# Experimental infections reveal that common Thai crustaceans are potential carriers for spread of exotic Taura syndrome virus

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ABSTRACT: Taura syndrome virus (TSV) was first reported as a serious cause of shrimp mortality limited to reared *Penaeus* (*Litopenaeus*) *vannamei* in the Americas, where it spread principally through regional and international transfer of live post larvae (PL) and broodstock. Subsequently, through importation of infected broodstock, TSV outbreaks spread to Asia, first to Taiwan and China and then to Thailand, Indonesia and Korea. Since its introduction to Thailand, outbreaks have occasionally been reported from rearing ponds stocked with batches of specific pathogen free (SPF) *P. vannamei* PL that tested negative for TSV by nested RT-PCR assay. Since it was possible that the outbreaks may have occurred via horizontal transfer of TSV from wild carrier species, we tested 5 common native crustaceans that live in and around shrimp ponds (2 palaemonid shrimp species, *Palaemon styliferus* and *Macrobrachium lanchesteri*, and 3 species of crabs, *Sesarma mederi*, *Scylla serrata* and *Uca vocans*) for susceptibility to TSV in experimental challenges. We found that *U. vocans*, *S. serrata* and *S. mederi* did not die but, respectively, gave strong RT-PCR reactions indicating heavy viral load at 5 , 10 and 15 d post-injection of TSV and 10, 15 and up to 50 d after feeding with TSV-infected *P. vannamei* carcasses. Also after feeding, *P. styliferus* did not die, but a high proportion gave strong RT-PCR reactions at 5 d post-challenge and no reactions at 15 d. Similarly after feeding, *M. lanchesteri* showed no mortality and gave only light RT-PCR reactions at 2 d, moderate reactions at 5 d and no reaction at 15 d. By contrast, transmission experiments from the TSV-infected crabs and palaemonid shrimp via water or feeding resulted in death of all the exposed *P. vannamei* from 8 to 12 d post-challenge and all were positive for heavy viral load by RT-PCR assay. Despite the results of these laboratory challenge tests, natural TSV infections were not detected by nested RT-PCR in samples of these species taken from the wild. These results indicated that transmission of TSV from infected crabs and palaemonid shrimp via water or feeding might pose a potential risk to shrimp aquaculture.

KEY WORDS: TSV · Taura syndrome virus · *Penaeus vannamei* · Crab · Palaemonid shrimp · Carrier · RT-PCR

## INTRODUCTION

Taura syndrome (TS) was first reported from Ecuador in 1992 (Jimenez 1992). It was a serious cause of shrimp mortality for reared *Penaeus* (*Litopenaeus*) *vannamei* in the Americas where it spread principally through the re-gional and international transfer of live post larvae (PL) and broodstock (Brock et al. 1995). Its viral etiology was discovered in 1995 (Brock et al. 1995, Hasson et al. 1995). Taura syndrome virus (TSV) is a small, non-enveloped icosahedral virus containing a single-stranded, positive-sense RNA genome of 10 205 nucleotides (Hasson et

al. 1995, Bonami et al. 1997, Mari et al. 2002). Its protein capsid consists of 3 major polypeptides (55, 40 and 24 kDa designated VP1, VP2 and VP3, respectively) and 1 minor polypeptide (58 kDa designated V0) (Bonami et al. 1997, Mari et al. 2002). TSV has been placed in the family Dicistroiridae (Mayo 2002, 2005).

TSV can infect several shrimp species including *Penaeus* (*Litopenaeus*) *stylirostris*, *Penaeus* (*Litopenaeus*) *setiferus* (Overstreet et al. 1997, Erickson et al. 2002), *Penaeus monodon* (Chang et al. 2004, Nielsen et al. 2005), *Penaeus* (*Marsupenaeus*) *japonicus* and *Macrobrachium rosenbergii* (Nielsen et al. 2005). A wider host range has been demonstrated for white spot syndrome virus (WSSV) by polymerase chain reaction (PCR) and *in situ* hybridization. It includes penaeid shrimp, crabs, palaemonid shrimp, krill, crayfish and copepods (Lo et al. 1996, Supamattaya et al. 1998, Kanchanaphum et al. 1998, Peng et al. 1998, Chen et al. 2000, Maeda et al. 2000, Jiravanichpaisal et al. 2001). There are also multiple hosts for yellow head virus (YHV), as shown by RT-PCR detection and immunohistochemistry. Hosts include 3 common palaemonid species (*Macrobrachium sintangense*, *Palaemon styliferus* and *Palaemon serrifer*) (Longyant et al. 2005).

Since TSV has been recently introduced to Thailand and has caused numerous disease outbreaks in shrimp ponds where *Penaeus* (*Litopenaeus*) *vannamei* is being reared, it is possible that native crustaceans have also become infected with the virus. If so, they could constitute a natural viral reservoir that presents some risk to shrimp farmers. Thus, it was of interest to use RT-PCR amplification to search for potential, native TSV carriers such as crabs and palaemonid shrimp that live in and around *P. vannamei* rearing ponds. We also tested some common crabs (*Sesarma mederi*, *Uca vocans* and *Scylla serrata*) and 2 palaemonid shrimp (*Palaemon styliferus* and *Macrobrachium lanchesteri*) for susceptibility to TSV in laboratory challenge tests. Detection by RT-PCR was confirmed by immunohistochemistry using a polyclonal antibody specific to the capsid protein VP1 of TSV. It was hoped that identification of carriers would assist shrimp farmers in implementing control measures to exclude them from the cultivation system and prevent horizontal transmission of TSV.

## MATERIALS AND METHODS

**Shrimp and crab specimens.** Two palaemonid shrimp species (*Palaemon styliferus* and *Macrobrachium lanchesteri*) (<1 g each) were collected from a *Penaeus* (*Litopenaeus*) *vannamei* farm and nearby canals in Samutsongkhram province. *Sesarma mederi* (~30 g each) specimens were collected from *P. vannamei* farms in Chantaburi and Samutsongsongkhram

provinces. *Uca vocans* (~30 g each) and *Scylla serrata* (~300 g each) were collected from mangrove forests near *P. vannamei* farms in Samutsongkhram and Samutsakorn provinces.

*Penaeus* (*Litopenaeus*) *vannamei* (~20 g each) used in TSV transmission tests were obtained from a shrimp farm in Samutsongsongkhram province. The collected crustaceans were acclimatized in the laboratory for 2 d in 65 l aquaria containing continuously aerated artificial seawater at 15 ppt and 30 to 35°C. Palaemonid shrimp were fed on a dry commercial shrimp feed diet and crabs were fed with uninfected TSV *P. vannamei* carcasses once daily. Challenge tests and transmission tests were carried out under the same conditions.

**TSV challenge tests.** A TSV viral stock solution was prepared from infected *Penaeus* (*Litopenaeus*) *vannamei* haemolymph and diluted with lobster haemolymph buffer (LHB) according to Boonyaratpalin et al. (1993). The TSV-infected shrimp were obtained from an outbreak farm in Samutsakorn province of Thailand. For each species of crab (*Sesarma mederi*, *Uca vocans* and *Scylla serrata*), 10 were injected with 100 µl of viral stock solution, 10 were fed with TSV-infected shrimp carcasses (15% body weight as described by Tsai et al. 2002) and 10 were not exposed to TSV and held as negative controls. The crustaceans were placed in 65 l aquaria containing continuously aerated artificial seawater at 15 ppt. They were then assayed at 2 d, 5 d, 10 d, 15 d, 40 d and 50 d by taking 150 µl of haemolymph from 5 arbitrarily selected individuals and mixing it individually with 300 µl of AC1 solution (450 mM NaCl, 30 mM Trisodium citrate, 26 mM Citric acid, 12.7 mM EDTA, 100 mM Glucose, pH 7.0). At termination of the experiment (Day 50), hemocytes of all remaining animals were tested for TSV infection by immunofluorescence using confocal microscopy.

Since the palaemonid shrimp (*Palaemon styliferus* and *Macrobrachium lanchesteri*) were too small (<1 g each) for injection challenge, 80 individuals from each species were fed with 12 g (15% body weight as described by Tsai et al. 2002) of chopped TSV-infected *Penaeus* (*Litopenaeus*) *vannamei* carcasses and 80 were fed with dry commercial shrimp feed diet as negative controls. The palaemonid shrimp were reared in 65 l aquaria containing continuously aerated artificial seawater at 15 ppt for 50 d. Whole bodies of 5 individuals were arbitrarily selected at each sampling interval and homogenized in 500 µl of TRI Reagent® (Molecular Research Center) for RNA extraction.

**RNA extraction.** Total RNA from crabs was extracted from 450 µl of haemolymph-AC1 mixture by vigorous mixing in 500 µl of TRI Reagent®, while total RNA from whole bodies of small palaemonid shrimp was extracted by grinding in 500 µl of TRI Reagent®. After incubation

for 5 min, 200 µl chloroform was added with vigorous mixing, and the tube was incubated for 10 min before centrifugation at $12\,000 \times g$ for 10 min. The aqueous phase was transferred to a fresh tube followed by addition of 500 µl of 100% isopropanol with gentle mixing. After incubation for 10 min at 25°C the tube was centrifuged at $12\,000 \times g$ for 10 min, and the pellet was washed with 70% (v/v) ethanol, air dried and dissolved in 50 µl of RNase-free water. RT-PCR amplification was carried out using 2 µl of this RNA solution as template.

**RT-PCR detection of TSV.** Extracted RNA was amplified by RT-PCR using the IQ2000™ TSV Detection and Prevention System (Farming IntelliGene Technology Corporation) according to the manufacturer's protocol. The nested PCR products were detected by 1.8% agarose gel electrophoresis with ethidium bromide with visualization on a UV transilluminator. Results were interpreted using the kit guidelines as follows: appearance of 3 amplicons at 284 bp, 476 bp and above 848 bp indicated a heavy viral load (+++); 2 amplicons at 284 bp and 476 bp indicated a medium viral load (++); 1 amplicon at 284 bp with or without the housekeeping band at 680 bp indicated a light viral load (+); 1 band only at 680 bp (housekeeping gene) was considered to be a negative test result for TSV (–) within the sensitivity of the test (20 copies of TSV genome per RT-PCR reaction vial).

**Confirmation of TSV infections by immunofluorescence and confocal microscopy.** Haemolymph of crabs at 50 d post-challenge with TSV was fixed with 4% formaldehyde in PBS (137 mM NaCl, 10 mM phosphate, 2.7 mM KCl, pH 7.4) for 30 min. They were washed 3 times with PBS, permeabilized with 0.1% Triton X-100 for 10 min and blocked with 10% normal bovine serum for 30 min. After incubation with a primary antibody specific to the capsid protein (VP1) as validated in a previous study (Chaivisuthangkura et al. 2006) for 1 h, they were washed with PBS-T (0.1% Tween 20 in PBS). They were incubated with GAM Alexa Fluor 546 (Molecular probe) (1:500) for 30 min and washed with PBS-T for 5 min. This was followed by a second fixation in 4% formaldehyde in PBS for 15 min and counterstaining with To-Pro 3 (Molecular probe) (1:500) nuclear stain for 1 h before a final wash with PBS. The cells were then examined by confocal microscopy. Positive controls consisted of stained hemocytes from TSV-infected *Penaeus* (*Litopenaeus*) *vannamei,* and negative controls consisted of stained hemocytes from *P. vannamei* that tested negative for TSV by RT-PCR.

**Preliminary experimental transmission tests.** To test whether TSV could be transmitted from crabs to *Penaeus* (*Litopenaeus*) *vannamei* by water, 3 experimentally infected *Sesarma mederi* (separate from the crabs described above) were placed (15 d post-injection with TSV inoculum) in a 65 l aquarium in a basket to separate them from 5 uninfected *P. vannamei*. Haemolymph was collected from each shrimp at 5 d and assayed by RT-PCR for TSV as described above. A similar protocol was used to test whether TSV could be transmitted from the palaemonid shrimp to *P. vannamei* by water. Five *Macrobrachium lanchesteri* fed with TSV-infected *P. vannamei* carcasses at 5 d post-challenge were placed in a basket in an aquarium containing 5 uninfected *P. vannamei*. For control groups, 5 uninfected *P. vannamei* were incubated under identical conditions without exposure to TSV.

In addition, to test whether TSV could be transmitted from infected crabs and palaemonid shrimp to *Penaeus* (*Litopenaeus*) *vannamei* orally, 15 g (15% total body weight *P. vannamei*) of carcasses of TSV-infected *Sesarma mederi* and infected *Macrobrachium lanchesteri* were fed to 5 uninfected *P. vannamei* (20 g each). Haemolymph was removed from each *P. vannamei* for RT-PCR testing at 5 d after feeding.

## RESULTS

### Natural infections

For each crustacean species, 10 to 40 individuals were used to detect natural TSV infection using the IQ2000™ Detection and Prevention System. None of the natural specimens of crustaceans collected gave positive test results for TSV infection (Table 1). Although there is no information available to indicate the epidemiological sensitivity and specificity of the

Table 1. Results of screening natural crustaceans for Taura syndrome virus (TSV) by nested RT-PCR using the IQ2000™ TSV detection kit. Assuming test sensitivity and specificity at 100% and populations of more than 100 000 (not necessarily valid assumptions), this would indicate freedom from TSV at the target prevalences (%) shown

| Species | Province | No. of TSV-negative samples | Target prevalence (%) |
|---|---|---|---|
| *Palaemon styliferus* | Samutsongkhram | 40 | 8 |
| *Macrobrachium lanchesteri* | Samutsongkhram | 40 | 8 |
| *Sesarma mederi* | Chantaburi | 20 | 14 |
| | Samutsongsongkhram | 15 | 19 |
| *Uca vocans* | Samutsongkhram | 20 | 14 |
| | Samutsakorn | 15 | 19 |
| *Scylla serrata* | Samutsongkhram | 10 | 26 |
| | Samutsakorn | 10 | 26 |

IQ2000 test, crude calculations on freedom from disease were calculated for each sample (Cameron 2002) assuming that sensitivity and specificity were both 100%, that the target populations in each area were over 100 000 and that the samples were random. The results for a total of 170 samples indicated freedom from TSV at 2% prevalence. To obtain a precise idea of the level of prevalence in these populations, a more rigorous sampling and testing protocol would be required. We did not test these wild specimens for the presence of other shrimp viruses since the intention was to determine the prevalence of TSV only.

## Experimental infections

RT-PCR analysis of haemolymph samples from crabs challenged with TSV by injection (Fig. 1, Table 2) revealed that all 5 *Sesarma mederi* specimens had light viral loads at 2 d post-challenge and heavy viral loads at 15 d post-challenge. All 5 *Uca vocans* had light

viral loads at 2 d post-challenge and heavy viral loads at 5 d post-challenge. *Scylla serrata* gave strong RT-PCR reactions indicating heavy viral loads at 10 d post-challenge.

RT-PCR assays of crabs orally challenged with TSV-infected *Penaeus* (*Litopenaeus*) *vannamei* carcasses (Fig. 2, Table 3) revealed that 3 of 5 *Sesarma mederi* had light viral loads at 5 d post-challenge, 4 of 5 had medium viral loads at 10 d post-challenge and 5 of 5 had heavy viral loads at 50 d post-challenge. At 5 d post-challenge, 4 of 5 *Uca vocans* showed light viral loads, and 3 of 5 showed heavy viral loads at 10 d post-challenge. At 15 d post-challenge, 4 of 5 *Scylla serrata* showed heavy viral loads. For control groups, all crabs survived and gave negative RT-PCR reactions for TSV at 2, 5, 40 and 50 d.

RT-PCR assays of the palaemonid shrimp *Palaemon styliferus* and *Macrobrachium lanchesteri* challenged by feeding with TSV-infected *Penaeus vannamei* carcasses (Fig. 3, Table 3) revealed light viral loads for all 5 individuals of both species at 2 d post-challenge. At
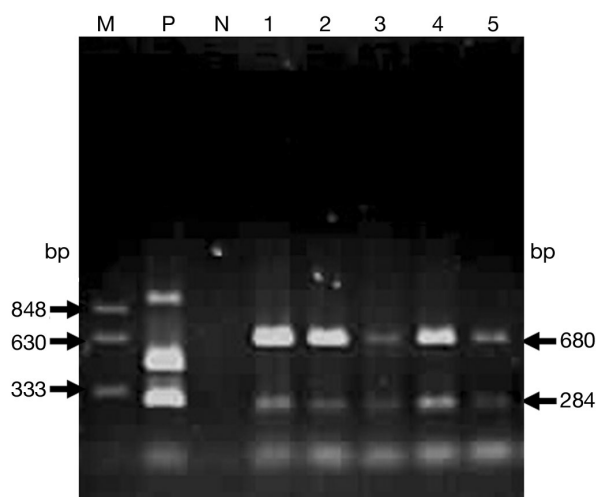


Fig. 1. Sample agarose gel of RT-PCR products (IQ2000™ TSV detection kit) from haemolymph of 5 *Sesarma mederi* at 2 d post-injection of TSV. Lane M: molecular weight marker. Lane P: positive control (TSV plasmid). Lane N: negative control. PCR products of 284 and 680 bp (housekeeping gene) indicate a light infection of TSV
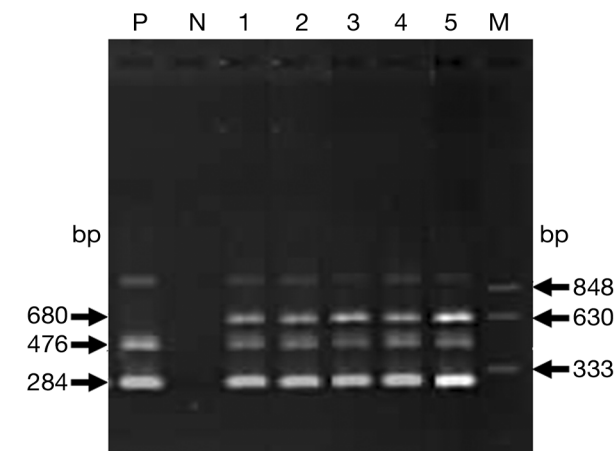


Fig. 2. Sample agarose gel of RT-PCR (IQ2000™ TSV detection) products from haemolymph of 5 *Sesarma mederi* at 40 d after feeding with TSV-infected *Penaeus* (*Litopenaeus*) *vannamei* carcasses. Lane M: molecular weight marker. Lane P: positive control (plasmid template with a TSV insert). Lane N: negative control (distilled water template). Lanes showing 2 PCR amplicons (284 and 476 bp) and a non-specific amplicon above 848 bp indicate heavy infections of TSV

Table 2. Results for RT-PCR detection of TSV in crabs using IQ2000™ detection system at various days post-challenge by injection. + = light infection; ++ = medium infection; +++ = heavy infection; – = negative infection; values in parentheses indicate no. positive/no. tested

| Species | 2 d | 5 d | 10 d | 15 d | 40 d | 50 d |
|---------|-----|-----|------|------|------|------|
| *Sesarma mederi* | + (5/5) | + (5/5) | + (5/5) | +++ (5/5) | +++ (5/5) | +++ (5/5) |
| *Uca vocans* | + (5/5) | +++ (5/5) | +++ (5/5) | +++ (5/5) | +++ (5/5) | +++ (5/5) |
| *Scylla serrata* | – (0/5) | ++ (5/5) | +++ (5/5) | +++ (5/5) | +++ (5/5) | +++ (5/5) |

Table 3. Results for RT-PCR detection of TSV in crabs and palaemonid shrimp using IQ2000™ detection system at various days post-challenge by feeding. + = light infection; ++ = medium infection; +++ = heavy infection; − = negative infection; values in parentheses indicate no. positive/no. tested; nd = not done

| Species | 2 d | 5 d | 10 d | 15 d | 40 d | 50 d |
|---|---|---|---|---|---|---|
| *Sesarma mederi* | − (0/5) | + (3/5) | ++ (4/5) | ++ (5/5) | ++ (5/5) | +++ (5/5) |
| *Uca vocans* | − (0/5) | + (4/5) | +++ (3/5) | +++ (4/5) | +++ (5/5) | +++ (5/5) |
| *Scylla serrata* | − (0/5) | − (0/5) | − (0/5) | +++ (4/5) | +++ (5/5) | +++ (5/5) |
| *Palaemon styliferus* | + (5/5) | +++ (4/5) | +++ (4/5) | − (0/5) | − (0/5) | nd |
| *Macrobrachium lanchesteri* | + (5/5) | ++ (4/5) | ++ (4/5) | − (0/5) | − (0/5) | nd |

5 d post-challenge, 4 of 5 *P. styliferus* showed heavy viral loads, while *M. lanchesteri* gave only medium viral loads. However, both species gave negative test results at 15 d post-challenge. For control groups all palaemonid shrimp survived and gave negative reactions for TSV at 2, 5, 15 and 50 d.

### Confirmation of TSV infections by immunofluorescence and confocal microscopy

To confirm the presence of TSV infections, 3 individuals of 3 species of crabs at 50 d post-challenge were processed for immunofluorescence and confocal microscopy using an antibody reagent specific to VP1 of TSV. Positive immunoreactions with the VP1 antibody (red fluorescence) was seen in the cytoplasm of haemocytes of 3 individuals of *Sesarma mederi* (Fig. 4A–C). No positive reactions were observed in negative control crabs (Fig. 4D). Similar positive red immunofluorescence was seen in the cytoplasm of hemocytes from
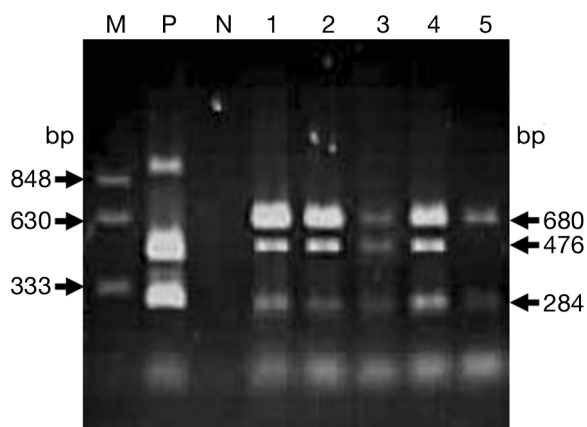


Fig. 3. Sample agarose gel of RT-PCR (IQ2000™ TSV detection) products from 5 *Macrobrachium lanchesteri* at 5 d after feeding with TSV-infected *Penaeus* (*Litopenaeus*) *vannamei* carcasses. Lane M: molecular weight marker. Lane P: positive control (TSV plasmid). Lane N: negative control. Specimens showing PCR products at 284, 476 and 680 bp (housekeeping gene) had medium infections of TSV. Specimens showing PCR products at 284 and 680 bp had light infections
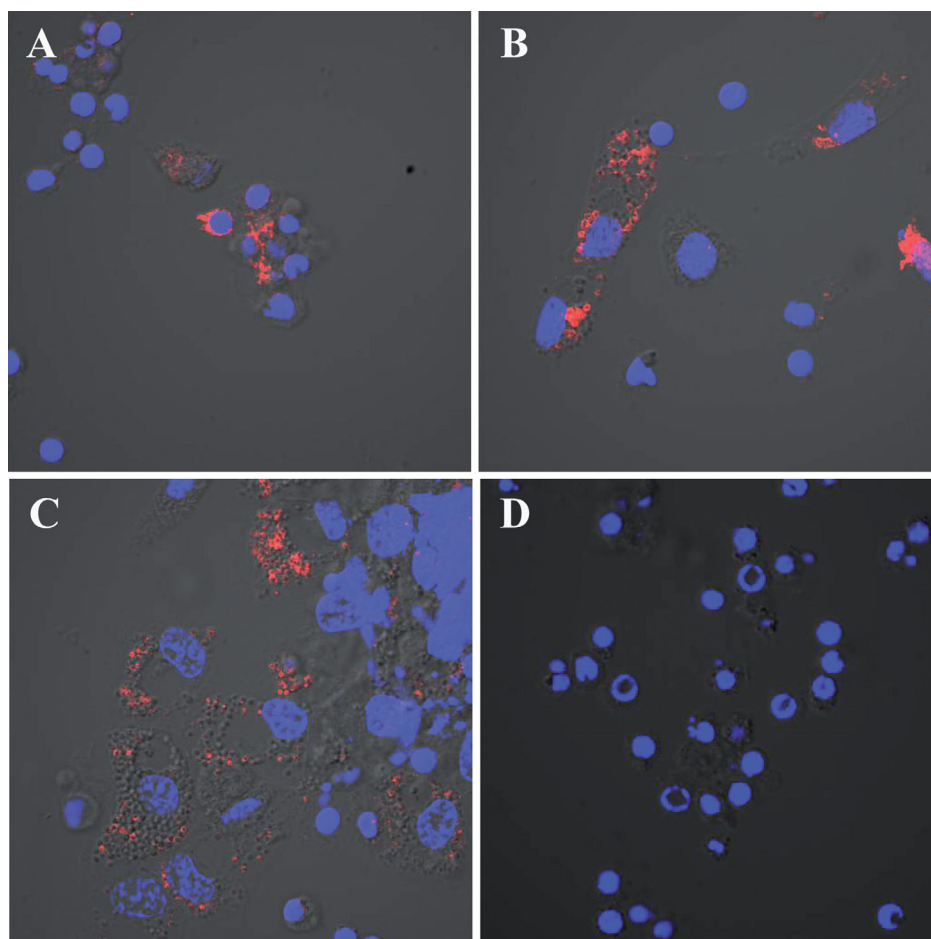
the positive control, TSV-infected *Penaeus* (*Litopenaeus*) *vannamei* (Fig. 5A), but not in the cytoplasm of hemocytes from *P. vannamei* negative for TSV by RT-PCR (Fig. 5B).

### Preliminary transmission experiments

To test whether TSV could transmit from crabs and palaemonid shrimp to *Penaeus* (*Litopenaeus*) *vannamei* by water, TSV-infected *Sesarma mederi* and *Macrobrachium lanchesteri* were placed in aquaria with uninfected *P. vannamei*. All 5 *P. vannamei* kept as cohabitants with TSV-infected *S. mederi* showed heavy TSV viral loads at 5 d (Fig. 6A) and died by 10 d after the start of cohabitation. All 5 *P. vannamei* cohabitants with infected *M. lanchesteri* showed light TSV viral loads at 5 d after the start of cohabitation and heavy viral loads at 10 d. All were dead by 12 d.

To test whether TSV could transmit from crabs and palaemonid shrimp to *Penaeus* (*Litopenaeus*) *vannamei* by the oral route, the carcasses of TSV-infected *Sesarma mederi* and *Macrobrachium lanchesteri* were fed to *P. vannamei*. All *P. vannamei* fed with TSV-infected *S. mederi* (Fig. 6B) and *M. lanchesteri* gave heavy viral loads by 5 d after feeding and all died by 8 d after feeding. However, *P. vannamei* not exposed to TSV in control aquaria gave negative RT-PCR reactions (Fig. 6C) and showed no mortality.

### DISCUSSION

The present study has very clearly shown that the 3 crab species studied can be infected with TSV by injection and by feeding with TSV-infected shrimp carcasses, and that the resulting infections can persist for up to 50 d. Similarly, it has been reported that *Sesarma mederi*, *Scylla serrata* and *Uca pugilator* are susceptible to WSSV infection and may carry it for life (Lo et al. 1996, Kanchanaphum et al. 1998, Peng et al. 1998). By contrast, we found that TSV infections in *Palaemon styliferus* and *Macrobrachium lanchesteri* could not be

Fig. 4. *Sesarma mederi.* Sample confocal microscope photographs of immunofluorescence from TSV-infected crabs. (A,B,C) 3 individuals of *S. mederi* showing positive red fluorescence for anti-VP1 of TSV in the cytoplasm of hemocytes. (D) Negative control for *S. mederi.* Blue signal shows TO-PRO-3 iodide staining of DNA for nuclei
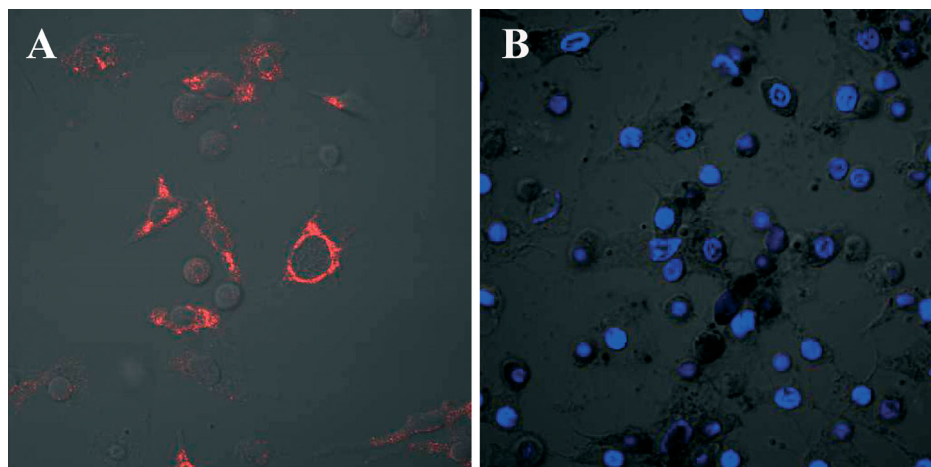


Fig. 5. *Penaeus* (*Litopenaeus*) *vannamei.* Confocal microscope photographs of immunofluorescence tests for TSV in hemocytes. (A) Positive red fluorescence for anti-VP1 of TSV in the cytoplasm of hemocytes of *P. vannamei* challenged with TSV. (B) Negative control for unchallenged *P. vannamei* that tested negative for TSV by RT-PCR and showed no reaction with anti-VP1. Blue signal shows TO-PRO-3 iodide staining of DNA in nuclei
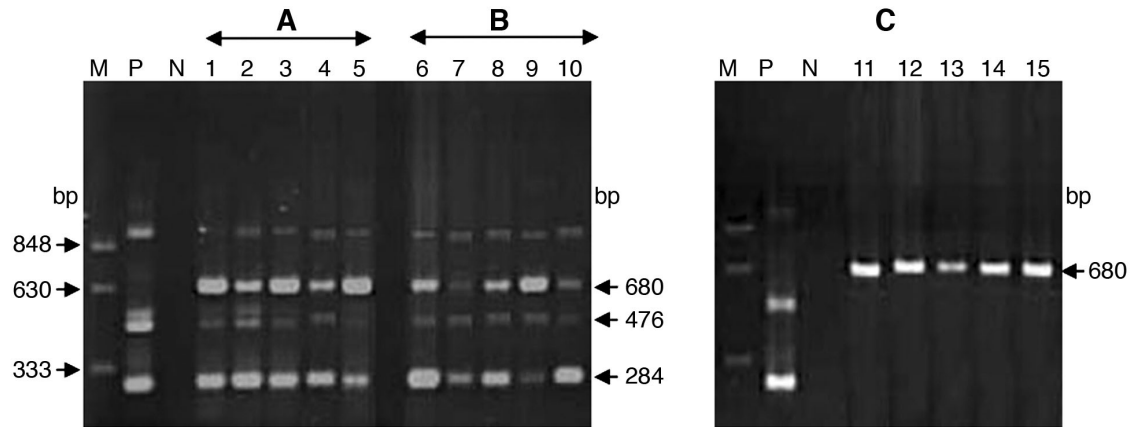
Fig. 6. *Penaeus* (*Litopenaeus*) *vannamei*. Example of assay results for TSV detection by RT-PCR in haemolymph. (A) Results for 5 individual *P. vannamei* 5 d after cohabitation with TSV-infected *Sesarma mederi* (Lanes 1 to 5). (B) Results for 5 individual *P. vannamei* 5 d after feeding with TSV-infected *S. mederi* carcasses (Lanes 6 to 10). (C) Results for 5 individual *P. vannamei* in the control tank (i.e. not exposed to TSV) (Lanes 11 to 15). Lane M: molecular weight marker. Lane P: positive control (plasmid containing target TSV fragment). Lane N: negative controls (distilled water template). Lanes showing 2 PCR amplicons (284 and 476 bp) and a non-specific amplicon above 848 bp indicate heavy TSV infections. PCR products showing a 680 bp band (housekeeping gene product) indicate absence of TSV at the detection limit of 20 genome copies per PCR reaction tube

detected from 15 d post-challenge onwards, suggesting that they were able to clear the virus from their systems. Similarly, Longyant et al. (2005) have reported that *M. lanchesteri* develops very mild yellow head virus (YHV) infections that are detectable at 3 d post-challenge but not after 10 d. The current study establishes the fact that crabs and to a lesser extent palaemonid shrimp may act as carriers of TSV. However, the degree of risk for disease transfer from these carriers to farmed shrimp would have to be determined by examining a number of other epidemiological factors.

It is interesting that RT-PCR test results for the palaemonid shrimp orally challenged with TSV initially indicated light or negative infections and later medium or heavy viral loads before becoming negative. This revealed that the virus multiplied in the shrimp (i.e. they are susceptible to TSV infection) but that they were able to clear it from their systems. The mechanism for this clearance would be of considerable interest to those studying crustacean immunity. Fortunately, these small shrimp species can be handled easily in the laboratory and would make excellent models for further study.

Although our challenge tests showed that native Thai crustaceans are potential carriers of TSV, transmission studies are needed to determine the degree of danger they might pose for shrimp farmers. Our very preliminary transmission experiments in aquaria revealed that transmission from infected crabs or palaemonid shrimp to *Penaeus* (*Litopenaeus*) *vannamei* is possible through the water or by ingestion of infected carcasses. However, our inability to find wild crustaceans infected with TSV in farm areas where

TSV outbreaks had previously occurred suggests that the prevalence of infected carriers there was not high. Altogether, it would appear that the most dangerous scenario would be for a TSV outbreak to occur in a pond of farmed *P. vannamei* and for wild crustaceans (especially crabs) to have the opportunity to eat infected shrimp carcasses. They would have a high chance of becoming infected and could possibly carry the virus to an uninfected pond where the risk of transmission would be high if they died and were eaten by *P. vannamei*.

In conclusion, it is possible that Thai farmers who buy SPF stocks of *Penaeus* (*Litopenaeus*) *vannamei* certified to be free of TSV could experience TSV outbreaks in their ponds as a result of horizontal transmission from wild, native crabs infected with TSV. However, the normal Thai practice of eliminating wild crustaceans from rearing ponds prior to stocking PL at the beginning of each crop cycle, filtration to eliminate potential vectors or carriers from exchange water during culture and the use of crab exclusion nets should constitute effective control measures.

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