Annals of Tropical Research 34[1]:96-113(2012)

Quantification of the Relative Virulence of White Spot Syndrome Virus (WSSV) in the Penaeid Shrimps *Litopenaeus vannamei* (Boone, 1931) and *Farfantepenaeus duorarum* (Burkenroad, 1939) by Quantitative Real Time PCR

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ABSTRACT

The relative virulence of the China isolate of white spot syndrome virus (WSSV-CN) in the penaeid shrimps *Litopenaeus vannamei* and *Farfantepenaeus duorarum*, was assessed by a comparison of 7-d median lethal dose (LD_{50}), survival curve, and mean lethal load after exposure by injection. Shrimps were injected intramuscularly with known WSSV dose. Median lethal dose of *L. vannamei* was lower than that of *F. duorarum*. Log LD_{50} in *L.vannamei* was 4.20 WSSV genome copies μg^{-1}total DNA. Log LD_{50} in *F.duorarum* was 5.32 WSSV genome copies μg^{-1}total DNA. Median survival times of *L. vannamei* and *F. duorarum* injected with 10^4 and 10^5 WSSV genome copies were 54.17 h and 38.91 h, respectively for *L. vannamei* whereas they were 119.58 h and 82.67 h, respectively for *F. duorarum*. Mean log of the WSSV lethal load for *L. vannamei* was 9.34(SE ± 9.09) copies μg^{-1} of total DNA and for *F. duorarum* was 11.80 (SE ± 11.55). No significant difference was noted in lethal load for the shrimp species using Student's t-test. Overall mean WSSV lethal load was 2.86×10^{11} (SE±1.63×10^{11}) genome copies μg^{-1} of total DNA. In conclusion, WSSV was found to be less virulent in *F.duorarum* than in *L. vannamei* by LD_{50} and mean survival time but not in mean lethal load. This suggests that shrimp resistance is imparted by controlling WSSV loads rather than by tolerating higher loads.

Key Words: WSSV-China isolate, median lethal dose (LD_{50}), median survival times, lethal loads

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INTRODUCTION

White spot syndrome virus (WSSV) is the causative agent of White Spot Disease (WSD)(OIE 2003). It is an important shrimp pathogen that has been devastating the shrimp farming industry worldwide, causing up to 100% mortality of farmed shrimps within 3 to 10 days (Lightner, 1996, Chou *et al.*, 1995). WSSV was first recognized in cultured penaeid shrimps in Asia and the Indo-Pacific Region in 1993 (Chou *et al.*, 1995; Wongteerasupaya *et al.*, 1995; Inouye *et al.*, 1994; Inouye *et al.*, 1996). The virus spread rapidly and reached the western hemisphere in 1995 in pond reared *Litopenaeus setiferus* in a Texas farm (Lightner, 1996).

In *L. vannamei*, WSSV infection starts with an initial short asymptotic prepatent state. In this state, the virus multiplies and in due time, causes a symptomatic acute infection. Acute infections then may progress and cause death of the shrimp or the shrimp may survive and develop chronic infections (Lotz & Soto, 2002). WSSV transmission in *L. vannamei* is more effective through ingestion of infected cadavers than through contact with living infected hosts (Soto & Lotz, 2001).

Virulence of a pathogen is the strength of the ability to cause disease. It can be measured in a number of ways, e.g., the LD_{50}, the lethal load (quantity of a pathogen that causes death), or the survival curve (median time were 50% of the population that survives). The LD_{50} of WSSV for different viral strains and shrimp species has been reported (Prior *et al.*, 2003; Escobedo-Bonilla *et al.*, 2005; Escobedo-Bonilla *et al.*, 2006). However they have been reported in units of dilution, rather than the number of DNA copies. Viral load is one of the most important factors in the progression and transmission of disease (Durand and Lightner, 2002).

Quantitative PCR has been applied to quantify WSSV lethal loads in shrimps (Durand and Lightner, 2002; Durand *et al.*, 2003; Dhar *et al.*, 2001) and other penaeid virus (Tang and Lightner, 2001; Tang *et al.*, 2004; Dhar *et al.*, 2002). WSSV virulence in *L. vannamei* and *F. duorarum* has also been reported (Chou *et al.*, 1995; Lightner 1996; Lightner *et al.*, 1998; Wang *et al.*, 1999). *F. duorarum* displayed a degree of resistance to infection and disease, in response to WSSV challenge than *L. vannamei* (Lightner *et al.*, 1998; Wang *et al.,* 1999). Although *F. duorarum* was shown to be more resistant than *L. vannamei*, so far, no study has been conducted to compare their LD_{50}s and lethal loads and median survival times. In this study, we compared the LD_{50} of WSSV in the penaeid shrimps *Litopenaeus vannamei*

and *Farfantepenaeus duorarum* using quantitative real time polymerase chain reaction, compared the survival curves of the two shrimp species, and determined the lethal loads of WSSV in the two species of shrimps using q-RTPCR.

MATERIALS AND METHODS

Experimental shrimps

The *L. vannamei* were SPF Kona stock obtained from the Oceanic Institute in Hawaii. *Farfantepenaeus duorarum* stock was derived from Specific Pathogen Free (SPF) stock acquired from Texas A&M Corpus Christi. The shrimp were solely intended for laboratory experiments and stocked in an isolated and biosecure facility. Shrimp selected for the experiments weighed 3-4 g of the same age.

Viral stock production

The WSSV viral stock was originally obtained from China, and it has been passed once in *L. vannamei* stocks. To obtain virus for the study, infection bioassay was conducted in a 500-L circular fiberglass tank containing UV treated 15 ppt artificial salt water at a temperature of 26 – 27 °C. One hundred SPF healthy *L. vannamei* shrimp (8-10 g) were stocked into the tank and allowed to acclimate for 48 h. The shrimp were fed with commercial shrimp pellets. After acclimation, shrimp were exposed to the WSSV China isolate by intramuscular injection at 0.02ml of WSSV inoculum g^{-1}body weight (BW) between the second and third tail segment. Moribund and freshly dead shrimp were collected and frozen at -80°C (Prior et al. 2003). Quantitative real time PCR was done to confirm that these shrimps were infected with WSSV.

In the preparation of the WSSV inocula, gills from infected shrimp were aseptically removed and finely chopped using a sterilized surgical blade. This was suspended in a 0.9% NaCl solution at a 1:10 (w/v) dilution of gill tissues to saline solution and was mixed thoroughly using a vortex mixer. The resulting mixture was centrifuged for 20 min at 3000 × *g* at 4°C. The supernatant was collected and centrifuged again for 13,000 x *g* at 4°C for 20 min (Escobedo-Bonilla *et al.,* 2005). The final supernatant was filtered in a 0.45um filter and divided into 1.5-ml aliquots, stored at -80°C, and served as the viral source for the assays (Prior *et al.,* 2003).

Bio assay system

The life support system that was used for the bio-assays was the Aquatic Habitat ZF 0601 (Aquatic Habitats™) stand-alone recirculation system with aeration, filtration and UV light. The system utilizes 60 rectangular, 3-L containers. Two systems were used to accommodate the 120 shrimp for each trial with ten shrimp stocked in each container. The systems were filled with 15 ppt seawater by mixing artificial salt (Marine Environment) and filtered fresh tap water. A submersible heater was used to maintain the temperature at 26 ± 0.5 °C.

Prior to injection, shrimp were placed in a 500-L cylindrical tank and acclimatized to a salinity of 15 ppt for 48 h inside the bioassay room. Polyester filters inoculated with nitrifying bacteria were placed in the tank for biofiltration. The water was maintained at 26 ± 0.5 °C.

Shrimp were transferred individually to the aquatic habitat containers. Polyester filters inoculated with nitrifying bacteria were placed in the systems' sump for biofiltration. Ultraviolet light was used to prevent virus contamination among tanks. The shrimp were individually weighed before they were transferred. Temperature and salinity were checked daily and adjusted when necessary.

Experimental infection

Preliminary injection assay using serial dilutions from 1: 10 through 1: 1 x 10^{15} was conducted to determine the viral dilutions to be used to estimate the LD_{50} in the final experiment. Specific pathogen free *L.vannamei* was used in the preliminary WSSV injection assay. Based on the result of the assay, the final viral dilutions for the determination of LD_{50} were 1: 1.0 x 10^4, 1: 5.5 x 10^4, 1: 1.0 x 10^5, 1: 5.5 x 10^5 and 1: 1.0 x 10^6

Two trials were conducted for *L.vannamei* to determine WSSV LD_{50} and one trial for *F.duorarum*. A total of 20 shrimp were used for each viral dilution in every experiment. A sample of the WSSV inoculum used in every trial was collected and q-PCR was done to determine WSSV genome copies. The result of this was used to determine the total number of virus injected per dilutions for every trial. Saline solution (0.9%NaCl) was used to inoculate the negative control shrimp. Shrimp were observed for 7 d after inoculation. Moribund and dead shrimp were recorded and collected. Hemolymph and pleopods were collected and placed in 1.5-ml test tubes and stored at -80°C until they were analyzed for WSSV by quantitative real

time PCR (q-PCR). Surviving and negative control shrimp were sacrificed after 7d and hemolymph and pleopods were stored at -80°C until they were analyzed for WSSV by PCR as described by Lo *et al.* (1996).

DNA extraction

Using the hemolymph of the shrimp, total DNA was extracted using the High Pure PCR template preparation kit following the protocol of the manufacturer (Roche Applied Science, Mannheim Germany). Immediately after extraction, the concentration of total DNA in the sample was estimated using a spectrophotometer.

Quantitative PCR for WWSV

The sequences of q-PCR primers and *Taq*-Man probe specific for WSSV were those of Durand et al. (2003). Total volume for q-PCR reaction per sample was 25 µl. The reaction mixture consisted of 12.5 µl of TaqMan Universal Mix (Applied Biosystems, Foster City USA) that contained Amplitaq Gold DNA polymerase, AmpErase UNG, dNTPs with dUTP and optimized buffer components, 2.5 µl of 3 µM forward primer (Invitrogen), 2.5 µl of 3.0 µM reverse primer (Invitrogen), 2.5 µl of 1.5 µM*TaqMan*Probe (Integrated DNA Technologies) and 5 µl of sample DNA.

Quantitative polymerase chain reaction amplification was conducted following the procedure of Durand and Lightner (2002) and Durand *et al.* (2003) using an iCycler Thermal Cycler (BioRad). All samples were tested in triplicate.

The standard for WSSV q-PCR is an oligonucleotide with 5'-CAA TGG TCC CGT CCT AGA AGC CAT GAA GAA TGC CGT CTA TCA CAC ACT AAT TTC CGG CAA AGC TCG– 3' sequence of 75 bp (Trilink Biotechnologies) corresponding to nucleotides 1008 to 1082. This standard was stored at -20 ºC, serially diluted prior to q-PCR analysis to generate standard curves.

Statistical analysis

The LD_{50} was calculated using the Probit Analysis or Logistic Regression of SPSS software, version 16 and the relative median potency was calculated as the ratio of WSSV LD_{50}s for *L. vannamei* and *F.duorarum.* Logistic regression was used to compare *L. vannamei* and *F. duorarum*

susceptibility to WSSV. Kaplan-Meir analysis was used to compare survival curves of the shrimp exposed to different WSSV viral doses. Student's *t*-test was performed to to determine the differences in viral loads. Alpha level was set to 0.05 for all statistical tests.

RESULTS

Quantification of WSSV content in experimental bioassays

Based on q-PCR, the inoculum used in *L. vannamei* trials 1 and 2 contained 1.58 x 10^8 WSSV genome copies μg^{-1} of total DNA or 3.13 x 10^6 WSSV genome copies $\mu g^{-1}$of inoculum and 4.92 x 10^8 WSSV genome copies μg^{-1} of total DNA or 5.0 x 10^6 WSSV genome copies μg^{-1} of inoculum respectively. The inoculum in the *F. duorarum* trialcontained 8.77 x 10^{10} WSSV genome copies μg^{-1} of total DNA or 1.42 x 10^9 WSSV genome copies μg^{-1} of inoculum. Analysis of variance detected no significant differenceamong the inocula(P = 0.094).

Student's *t*-test did not show significant differences in the concentration of virus in the inocula used for *L. vannamei* 1 and *L. vannamei* 2 (p= 0.30), *L. vannamei* 1 and *F. duorarum* (p=0.23) and *L. vannamei* 2 and

Table 1. Doses of WSSV intramuscularly injected to *Litopenaeus vannamei* and *Farfantepenaeus duorarum*.

WSSV dose (genome copies μg^{-1}total DNA) /shrimp	WSSV Log Dose
L. vannamei trail 1	
2.53 x 10^5	5.40
4.60 x 10^3	3.66
2.53 x 10^3	3.40
4.60 x 10^2	2.66
2.53 x 10^2	2.40
L. vannamei trail 1	
4.04 x10^5	5.61
1.24 x 10^4	4.09
7.35 x 10^3	3.87
5.21 x 10^3	3.72
4.04 x 10^3	3.61
F. duorarum trial	
8.58 x 10^6	6.93
1.56 x 10^6	6.19
8.58 x 10^5	5.93
1.56 x 10^5	5.19
8.58 x 10^4	4.93

Median lethal dose

The log LD_{50} estimate for *L. Vannamei* Trial 1 was 4.16 WSSV genome copies μg^{-1} total DNA (95% CI: 2.23 to 5.24). The log LD_{50} estimate for *L. vannamei* Trial 2 was 4.21 WSSV genome copies μg^{-1}total DNA(95% CI: 3.22 to 5.02). Combined log LD_{50} value for *L. vannamei* was 4.20 WSSV genome copies μg^{-1}total DNA(95% CI: 3.34 to 4.84).

The log LD_{50} estimate for *F. duorarum* was 5.32 WSSV genome copies μg^{-1}total DNA (95% CI: 3.78 to 5.96). The relationship of the LD_{50}s for the two species is shown in the logistic curves (Figures 1).

The relative median potency (ratio of LD_{50}s) of *F. duorarum* to *L. vannamei* was 13.37. The odds ratio of mortality for *F. duorarum* in reference to *L. vannamei* for the same dose of WSSV virus was 0.121. This reflects a 12% reduced risk of WSSV mortality for *F. duorarum* compared to *L. vannamei* exposed to the same dose of WSSV.

The log dose estimates represent the effect of increased dose of WSSV to shrimp, regardless of the species. In this experiment, the estimate of this parameter was 1.791. This means that for every log dose increase of WSSV, the effect on the shrimp mortality is almost doubled. Similarly, the odds ratio of log dose indicates the increase in risk of mortality from an increase in WSSV log dose, regardless of the species of shrimp. In this study, the odds ratio is 5.994, which means the risk of mortality for the shrimp, regardless of species, is 6 times greater as the log of dose increases by one log.

Survival Curve

Litopenaeus vannamei trials

Shrimp survival and median survival time showed a decreasing trend as WSSV dose increased. In *L. vannamei trial* 1, survival for the respective WSSV dose was 20, 30, 100, 90 and 90%. While in *L. vannamei* trial 2 survivals at respective WSSV dose was 5, 25, 50, 90 and 95%. In *L. vannamei* trial 1, mortality due to WSSV infection began 27.83 h post injection and mortality stopped 51.25 h post injection while in *L. vannamei* trial 2, mortality due to WSSV infection began 28.97 h post injection and ended 69.12 h post injection. No mortality was observed in the negative control shrimps. Combining doses of trial 1 and 2 resulted in a median survival time and doses for *L. vannamei* was 51.25 h at 2.53 x 10^2WSSV genome

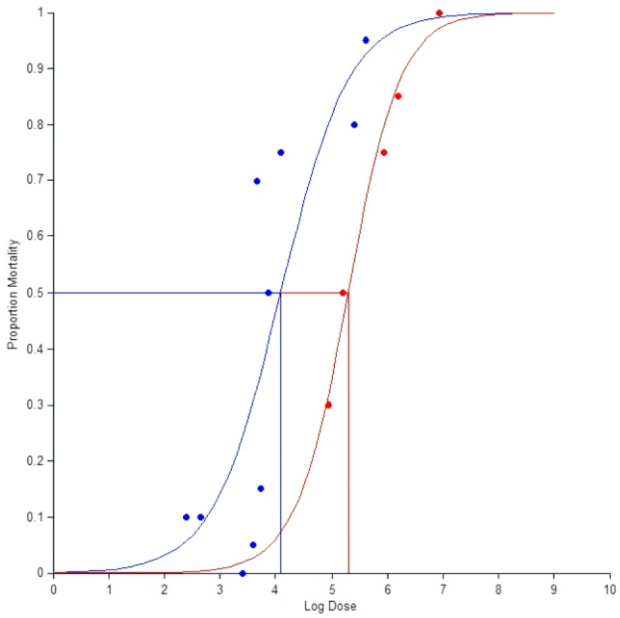

Figure 1. Dose-response curves (Logistic analysis) of *L. vannamei* (bold circle) andF. duorarum (open circle) with the corresponding WSSV LD_{50} estimates.

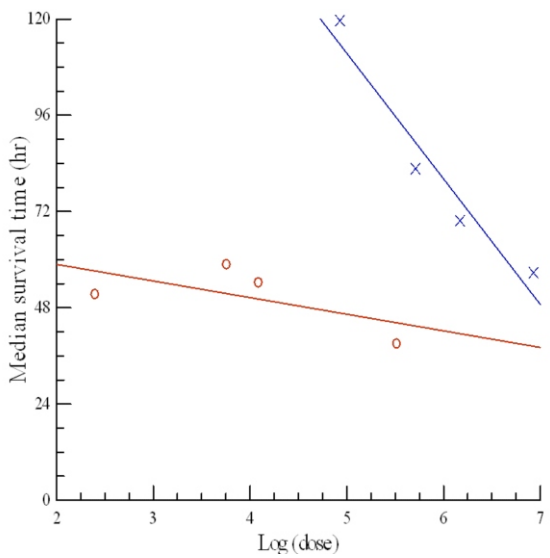

Figure 2. Median survival time of *Litopenaeus vannamei* (o) and *Farfantepenaeus duorarum* (x) as a function of WSSV dose.

copies μg^{-1} total DNA, 58.68 h at 5.70 x 10^3WSSV genome copies μg^{-1} total DNA, 54.17 h at 1.24 x 10^4WSSV genome copies μg^{-1} total DNA, and 38.91 h at 3.30 x 10^5WSSV genome copies μg^{-1} total DNA. The result showed that medium survival time increases with decreasing WSSV dose

In *F. duorarum* trial, decreasing trend in shrimp survival and in median survival time as WSSV dose increase was observed. Survival at the respective doses was 0, 15, 25, 50 and 70%. Mortality due to WSSV infection began 29.63 h post injection and ended 119.58 h post injection. No mortality was observed in the negative control shrimps.

Median survival time was longer in *F. duorarum* than in *L. vannamei* given the same WSSV dose. To compare the median survival time of the two species of shrimp, we compared the survival curves of shrimp inoculated with doses of 10^5 and 10^4WSSV genome copies μg^{-1} total DNA because those doses were common to both species. The means of 10^5 and 10^4doses were 1.24 x 10^4WSSV genome copies μg^{-1} total DNA and 3.30 x 10^5WSSV genome copies μg^{-1} total DNA for *L. vannamei.* For *F. duorarum* were 8.58 x 10^4WSSV genome copies μg^{-1} total DNA and 5.07 x 10^5WSSV genome copies μg^{-1} total DNA. The median survival times for *L. vannamei* were 54.17 h at 1.24 x 10^4WSSV genome copies μg^{-1} total DNA and 45.60 h at 3.30 x 10^5WSSV genome copies μg^{-1} total DNA. For *F. duorarum*, the median survival time was 119.58 h at 8.54 x 10^4WSSV genome copies μg^{-1} total DNA and 82.67 h at 5.07 x 10^5WSSV genome copies μg^{-1} total DNA. Therefore *F. duorarum* showed resistance to WSSV compared with *L. vannamei* since it took longer for WSSV in *F. duorarum* to cause 50% mortality at the same dose.

WSSV lethal loads

No significant difference existed between lethal loads of the trials. Mean log of WSSV lethal load in *L. vannamei* trial 1 was 9.72(SE ± 9.46) with a range from5.02 to 10.92 copies μg^{-1} of total DNA. Meanlog lethal load in *L. vannamei* trial 2 was 7.65 (SE ± 7.15) with a range from4.58 to 8.77 copies μg^{-1} of total DNA. Combined *L. vannamei* mean log WSSV lethal load was 9.34(SE ± 9.09) copies μg^{-1} of total DNA with a range from 4.58 to 10.92 copies μg^{-1} of total DNA. For *F. duorarum* the mean log lethal load was 11.80 (SE ± 11.55) with a range from 3.52 to 13.30 WSSV copies μg^{-1} of total DNA. Although close, Student's T-test showed no significant difference in mean lethal load for the two species (P=0.056). The overall mean of log WSSV lethal load in fresh dead and moribund shrimps regardless of species or

trial was 11.45(SE±11.21) genome copies μg^{-1} of total DNA with a range from 3.52 to 13.30 genome copies μg^{-1} of total DNA.

DISCUSSION

Prior to the development of q-PCR for WSSV, the amount of WSSV used in an assay was standardized by dilution, not by viral concentration. Subsequently, q-PCR has been used to determine concentration of WSSV (Durand and Lightner, 2002: Durand *et al.,* 2003). In this study, we evaluated the relative virulence of WSSV-China isolate in the penaeid shrimp, *L. vannamei* and *F. duorarum* by employing q-PCR to obtain the WSSV dose in terms of the number of genome copies. This is the first study to estimate the WSSV LD_{50} in *F. duorarum* for any isolate and the first to use q-PCR to estimate the LD_{50} in either *F. duorarum* or *L. vannamei*. Although it has been reported that there are differences in virulence of WSSV geographic isolates (Wang *et al.,* 1999), it is not possible to compare our isolate with others because of the lack of knowledge of the precise dose used in studies of other WSSV isolates. The study of Prior *et al.* (2003) reported LD_{50}s of 1: 4.44 x 10^6 and 1: 4.50 x 10^6 in *L. vannamei* using WSSV South Carolina isolate. Escobedo-Bonilla *et al.* (2005) reported median virus infection titers in 60 and 135 d old juvenile *L. vannamei* at $10^{6.8}$ and $10^{6.5}$ $SID_{50}ml^{-1}$ using WSSV Thailand isolate. Durand *et al.* (2002) reported that a viral load of 10^4 and 10^5 copies of WSSV China isolate resulted in disease and mortality in *L. vannamei* when injected intramuscularly. In the same study they found that a WSSV viral load of 10^5 was needed for transmission and mortality in immersion assays. Tan *et al.* (2001) reported that if the viral load in *P. monodon* exceeded 10^6 copies then clinical signs of WSSV and mortality were observed.

Although *F. duorarum* showed greater resistance to WSSV infection than *L. vannamei*, it is possible that the result would have been different if another method of inoculation was used. Injection trials place virus directly into the shrimp's muscle (Prior *et al.,* 2003) avoiding many host barriers that might prevent pathogen entry (Escobido-Bonilla *et al.,* 2006). Injection assay has been shown to result in more virulent infections that lead to increased mortality in shorter times (Rajendran *et al.,* 1999; Escobido-Bonilla *et al.,* 2005). In the study of Rajendran *et al.,* (1999) cumulative mortality of 100% was observed within 5-7d in shrimp injected with WSSV and 7-9 d in shrimp fed infected tissue. Escobedo-Bonilla *et al.,*

(2005) reported that virus titers were reduced ten-fold in oral intubation compared to intramuscular inoculation. Likewise, Durand *et al.* (2002) reported that a minimum of 10^5 WSSV copies is necessary to transmit disease in shrimp by immersion but only 10^4 WSSV copies by injection resulted in an acute infection and disease. In this study, a dose of a little as 10^2 WSSV genome copies per μg^{-1} of total DNA was found to transmit WSSV virus and cause infection and mortality in *L. vannamei.* Possibly the same amount of virus is enough to transmit WSSV virus and cause infection in *F. duorarum,* but the length of time to mortality might be longer and cumulative mortality will be lower.

Analysis of the median survival time of shrimps injected with $10^5$and 10^4 WSSV genome copies showed that *F. duorarum* has longer median survival times compared with those for *L. vannamei*after 7 d. The median survival time at 10^5 and 10^4 WSSV copies μg^{-1} of total DNA (45.60 -54.17 h) for *L. vannamei* is within the range reported by Durand *et al.* (2002) of 49-52 h, which is a highly acute infection compared to *F. duorarum* which almost doubled the median survival time (82.67-119.58 h) when injected with the same amount of WSSV dose. In addition, at a dose of 10^5 WSSV genome copies, mortality was higher in *L. vannamei* at 80-95% compared to *F. duorarum* , with 50-75% mortality. These findings confirm the study of Lightner *et al.* (1998) in which *F. duorarum* displayed resistance to WSSV. Lightner *et al.* (1998) experimentally infected *L. vannamei*, *L. setiferus*, *F. duorarum* and *F. aztecus* with a WSSV isolate from China by feeding and found that WSSV challenge of postlarval shrimp resulted in severe infections in *L. vannamei* and *L. setiferus* and 100% cumulative mortality and 27% cumulative mortality in *F. aztecus* and no signs of clinical infection and 0% cumulative mortality in *F. duorarum.* Likewise, Wang *et al.* (1999) using oral inoculation of six WSSV geographic isolates reported that all the geographic isolates were more virulent to *L. vannamei* than to *F. duorarum*. *Litopenaeus vannamei* challenged in this study died 14 d after feeding while *F. duorarum* juveniles showed resistance to WSSV with 40% to 65% shrimp surviving after 18 d challenge. The findings of this study however differed from that of Soto *et al.* (2002) who reported no statistical difference detected in final mean mortality rates between *L. vannamei* and *F. duorarum.*

The taxonomic differences between *L. vannamei* than to *F. duorarum* might explain the difference in virulence. *Litopenaeus vannamei* belongs to genus *Litopenaeus* and *F. duorarum* belongs to the genus *Farfantepenaeus*

(Lightner *et al.,* 1998). Shrimp of genus *Farfantepenaeus* have been reported to be less susceptible when challenged with other penaeid viruses. Overstreet *et al.* (1997) demonstrated that *L. setiferus* and *L. vannamei* but not *F. duorarum* or *F. aztecus* could be killed by Taura syndrome virus (TSV). Erickson *et al.* (1996) reported that juvenile specimens of *L. setiferus*, *L. aztecus* and *F. duorarum* were not killed when fed 15% body weight TSV infected *L. vannamei* but over 90% of *L. vannamei* were. This shows that *L. vannamei* is the most susceptible species of the subgenus *Litopenaeus*. Studies of experimental WSSV infections in *P. monodon*, *M. japonicus*, *F. penicillatus, F. chinensis,P. semisulcatus, F. indicus, M. monoceros and M. dobsonii* have shown no greater resistance for these species than for *L. vannamei* with 100% mortality as soon as 2d after exposure (Chou *et al.* 1995; Tan *et al.,* 2001; Rajendran *et al.,* 1999; Zhan *et al.,* 1998; Hasson *et al.* 2006). *Farfantepenaeus duorarum* may be the most resistant penaeid shrimp identified to date. This doesn't mean however that they will not develop white spot syndrome disease when infected with the virus.

The study found no significant difference in the lethal load of WSSV in the two shrimp species as determined by q-PCR. That is to say that although *F. duorarum* is more resistant to WSSV than *L. vannamei*, the resistance is not imparted by tolerating a greater load of virus by *F. duorarum*. However, the variability in WSSV lethal load among shrimp of the same species suggests that other factors are involved. For example, the state of shrimp health at the time of an experiment, the immune response of a shrimp, and the dose of virus injected (Durand and Lightner, 2002), cannibalism, shrimp density and rearing conditions (Wu *et al.,* 2001).The mean WSSV lethal load in the two species of shrimp in this study was 2.86×10^{11}genome copies μg^{-1} of total DNA. Using WSSV- China isolate, Durand *et al.* (2002) found that the WSSV lethal load in moribund penaeid shrimps *P. monodon*, *L. stylirostris* and *L. vannamei* had the mean WSSV lethal load of 3.0×10^{10} WSSV genome copies μg^{-1} of total DNA, while for naturally infected *P. monodon*, the WSSV mean lethal copy number was 2.10×10^{6}genome copies μg^{-1} of total DNA and for *L. vannamei* the WSSV mean lethal load was 1.60×10^{9}genome copies μg^{-1} of total DNA. This implies that using WSSV-China isolate, WSSV mean lethal load could reach very high numbers of WSSV, regardless of whether they are naturally or experimentally infected and or shrimp species. Quantification of lethal viral loads have also been done in other penaeid viruses like infectious hypodermal and

hematopoietic necrosis virus (IHHNV) and Taura syndrome virus (TSV) using quantitative real time PCR tools. Tang and Lightner (2001) reported the viral loads IHHNV in naturally infected *L. stylirostris* juveniles from the Gulf of Mexico, hatchery raised small juveniles *L. stylirostris* from Guam and in farm raised post larvae *P. monodon* from the Philippines. They reported maximum level of IHHNV in these shrimps in the order of 10^9 copies μg^{-1} of total DNA with observed high level of mortalities. Tang *et al.* (2004) used q-PCR in the quantification of TSV in infected juvenile *L. vannamei*. Results showed that acute and chronically infected shrimp contained 10^6-10^8 copies of TSV per microgram of RNA in both gills and pleopods with cumulative mortality in acute infected shrimp of 71%.

CONCLUSION

In conclusion, the present study demonstrates that WSSV virus is more virulent to *L. vannamei* than to *F. duorarum* as measured by LD_{50} and survival curves. However, the resistance is not provided by the greater ability of *F. duorarum* to tolerate a greater viral loads present. This observation may indicate that other factors, such as, but not limited to stocking density, stocking condition (individually and collectively reared shrimp), mode of transmission (Wu *et al.,* 2001) and size of host (Wu *et al.,* 2001; Lotz, 1997) in the rearing systems plays a role in the virulence.

ACKNOWLEDGMENT

Partial funding for this research was provided by a grant from the United States Department of Agriculture, CSREES grant number 2002-38808-01381. Rey J. dela Calzada is supported by the Fulbright-Philippine Agriculture Scholarship Program. The authors would like to thank the anonymous reviewers for comments regarding the manuscript.

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