (with H&E stains). The crabs died at 60 and 71 hours post-exposure. One of the 15 crabs exposed *per os* was found to be histologically positive for WSSV. No WSSV associated lesions were observed in crabs used as negative control animals. Tissue tropism was similar to WSSV infections of penaeid shrimps (Lightner 1996). In infected crabs WSSV intranuclear inclusions were observed in cuticular epithelium, connective tissue, antennal gland, hematopoietic tissue, heart, and gill. With *in situ* hybridization, light reactions were observed in WSSV infected cells from crabs despite getting dark reactions in WSSV infected cells from the positive control shrimp.

In addition to the inclusions typically caused by WSSV, intranuclear inclusions were found in hypertrophied nuclei in cells of the hepatopancreas. Affected nuclei

were usually large but varied in size, contained a single basophilic to lightly eosinophilic inclusion, and displayed marginated chromatin. No occlusion bodies were observed in the inclusions. The inclusions were found in crabs exposed to WSSV, and in crabs used as negative control animals. Further, the inclusions were present in one of the two crabs that died during the experiment. Ten of the 24 crabs used in the experiment were positive for this hepatopancreas inclusion.

DISCUSSION

The results suggests that juvenile *F. duorarum* are as susceptible to mortality from WSSV as juvenile *L. vannamei*. In contrast, Lightner et al. (1998) and Wang et al. (1999) exposed juvenile *F. duorarum* and *L. vannamei* per os to WSSV and found juvenile *F. duorarum* to be more resistant to WSSV than juvenile *L. vannamei*. However in the study by Wang et al. (1999), they mention a preliminary experiment (unpublished) in which juvenile *L. vannamei* and *F. duorarum* were challenged per os to WSSV. In that study no difference in mortality rates was detected which corroborates our findings.

In a previous study the procedure for estimating transmission rate (β) used in this study was used to compare the transmission rates of WSSV by ingestion of an infected cadaver of *L. vannamei* to the transmission by ingestion of an infected cadaver of *L. setiferus* (Soto and Lotz 2000). In that study most shrimp were examined histologically to determine infection status. They determined that mortality rate was a good measure of transmission rate. In this study, since no histological examination has been performed on *F. duorarum*, it is possible that the transmission rate is even greater than the mortality rate.

Menippe adina were found to be susceptible to WSSV. *Callinectes sapidus* has also been found to be susceptible to WSSV (Flowers et al. 2000). Moreover, all three species of commercially important penaeid shrimps found in the Gulf of Mexico have been found to be susceptible to WSSV (Lightner et al. 1998, Wang et al. 1999, Soto and Lotz 2000). If successfully introduced, WSSV may pose a threat to the most economically important shrimp and crab fisheries in the Gulf of Mexico.

Tissue tropism of WSSV to *M. adina* was similar to WSSV infections of penaeid shrimps (Lightner 1996). WSSV typically infects cuticular epithelial cells, connective tissue cells, antennal gland epithelium, lymphoid organ sheath cells, hematopoetic tissues, and fixed phagocytes of the heart (Lightner 1996, Chang et al. 1996). WSSV has not been found to infect hepatopancreatic cells. Some researchers have found the hepatopancreas to be PCR positive for WSSV (Lo et al. 1997), but the hepatopancreas is composed of other types of cells including cuticular epithelial and connective tissue cells. The PCR positive results were probably due to the test reacting to infections in these cells.

It is possible that the inclusions observed in hepatopancreatic cells of *M. adina* were caused by another virus. The inclusions are similar to inclusions caused by Baculo-A virus described from *Callinectes sapidus* (Johnson 1980, 1983) and to Baculoviral Midgut Gland Necrosis (BMN), a virus that infects some penaeid

shrimps from south and southeast Asia, primariliy *Penaeus japonicus* and *P. monodon* (Lightner 1996).

It is important to note that the *M. adina* used in the experiments were kept in holding tanks for up to one year prior to the start of this experiment. In these tanks, *M. adina* were cohabitating with other crabs, specifically *C. sapidus*, *Panopeus simpsoni*, and *Eurypanopeus depressus*. Therefore, little can be said of the prevalence (41.6%) of the inclusion in *M. adina* in wild populations.

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