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Taura Syndrome Virus Disease in Farm-Reared *Penaeus monodon* in Thailand

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ABSTRACT

Taura syndrome virus (TSV) has caused major economic losses to shrimp aquaculture throughout the world. TSV has been reported to infect a number of penaeid species as hosts. In this study, we reported the natural infection of TSV in farm-reared *Penaeus monodon* from eastern provinces of Thailand between June to September 2004. There were different degrees of disease outbreak severity. In some cases large number of shrimp died and caused great losses to farmers. However, in most cases only small number of shrimp died and the farmers could control the situation enough to raise the majority to marketable size. Diseased shrimp varied in size from aged 40-50 days (4 g) to 20 g. Infected shrimp was characterized by black cuticular lesions and loose shell. Histopathological changes in infected shrimp showed multifocal to extensive areas of necrosis in the sub-cuticular epithelium, connective tissue and adjacent striated muscle. Affected cells often displayed an increased cytoplasmic eosinophilia, nuclear pyknotic and karyorrhexis. *In situ* hybridization tests gave positive results with the tissues of shrimp collected from the TSV outbreaks. In addition to TSV infection, most moribund shrimp also had dual infections with microsporidians in the hepatopancreas and/or gregarines in the gut.

Key words: Taura syndrome virus, *Penaeus monodon*

INTRODUCTION

Taura syndrome was first recognized as a shrimp disease in farms near the mouth of the Taura river, Ecuador, in June 1992 (Jimenez, 1992; Rosenbery, 1993; Lightner *et al.*, 1994). The infectious agent was named Taura syndrome virus or TSV in 1994 (Hasson *et al*., 1995; Lightner *et al*., 1995). TSV was first isolated from *Litopenaeus vannamei* and characterized as a non-enveloped, icosahedral particle, 31-32 nm in diameter, with a density of 1.338 g/ml in CsCl. Its genome consists of a linear, positive sense ssRNA molecule of approximately 10.2 kb and it is classified as a Picornavirus (Bonami *et al*., 1997; Brock *et al*., 1997). From nucleotide sequence data, TSV is more closely related to the cricket paralysis- like viruses (Mari *et al*., 2002).

The occurrences of TSV outbreaks in *L. vannamei* include cultured shrimp stocks in Hawaii, Peru, Ecuador, Colombia, Panama, Costa Rica, Nicaragua, El Salvador, Honduras, Guatemala and Mexico (Lightner 1996). The outbreaks were reported for *P. stylirostris*, *P. setiferus* and *P. schmitti* in Ecuador and Peru (Lightner *et al.*, 1995; Brock *et al*., 1997). In Asia, TSV was first reported from Taiwan in 1999 (Tu *et al*., 1999).

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In Thailand, since early 2000, the cultivation of black tiger shrimp, *Penaeus monodon*, had suffered slow growth, leading shrimp farmers to shift to the cultivation of *L. vannamei*. Most of the nauplii were illegally imported from China and Taiwan. Alarmed by the possibility of TSV introduction, the Thai Department of Fisheries permitted legal importation of *L. vannamei* from March 2002-February 2003, if the imported stocks were certified free of TSV by RT-PCR testing. However, in early 2003, TSV outbreaks occurred in inland farm-reared *L. vannamei* (Limsuwan, 2003; Nielsen *et al*., 2005). Since then more TSV outbreaks were reported in *L. vannamei* in most areas of cultivation. Shortly thereafter, in early 2004, mortalities were observed in *P. monodon* intensive culture ponds. Diseased shrimp were PCR-negative for both white spot syndrome virus (WSSV) and yellow-head virus (YHV) but positive for TSV. This disease was widespread and caused heavy mortalities to some farms.

This paper describes an epizootic of TSV including gross signs, histopathology and *in situ* hybridization in intensively reared *P. monodon* in Thailand.

MATERIALS AND METHODS

Penaeus monodon samples were collected from TSV-affected farm ponds in the eastern provinces of Thailand during June to September 2004. The shrimp samples weighing of 4-20 g 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). A commercially available *in situ* hybridization probe for TSV (Diagxotics Inc.) was used according to the manufacturer's instructions. The protocols have been outlined by Lightner (1996) and Mari *et al*. (1998).

RESULTS AND DISCUSSION

Taura syndrome virus has been reported to infect a number of penaeid species as hosts. However, only *Litopenaeus vannamei* appears to be highly susceptible to the disease (Lightner 1996). Overstreet *et al*. (1997) and Lightner (1996) reported natural TSV infections in *P. setiferus* and experimental infections have been reported in *P. schmitti*, *P. aztecus*, *P. duororaram*, *P. chinensis*, *P. monodon*, and *P. japonicus*. TSV may be transmitted horizontally by co-habitation or cannibalism (Lotz *et al*., 2003). In Thailand, TSV was first reported from intensive farm-reared *P. monodon* in June 2004. Moribund shrimp aged 40-50 days were found in scattered areas around the edges of the pond. Although in some farms it could be found in younger or older shrimp as well. Diseased shrimp was characterized by black cuticular lesions and loose shell. Shrimp with these black lesions are at some risk of mortality during the succeeding molt, but if they survive, lesions disappear from the cuticle and shrimp look normal. However, affected shrimp did not display signs of red body or tail (Figure 1 and 2) which was different from the report of Lightner *et al.* (1995) indicated that the expansion of red chromatophores in the appendages, especially of the uropods, telson, and pleopods of *L. vannamei* infected with TSV. There were different degrees of disease outbreak severity in cultured *P. monodon*. In some cases large number of shrimp died quickly and caused great losses to farmers. However, in most cases only small number of shrimp died and the farmers could control the situation enough to raise the majority to marketable size and harvest them for sale.

Histopathology of moribund shrimp showed multifocal to extensive areas of necrosis in the sub-cuticular epithelium, connective tissue and adjacent striated muscle (Figure 4). Affected

cells often displayed an increased cytoplasmic eosinophilia, nuclear pyknosis and karyorrhexis (Figure 5). Some samples also showed necrosis in the cells of haematopoietic tissue corresponded to those previously described for TSV infections (Lightner *et al*., 1995). *In situ* hybridization tests also gave positive results with the tissues of shrimp collected from the TSV outbreaks (Figure 6). In addition to TSV infection, most moribund shrimp also had infections with microsporidians in the hepatopancreas (Figure 7) and gregarines in the gut (Figure 8). These protozoans are highly pathogenic and frequently cause epizootics in crustacean populations (Overstreet,1973; Sindermann, 1990). Sprague and Couch (1971) indicated that in addition to microsporidians, shrimps in the ponds often harbor cephaline gregarines, similar to the results in this report. Brock *et al*. (1997) reported experimental infection of *P. monodon* with TSV and indicated that *P. monodon* was susceptible to TSV but suffered few mortalities. To avoid TSV infections or a significant outbreak of the disease, farmers must have sufficient reservoir ponds available and only refill the shrimp ponds or stocking postlarvae into the pond with water that has been left to rest for at

Figure 1 Moribund shrimp with TSV during the first 2 months of culture with multiple melanized cuticular lesions.

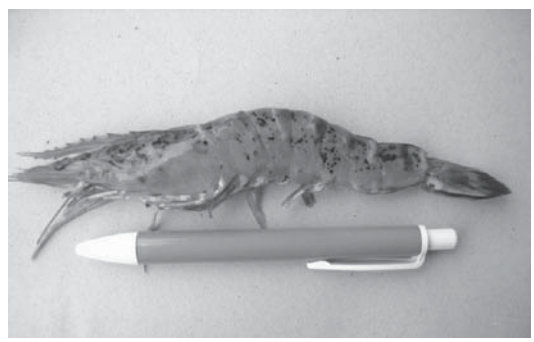

Figure 2 Affected shrimp at harvest with multiple black melanized cuticular lesions.

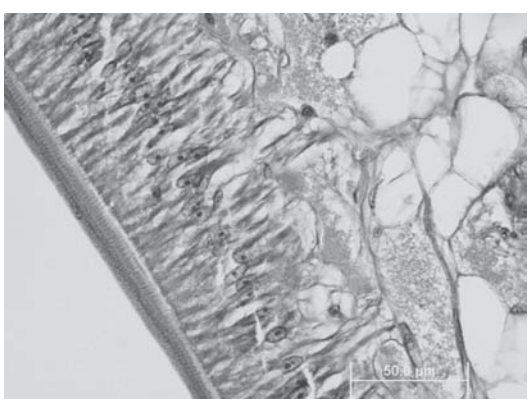

Figure 3 Normal subcuticular epidermal and connective tissue (H&E).

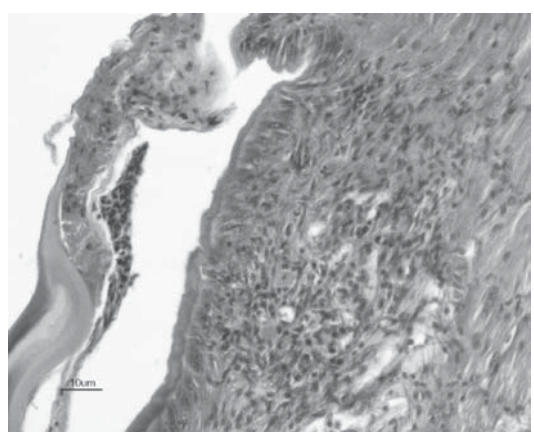

Figure 4 Typical TSV lesion showing area of extensive subcuticular epidermal and connective tissue necrosis (H&E).

least 15 days (Chuchird and Limsuwan, 2005). It will then be less likely that the virus will be alive in the water and the farmers will have a greater chance of rearing a good harvest of shrimp.

CONCLUSION

Gross sign of TSV in *P. monodon* was characterized by black cuticular lesions and loose shell. Histologically, sub-cuticular lesions were characterized by large numbers of spherical eosinophilic to densely basophilic inclusions and gave the tissue a kind of "buck-shot" appearance. Most moribund shrimp had dual infections with microsporidians in the hepatopancreas and/or gregarines in the gut.

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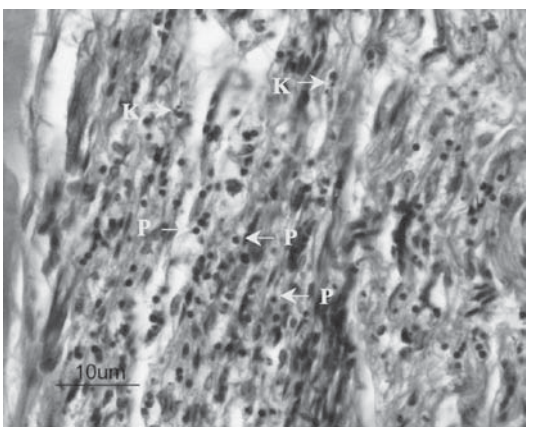

Figure 5 Higher magnification of TSV lesion with numerous nuclear pyknosis (P) and karyorrhexis (K), (H&E).

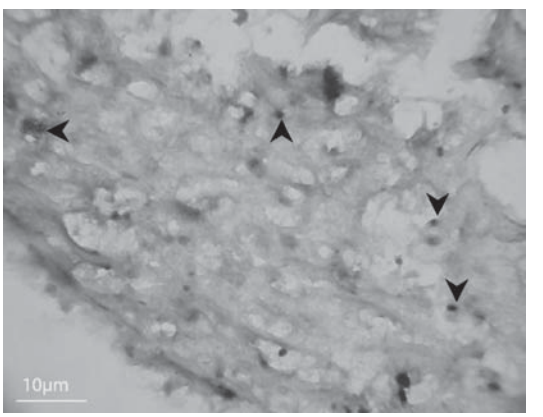

Figure 6 Tissue section of cuticular epithelium with positive *in situ* hybridization reaction for TSV (arrows).

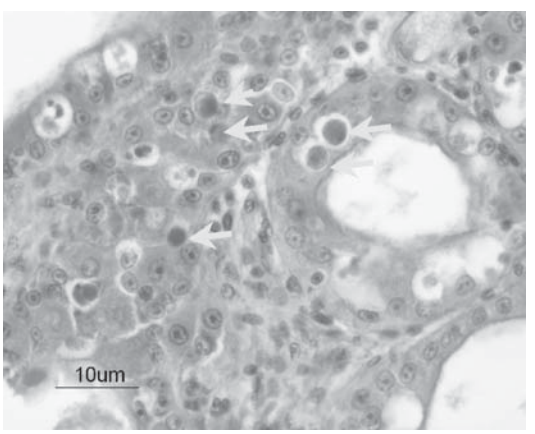

Figure 7 Microsporidians (arrows) infection in the hepatopancreas of TSV infected shrimp (H&E).

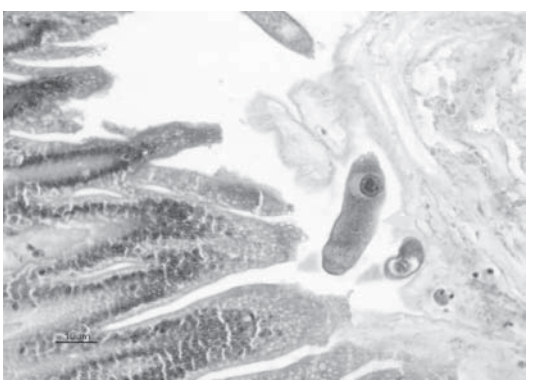

Figure 8 Gregarine (arrow) in the gut of TSV infected shrimp (H&E).

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