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The Viability of Taura Syndrome Virus in Low-salinity Water

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ABSTRACT

Taura syndrome virus (TSV) could survive up to 10 days in water at the salinity of 5- 10 parts per thousand (ppt) and could infect Pacific white shrimp, causing mortalities. Histopathological investigations of TSV-infected shrimp showed necrosis of the cuticular epithelium, marked by nuclear pyknosis and karyorrhexis. Basophilic cytoplasmic inclusion bodies could be seen in the cuticular epithelium of the shrimp's body, appendages, gills, esophagus, stomach, hindgut and rarely in the epithelium of antennal gland, giving them the characteristic "peppered" or "buckshot" appearance. The studied results indicated that shrimp farmers should eliminate all carriers of TSV, such as shrimp and crabs from the rearing ponds and allow water to sustain for at least 15 days before releasing shrimp into the pond.

Key words: Pacific white shrimp, Taura syndrome virus, low-salinity water

INTRODUCTION

As many shrimp farmers raising black tiger shrimp (*Penaeus monodon*) experienced problems with slow growth rates and economic losses, the Department of Fisheries (under the jurisdiction of the Ministry of Agriculture and Agricultural Cooperatives) authorized the importation of breeding pairs of specific pathogen-free (SPF) Pacific white shrimp (*Litopenaeus vannamei*) for testing. Shrimp farmers who purchased disease-free Pacific white shrimp larvae found that they were easy to culture, grew quickly and gave good harvests. Expenses were also much lower than raising black tiger shrimp. In 2004, the production of Pacific white shrimp is expected to exceed that of black tiger shrimp in Thailand. There is a great demand for Pacific white shrimp larvae and it appears that more and more farmers will switch from raising black tiger shrimp to Pacific white shrimp. However, there are only limited numbers of SPF Pacific white shrimp available for breeding. Although the Department of Fisheries gave permission for additional imports in June 2004, many farmers are still illegally purchasing the larvae from China and Taiwan. These contraband shrimp larvae are not tested for diseases. In May 2003 there was an outbreak of Taura syndrome virus (TSV), which caused the death of many Pacific white shrimp and heavy losses for farmers. TSV is most common in shrimp aged 20-60 days (Flegel *et al.*, 2003) and the death rate is quite high at 50-80 %. The most effective and popular method of preventing TSV is stocking with SPF shrimp and increase biosecurity measures to prevent further introductions of virus (Lotz *et al*., 1995). In the regions where the virus is already established, such methods may not be applicable and management strategies must be employed that are effective in the presence of the virus. One possible method of preventing TSV that ought to be effective is to change the shrimp raising method from an open system with frequent water changes to a closed or semi-closed method with little or no

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water replacement, along with killing the virus and carriers before introducing the shrimp.

This project was undertaken to study the viability of TSV in low-salinity water. The results of which can be used to inform shrimp farmers on how to prevent the severity of the TSV disease to insure that Thai shrimp industry will continue to prosper.

MATERIALS AND METHODS

Taura syndrome virus was obtained from naturally infected Pacific white shrimp collected from Nakhon Pathom province in April 2004. Thirty normal Pacific white shrimp were put into two 300 l fiberglass tanks containing water with a salinity of 5-10 ppt. Pacific white shrimp infected with TSV were cut into pieces and fed to the shrimp in the tanks in the morning and in the afternoon. After the normal shrimp began to display clinical signs of the disease, 150 l of water from the tanks was taken and transferred to ten 50 l glass aquariums. Aerators were turned on continuously in all ten aquariums. Ten normal Pacific white shrimp weighing about 8 g each, which had been tested using the nested RT-PCR technique and found to be free of TSV, were put into the test aquariums at 0, 5, 10, 15 and 20 days after the TSV-infected water was added. Three aquariums were used for each length of time tested.

Shrimp were observed and ones that showed clinical signs of TSV were selected for nested RT-PCR testing . The death rate was recorded for 15 days. Dead or moribund shrimp were removed immediately from the aquariums to prevent the water from being contaminated. Some moribund shrimp were fixed with Davidson's fixative for histopathological study. Throughout the experiment, shrimp were fed with commercial shrimp feed diet at a rate that would avoid deteriorating water quality.

RESULTS AND DISCUSSION

The mortality rate of TSV infected shrimp are shown in Table 1. It was indicated that TSV could remain viable in water with a salinity of 5-10 ppt and could be transmitted horizontally to other organisms. TSV could live without a carrier in low-salinity water up to 10 days. The virus remained infectious and caused death in the test shrimp. Nested RT-PCR tests on the shrimp that were put in the test aquariums 10 days or less after the TSV-infected water was added were determined to be positive for TSV. However the shrimp that were put in the test aquariums 15 days or later were negative and the shrimp did not die (Figure 1).

Lotz *et al*. (2003) reported that TSV could be horizontally transmitted to other kinds of aquatic animals through co-habitation or cannibalism. The laughing gull could also be a carrier of TSV. Bioassays of live shrimp found that TSV could live and remain infectious in the droppings of this bird (Garza *et al.*, 1997). Flegel *et al*. (1997a) reported that water from white spot syndrome virus (WSSV) outbreak ponds remains infectious for approximately 4 days and yellow-head virus (YHV) extracts in water remain infectious more than 72 hours (Flegel *et al*., 1997b).

The results of this study provided some fundamental information for shrimp farmers on the prevention of TSV outbreaks. Before starting to culture each new batch of shrimp they should first eliminate any possible carriers, such as other crustaceans, in the water and let the water sustain for at least 15 days before introducing the new shrimp larvae. This will help prevent TSV infection.

In this study it was found that TSV-infected shrimp had red bodies, tails and swimming legs (Figure 2). The clinical signs and pathological changes became apparent within 3-4 days of infection. The shrimp died 2-3 days later. In histopathological studies of shrimps contracted with TSV, there was necrosis of the cuticular epithelial tissues along with nuclear pyknosis and

karyorrhexis (Figure 3). Dark colored spots caused by cytoplasmic inclusion bodies gave the cells a characteristic "peppered" or "buckshot" appearance. The inclusion bodies stained basophilically and could be observed in the cuticular epithelial tissues of the thorax, antennae, gills, hindgut, esophagus, stomach and antennal gland epithelium. The affected shrimp also had soft exoskeletons and no food could be found in their digestive tracts. They usually die during molting.

Lightner (1996) reported that, in general,

Table 1 TSV infection rates in low-salinity (5-10 ppt) water.

Time of introduction		Number of shrimp	Death rate	PCR test
Days	Batch		(%)	
0	1	10	100	+
	2	10	90	+
	3	10	80	+
5	1	10	80	+
	2	10	80	+
	3	10	100	+
10	1	10	70	+
	2	10	80	+
	3	10	60	+
15	1	10	0	_
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	3	10	0	_
20	1	10	0	_
	2	10	0	_
	3	10	0	_

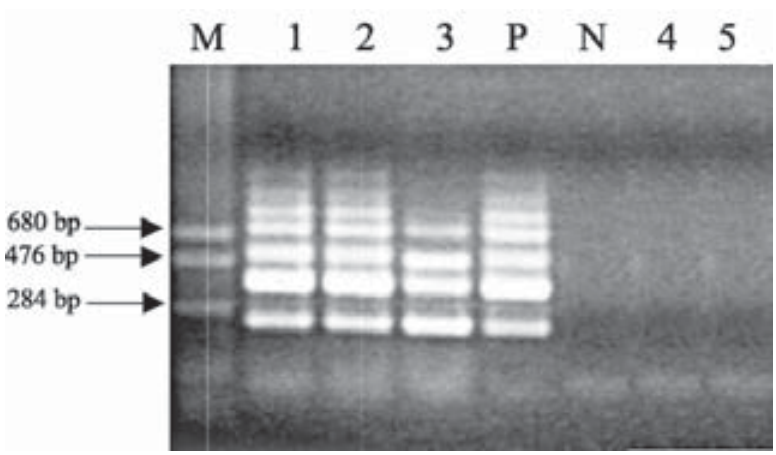

Figure 1 Results of nested RT-PCR tests for TSV infected Pacific white shrimp using 1% agarose gel electrophoresis.M Marker; P positive control; N negative control (normal shrimp); lane1-5 shrimp infected with TSV after being placed in TSV-infected water at 0, 5, 10, 15 and 20 days, respectively.

TSV infects cells generated by the ectodermal and mesodermal layers. In the acute stage, the cuticular epithelium is severely impacted by the infection. The lymphoid organ is only infected in the chronic stage of the disease. In the acute stage, necrosis of the cuticular epithelium and subcuticular connective tissue is apparent; nearby striated muscle fibers may also be infected. The epithelial cells of the antennal gland tubules are almost never affected, however. The cytoplasm of infected cells can be stained eosinophilically. Nuclear pyknosis or karyorrhexis may be observed. Particles of the residual cytoplasm from the necrotic cells appear as eosinophilic or basophilic round balls 1-20 microns in diameter. Changes such as nuclear pyknosis and karyorrhexis occur in the acute stage of the disease. The term for describing the appearance of the affected cells is "peppered" or "buckshot-riddled." It is a defining characteristic that is specific for TSV. There is no sign of hemocytic infiltration or any other inflammatory response. This is one way of differentiating cases of acute TSV infection from the intermediate, chronic or recovery stages.

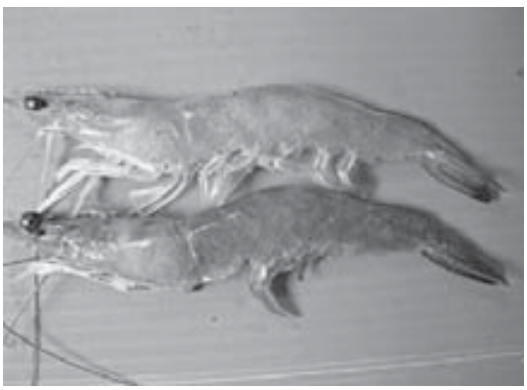

Figure 2 The external appearance of a TSV-infected shrimp with a red body, tail and legs (lower) comparing with normal shrimp (upper).

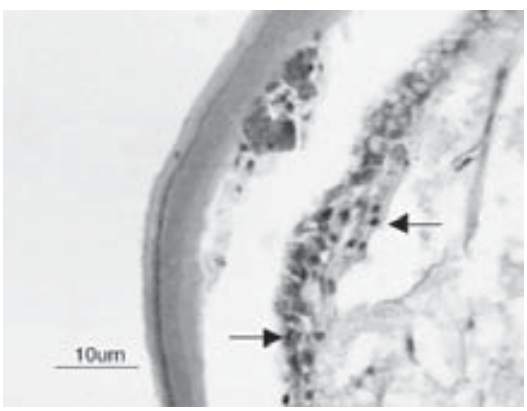

Figure 3 Histopathological change of Pacific white shrimp infected with TSV, showing extensive necrosis of subcuticular epithelial tissue with prominent nuclear pyknosis (arrows). (H&E x800)

CONCLUSION

The studied results indicated that shrimp farmers should eliminate all carriers of TSV, such as shrimp and crabs from rearing ponds and allow water to sustain at least 15 days before releasing shrimp into the pond.

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LITERATURE CITED

Flegel, T.W., S. Boonyaratpalin and B. Withyachumnarnkul. 1997a. Progress in research on yellow-head virus and white-spot virus in Thailand, pp. 285-296. *In* T.W Flegel. and I. MacRae (eds.). **Diseases in Asian Aquaculture III**. Fish Health Section, Asian Fisheries Society, Manila.

Flegel, T.W., S. Sriurairatana, C. Wongteerasupaya, V. Boonsaeng, S. Panyim and B. Withyachumnarnkul. 1997b. Progress in Characterization and Control of Yellow-Head Virus of *Penaeus monodon*, pp. 71-78.

In **Shrimp Biotechnology in Thailand**.

Flegel, T.W., L.Nielson and W. Sang-oum. 2003. **Outbreaks of Taura Syndrome Virus (TSV) with Exotic *Penaeus vannamei* Cultivated in Thailand JSPS-NRCT International Symposium**. Faculty of Fisheries, Kasetsart University, Bangkok, Thailand.

Garza, J.R., K.W. Hasson, B.T. Poulos, R.M. Redman, B.L. White and D.V. Ligthner. 1997. Demonstration of infectious Taura syndrome virus in the feces of sea gulls collected during an epizootic in Texas. **J. Aquat. Anim. Health** 9: 156-159.

Lightner, D.V. 1996. **A Handbook of Pathology and Diagnostic Procedures for Diseases of Penaeid Shrimp**. World Aquaculture Society, World Aquaculture Society.

Lotz,J.M., C.L. Browdy, W.H. Carr, P.F. Frelier, and D.V. Lightner. 1995. USMSFP suggested procedures and guidelines for assuring the specific pathogen status of shrimp broodstock and seed, pp. 66-75. *In* C.L. Browdy and J.S. Hopkins (eds.) **Swimming through Troubled Waters**. Proceedings of the Special Session on Shrimp Farming. World Aquaculture Society, Baton Rouge, LA.

Lotz, J.M., A.M. Flowers and V. Breland. 2003. A model of Taura syndrome virus (TSV) epidemics in *Litopenaeus vannamei*. **J. Invertebr. Pathol**. 83: 168-176.