

Experimental Infection of Taura Syndrome Virus (TSV) to Pacific White Shrimp (*Litopenaeus vannamei*), Black Tiger Shrimp (*Penaeus monodon*) and Giant Freshwater Prawn (*Macrobrachium rosenbergii*)

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ABSTRACT

Laboratory infectivity of Taura syndrome virus (TSV) to Pacific white shrimp (*Litopenaeus vannamei*), black tiger shrimp (*Penaeus monodon*) and giant freshwater prawns (*Macrobrachium rosenbergii*) was investigated. All the infected *L. vannamei* died, while some *P. monodon* and *M. rosenbergii* survived. However, RT-PCR and *in situ* hybridization revealed TSV-positive results from surviving *P. monodon* and *M. rosenbergii*. Histopathological changes were observed in the subcuticular epidermis of infected *L. vannamei*. Extensive necrosis with prominent nuclear pyknosis and karyorrhexis of membranous tissues, including the abdominal segments and hindgut, were observed. Histopathological changes in *P. monodon* showed necrosis in the cuticular epithelium of the body surfaces; some of the infected cells showed pyknotic nuclei and melanization in the subcuticular layer tissues. In *M. rosenbergii* no histopathological changes of the cuticular epithelial layer were observed, only striated muscle cell necrosis.

Key words: Taura syndrome virus, Pacific white shrimp, black tiger shrimp, giant freshwater prawn

INTRODUCTION

Pacific white shrimps (*Litopenaeus vannamei*) were first introduced to Thailand on a limited scale in 1998 (Limsuwan and Chanratchakool, 2004). Due to slow growth and a wide disparity in the sizes of black tiger shrimp (*Penaeus monodon*) which led to meagerly harvests, *L. vannamei* has been cultured in both inland and coastal areas in Thailand. This increased the potential for disease transfer to several commercially important native species in

Thailand. Although at that time importation and rearing of Pacific white shrimp were prohibited by the Thai Department of Fisheries (DoF), the demand and price of postlarvae (PL) were increased, stimulating further illegal importation of stocking and led to the possibility of introducing Taura syndrome virus (TSV). Later in March 2002, the DoF permitted the legal importation of *L. vannamei* if the imported stocks were certified to be free of TSV by RT-PCR testing. In spite of these measures, TSV outbreaks occurred in Thailand in early 2003 (Nielsen *et al.*, 2005).

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Taura syndrome virus is a cytoplasmic, non-enveloped icosahedral virus (Hasson *et al.*, 1995; Lightner *et al.*, 1995). It has been characterized as a picornavirus based on physical and chemical characteristics (Bonami *et al.*, 1997; Robles-Sikisaka *et al.*, 2001). However, nucleotide sequence data suggested that TSV is more related to cricket paralysis viruses (Mari *et al.*, 2002). TSV was first recognized in farms near the mouth of the Taura river, Ecuador, in mid-1992 (Brock *et al.*, 1995; Lightner *et al.*, 1995). The occurrences of the disease including cultured shrimp stocks were reported in Ecuador, Peru, Colombia, Honduras, Jamaica, Guatemala, EL Salvador, Brazil, Nicaragua, Belize, USA and Mexico (Lightner, 1996). In Asia, TSV was first reported in Taiwan (Tu *et al.*, 1999) and it later appeared in Thailand in 2003. Brock *et al.* (1997) indicated that *P. monodon* could be infected with TSV but with low mortality. However, TSV is an RNA virus that mutates rapidly. Nielsen *et al.* (2005) revealed that Thai, Burmese and Chinese TSV types are more related to each other and are distinct from TSV types from USA. TSV is known to infect a number of Penaeid species, including *P. stylirostris*, *P. schmitti*, *P. chinensis*, *P. setiferus*, *P. aztecus*, *P. japonicus* and *P. duorarum* (Lightner, 1996; Overstreet *et al.*, 1997).

In this study, three major cultivated species, *L. vannamei*, *P. monodon* and *M. rosenbergii*, were infected with TSV and the susceptibilities to this virus were determined. The results from this study could be used as a guideline to prevent TSV outbreak in inland shrimp culture especially when these three species are co-cultured in the same farm.

MATERIALS AND METHODS

Shrimp specimen

P. monodon and *M. rosenbergii* (size 3-5 g) were obtained from a commercial farm in Ratchaburi province, Thailand. For positive and

negative controls, specific pathogen-free (SPF) *L. vannamei* of the same size were obtained from broodstock imported from the Oceanic Institute (OI). They were held at 25-28°C in 100-liter tank (10 shrimp/tank) with a constant air supply and fed daily on a commercial pelleted feed for 14 days.

Virus stock and infection trials

The TSV used in this study was collected from naturally infected *L. vannamei* reared in a commercial shrimp farm located in Nakhon Pathom province, Thailand, on March 2003. The TSV disease status of the shrimp was confirmed by RT-PCR. A cell-free viral suspension was prepared according to the protocol of Hasson *et al.* (1995). Experimental shrimps were exposed to the virus by feeding with triturated infected shrimp tissue or injected intramuscularly with a cell-free aqueous extract of infected shrimp tissue into the abdominal musculature according to the protocol of Lotz (1997).

For experimental TSV infection in *P. monodon* and *M. rosenbergii*, each species was divided into four groups as follows. Group 1, 30 shrimps were injected with 0.02 ml/g of a 1:10 dilution of the viral stock solution. Group 2, 30 shrimps were fed on TSV-infected shrimp tissues at the rate 7.5% of body weight. Groups 3 and 4 were controls that fed on uninfected shrimp tissues.

For positive control 1, 30 SPF *L. vannamei* from broodstock imported from the OI were injected with 0.02 ml/g of a 1:10 dilution of the viral stock solution. For positive control 2, 30 SPF *L. vannamei* were fed on tissues TSV-infected shrimp at the rate 7.5% of body weight. In negative control groups 1 and 2, SPF *L. vannamei* were exposed either by feeding or by injection of material that was prepared exactly as above except using uninfected shrimp tissues.

For the infectivity test, shrimp mortalities were recorded for 15 days. Specimens were preserved in Davidson's fixative solution and then

transferred to 70% ethanol after 48 h; all histological materials were prepared using standard histological procedures for shrimp and stained with haematoxylin and eosin (H&E) as described in Bell and Lightner (1988).

RT-PCR and *in situ* hybridization test

A commercially available *in situ* hybridization probe for TSV (Diagnosics Inc.) was used according to the manufacturer's instructions. The protocols have been outlined by Lightner (1996) and Mari *et al.* (1998).

RESULTS

Infection trials

The results of experimental infection of TSV to *P. monodon*, *M. rosenbergii* and *L. vannamei* are shown in Table 1. TSV-infected *P. monodon*, on day 15 post-exposure, by injection had a lower survival rate than those infected by feeding. However, the mortality rate of the infected *P. monodon* group was low and shrimp showed no abnormal clinical signs compared with the infected *L. vannamei* group which had a high mortality rate and severe clinical signs. These shrimp showed body cuticular lesions and all of them developed soft shells on 4 day post-infection.

Some *P. monodon* in the control group died during the experiment but after checking the presence of TSV by RT-PCR, they revealed negative results for virus infection. For TSV-infected *M. rosenbergii*, on day 15 post-exposure, no mortality was observed as well as in the control group by appearing their healthy and fed normally.

Histopathology

Moribund shrimp were investigated for histopathological study. In the control group, no abnormalities were found in the tissues of the *P. monodon* and *M. rosenbergii*. However, *P. monodon* infected with TSV showed necrosis in the body subcuticular epidermis (Figure 1 and 2); no abnormalities were found in the cuticular epithelium of the hindgut, esophagus and stomach. Some of affected cells showed pyknotic nuclei (Figure 1) and melanization in the tissues of the subcuticular layer (Figure 2). Similar histopathological changes were observed in the subcuticular epithelium of the body surface and hindgut of TSV-infected *L. vannamei* (Figure 3 and 4) Histopathology of *M. rosenbergii* infected with TSV showed necrosis in the striated muscle cells (Figure 5 and 6). The affected cells displayed an increased eosinophilia of the cytoplasm and pyknotic nuclei (Figure 6).

Table 1 Percent mortality and RT-PCR results of TSV infected *Penaeus monodon*, *Macrobrachium rosenbergii* and *Litopenaeus vannamei*.

Type of shrimp or prawns	Number of shrimp/ prawns	Cumulative % mortality on day 15 exposure by feeding infected shrimp tissue	Cumulative % mortality on day 15 exposure by intramuscular injection of TSV	RT-PCR results
<i>P. monodon</i>	30	6.67	13.33	+
<i>P. monodon</i> (control)	30	3.33	6.67	-
<i>M. rosenbergii</i>	30	0	0	+
<i>M. rosenbergii</i> (control)	30	0	0	-
<i>L. vannamei</i>	30	80	96.67	+
<i>L. vannamei</i> (control)	30	3.33	6.67	-

RT-PCR and *in situ* hybridization

RT-PCR of exposed shrimp samples confirmed the presence of TSV infection in all exposure groups (Figure 7). In addition to the use of RT-PCR to verify the presence of TSV, a specific DNA probe was used for *in situ* hybridization tests

with *P. monodon* and *M. rosenbergii* tissues. The probe reacted with the virus-laden cytoplasm of the necrotic cuticular epithelial cells and subcuticular connective tissue cells (Figure 8 and 9).

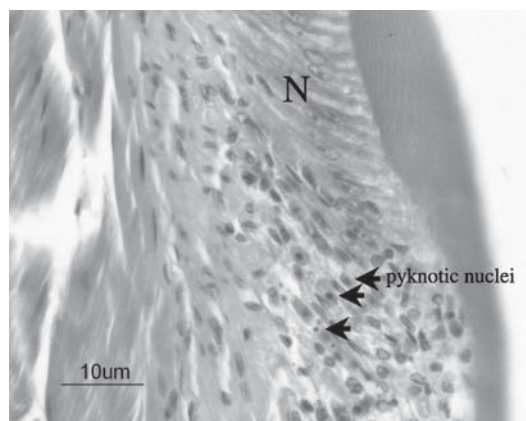


Figure 1 Histological section of TSV infected *P. monodon*, showing lesions of cuticular epithelium with pyknotic nuclei (arrows), and normal appearing epithelium cells (N) (H&E).

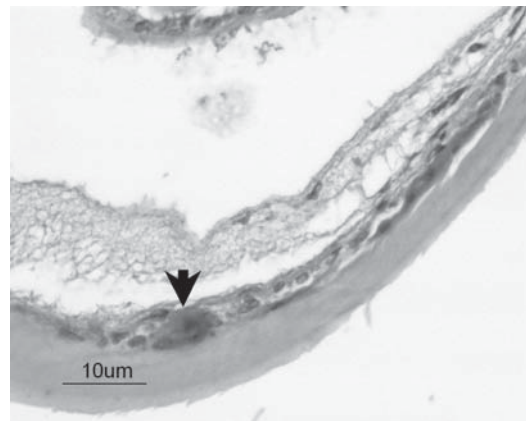


Figure 2 Histological section of TSV infected *P. monodon*, showing in the cuticular epithelium melanized foci (arrow) (H&E).

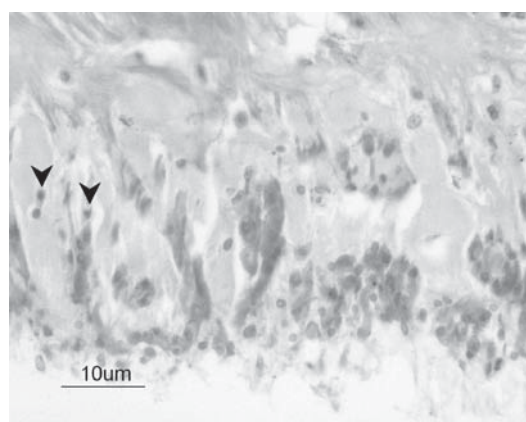


Figure 3 Histological section of TSV infected *L. vannamei*, showing lesions of cuticular epithelium with pyknotic nuclei (arrows) (H&E).

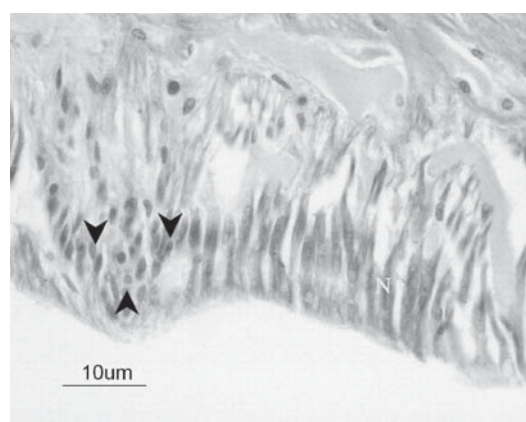


Figure 4 Histological section of TSV infected *L. vannamei*, showing disorganized arrangement of subcuticular epithelium (arrows), normal appearing cells (N) (H&E).

DISCUSSION

In this study, the susceptibility of two major local aquatic crustacean species, *P. monodon* and *M. rosenbergii*, to the infection of TSV were observed. The results showed that *P. monodon* and *M. rosenbergii* could be infected by TSV. In

cultivated areas where there has been a TSV outbreak, the farmers must eliminate any remaining diseased shrimp or carriers in the water and treated the water prior to the new rearing cycle. The PL should be tested for TSV using the RT-PCR method before they were released into the pond. TSV is known to infect a number of penaeid

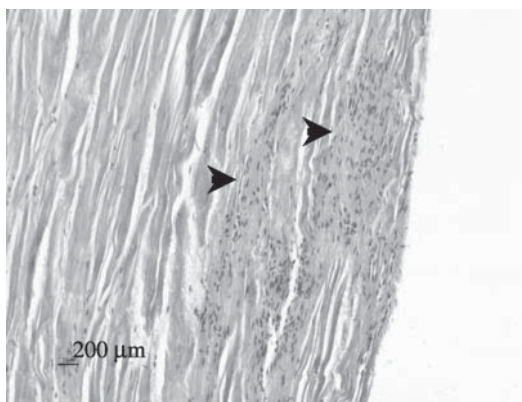


Figure 5 Histological section of TSV infected *M. rosenbergii*, showing lesions of striated muscle (arrows) (H&E).

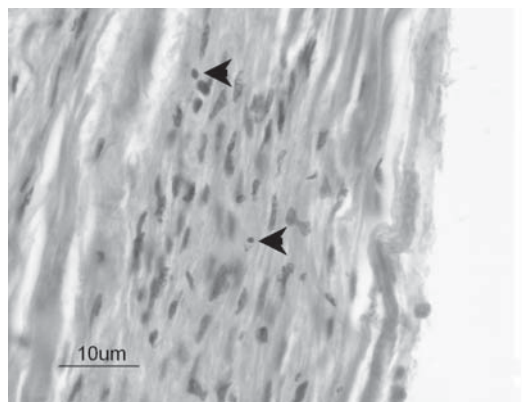


Figure 6 A high magnification of striated muscle of TSV infected *M. rosenbergii*, showing pyknotic nuclei in some affected cells (arrows) (H&E).

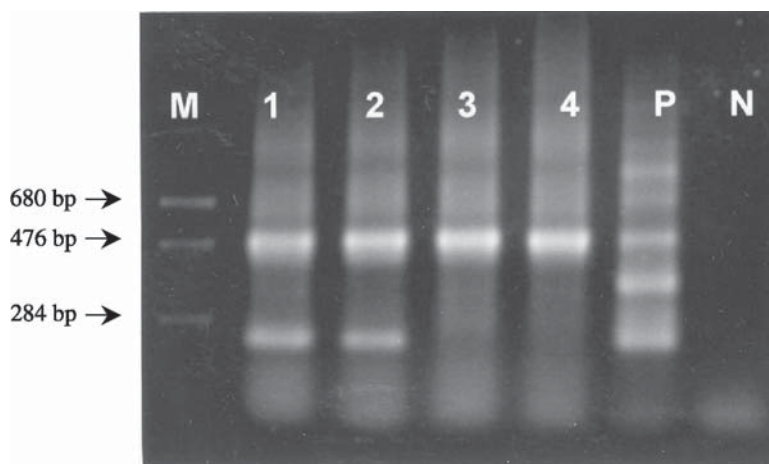


Figure 7 Detection of TSV by RT-PCR with TSV-specific primer after 15 days post infection. Lane 1: *P. monodon* fed with diseased shrimp, Lane 2: *P. monodon* intramuscular injection, Lane 3: *M. rosenbergii* fed with diseased shrimp, Lane 4: *M. rosenbergii*, intramuscular injection, Lane P: positive control.

species. It causes a serious disease in the juvenile and adult stages of *L. vannamei* as well as reported the infection of TSV in PL or juvenile stages of *P. schmitti*, *P. aztecus*, *P. duorarum*, *P. setiferus*, *P. chinensis*, *P. monodon* and *P. japonicus* (Lightner 1996; Overstreet *et al.* 1997). Brock *et al.* (1997) indicated that *P. monodon* could be infected with TSV but it suffered little mortality.

This bioassays suggested that *P. monodon* and *M. rosenbergii* could be infected with TSV although the infected shrimp showed no clinical signs of infection. Overstreet *et al.*

(1997) indicated that an infected shrimp might or might not exhibit clinical signs of TSV infection and mortalities, depending on the species and probably several other features involving the host, virus and environmental conditions.

Histopathological observation of TSV infected specimen showed a lower percentage of *P. monodon* with lesions than those of *L. vannamei*. The lesions in *P. monodon* appeared as necrosis in the cuticular epithelium of body surfaces and melanization of the subcuticular layer tissues. These results were different from those of TSV

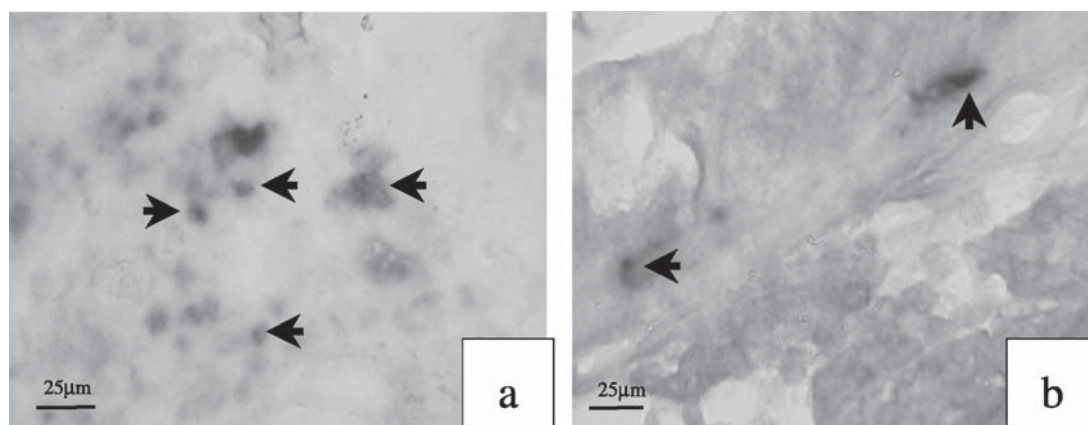


Figure 8 (a,b) *In situ* hybridization assays results from subcuticular connective tissue cells (a) and body subcuticular epidermis (b) of *P. monodon* infected with TSV. The infected areas reacted strongly to the TSV-specific probe seen as black precipitate (arrows).

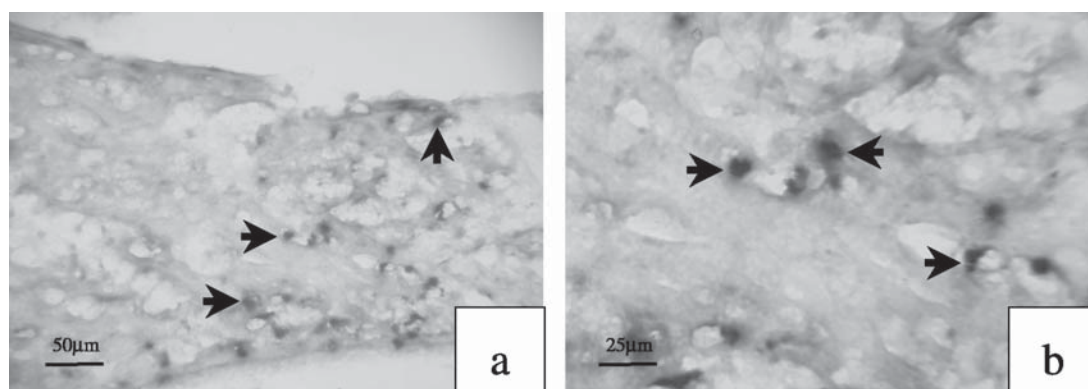


Figure 9 (a,b) *In situ* hybridization of TSV infected striated muscle cells. (a) A high magnification (b) of *M. rosenbergii* infected with TSV. The infected areas reacted strongly to the TSV-specific probe seen as black precipitate (arrows).

infected *M. rosenbergii* which showed no abnormalities in the cells of subcuticular epithelium, but some necrosis in striated muscle cells was observed. Lightner (1996) reported that TSV infects cells in the tissues of ectodermal and mesodermal origin. The cuticular epithelium is the most severely affected tissue. Cells of the subcuticular connective tissues and adjacent striated muscle fibers basal to affected cuticular epithelium are sometimes also be affected. Some of the cell nuclei in affected tissues are pyknotic or karyorrhectic.

Shrimp infected with infectious hypodermal and hematopoietic necrosis virus (IHHNV) and various forms shell disease can display a histopathology similar to those of TSV infection especially when shrimp in the recovery or chronic phases of disease are present in affected populations (Cipriani *et al.*, 1980). However, IHHNV can be readily distinguished from TSV by the presence of characteristic intranuclear inclusion bodies in affected cells. Further distinction between TSV infection, bacterial shell disease and IHHNV can be accomplished by using *in situ* hybridization assay with histological materials (Mari *et al.*, 1998). In this experiment, *in situ* hybridization assay was performed and the results confirmed that *P. monodon* and *M. rosenbergii* could be experimentally infected with TSV.

CONCLUSION

The two major local cultivated species, *P. monodon* and *M. rosenbergii* could be infected by TSV although the infected shrimp survived and showed no clinical signs of infection. In cultivated areas where there has been a TSV outbreak, shrimp farmers should eliminate potential virus carriers such as shrimps and crabs and treated the water before stocking postlarvae.

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