Detection and prevention of WSSV infection in cultured shrimp

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Outbreaks of white spot syndrome virus (WSSV), causing serious mortality to cultured shrimp, have been occurring in Asia since 1992. In order to prevent WSSV infection in cultured shrimp, we have been investigating detection and inactivation methods for WSSV in shrimp and other crustacea and ways to eradicate virus-carrying crustacea in shrimp culture ponds.

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WSS is a viral disease affecting most of the commercially cultured marine shrimp species in the world. WSSV infection is characterised by a rapid disease onset and high mortalities. The principal clinical sign of WSSV is the presence of white spots on the carapace (fig.1). The body colour of diseased shrimp becomes pale or reddish in colour (Takahashi *et al.*, 1994). The histopathological feature of WSS is the hypertrophied nuclei of curticular epidermis, connective tissue, lymphoid organ, antennal gland, hematopoietic tissue and nervous tissue (Momoyama *et al.*, 1994).

The causative agent of WSS is a rod-shaped virus (fig. 2), (Takahashi *et al.*, 1994, Inouye *et al.*,1994, Chu*et al.*, 1995, Lo *et al.*, 1996).

Detection of WSSV

In order to detect WSSV in the carrier state shrimp and other crustaceans, we designed a nested primer for a two-step PCR for epidemiological and carrier studies on shrimp in the natural environment (Takahashi *et al.*, 1996, Maeda *et al.*, 1998). We examined 374 wild caught kuruma shrimp, *Marsupenaeus japonicus* female spawners in Japan. The heart of each shrimp was used for the extraction of DNA as a template for the two-step PCR. Virus positive ▷

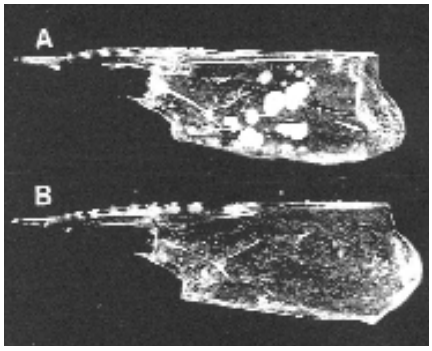

Figure 1: White spot on the removed carapace of diseased shrimp (A) and healthy shrimp (B).

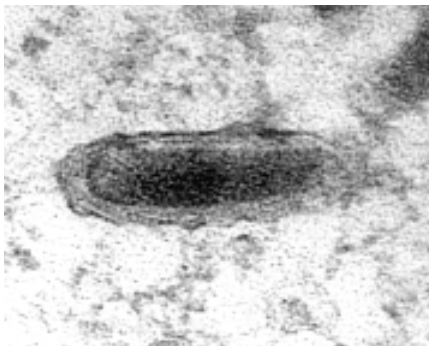

Figure 2: The causative agent of WSS is a rod-shaped virus.

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Table 1: Two-step PCR detection of WSSV in the wild-caught kuruma shrimp.

Shrimp	Body weight (g)	No. of PCR positive/No. tested	Positive rate (%)
Female spawners	71.0 – 140.0	91/374	24.3*
Immature female	13.3 – 28.0	18/132	13.6

*Significant different ($p < 0.05$)

Table 2: PCR detection of WSSV and IHHNV from parent shrimp of *Litopenaeus vannamei* in Panama.

	No. of PCR positive/No of tested %	
Sex	WSSV	IHHNV
Male	6/69 (8.7)	411/480 (85.6)
Female	7/63 (11.1)	397/460 (86.3)
Total	**13/132 (9.8)**	**808/940 (86.0)**

◁ rate by PCR was 24.3% (table 1).

The filtrate of the heart homogenate of the virus carrying shrimp killed healthy shrimp through the injection challenge. When we examined the hearts of the immature shrimp (body weight 13.3-28.0g) that were collected from the natural environment, 13.6% of the samples were virus positive by PCR (table 1).

These results suggest that the wild population of kuruma shrimp have been infected with WSSV. In this experiment, we found that the incidence of WSSV among wild caught females was significantly lower in immature females (13.6%) than in spawners (24.3%) ($P<0.05$). Interestingly, the frequency of the virus-positive shrimp increased in the mature shrimp.

However, it is not clear whether this was due to the sexual maturation process or to multiple spawning behaviour. In Panama, WSSV and infectious hypodermal and hematopoietic necrosis (IHHN) are now widespread, and we detected WSSV and IHHN virus (IHHNV) in white leg shrimp, *Litopenaeus vannamei* female spawner and male. WSSV and IHHNV positive rate were 9.8% (female: 11.1%, male: 8.7%) and 86.0% (female: 86.3%, male: 85.6%) respectively (table 2).

Thus, we would not recommend the import of the white leg shrimp from the Americas to Asian countries as it has a high rate of IHHNV positives.

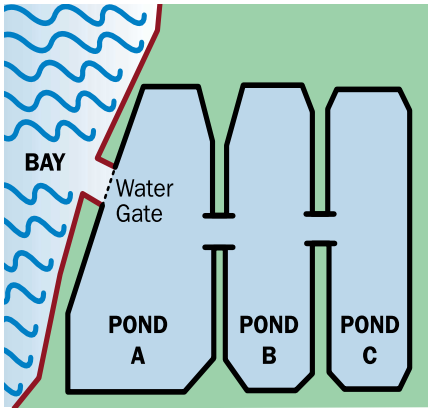

Figure 3: The culture ponds and storage pond in shrimp farm.

Fourteen species of crustacea were collected in shrimp ponds and examined by the two-step PCR (table 3)(Maeda *et al.*, 1998). We found that five species of shrimp and six species of crabs showed a positive reaction. The shore crab, Helice tridens showed an especially high rate of positive reactions (66.7%, 26 out of 39 specimens). Healthy kuruma shrimp were placed in a holding tank with untested shore crabs part of which were proven to be infected with WSSV when examined by the two-step PCR. All shrimp died in 28 days and 60% of the crabs died in 30 days.

Inactivation of WSSV

The inactivation of WSSV by chemicals, temperature and drying were examined (Nakano *et al.*, 1998, Maeda *et al.*, 1998). The pathogenicity of the treated virus was determined by injecting it into shrimp. The virus was inactivated by exposure to sodium hypochlorite at a concentration of 1ppm for 10 minutes. Povidone-iodine inactivated the virus at a concentration of 2.5ppm for 10 minutes. The virus lost its pathogenicity by heating at 50°C for 20 minutes in sterile seawater and by a drying treatment at 30°C for one hour. The virus was sensitive to ethyl ether and chloroform.

The virus suspended at a high concentration in sterile seawater maintained its pathogenicity for 120 days at 25ppt and for more than 120 days at 4ppt. However, the virus suspended at a low concentration (a 10˙7 dilution of the above solution) maintained its pathogenicity for only 10 days at 25ppt and for only 15 days at 4ppt.

Based on the above mentioned results, in order to prevent WSSV infection in cultured shrimp and shrimp larvae, we disinfected culture ponds, tanks and materials in the

Table 3: Detection of WSSV by two-step PCR in crustacea collected inside or outside shrimp ponds.

		No. of PCR positive/No. tested	
Species	Date of sampling	Inside	Outside
Alpheus brevicristatus	Nov 1995	2/4	
Alpheus lobidens	Oct 1996		1/8
Palaemon serrifer	Oct 1996		1/1
Penaeus semisulcatus	Nov 1995	2/5	
Charybdis japonica	Oct 1996		1/4
Gaetice depressus	Oct 1996		0/2
Helice tridens	Sep 1996	26/39	
	Oct 1996	2/22	0/1
	Nov 1996	1/10	
Hemigrapsus sanguineus	Jun 1996	3/4	
	Sep 1996	6/8	
	Oct 1996		0/1
Leptodius exaratus	Oct 1996		0/2
Ocypode stimpsoni	Nov 1995	1/4	
Parasesarma pictum	Oct 1996		0/1
Petrolististhes japnicus	Oct 1996		1/1
Portunus trituberculatus	Nov 1995	1/8	
Upogebia major	Jan 1996	1/12	

hatchery with sodium hypochlorite (20ppm for one day). We disinfected the spawns by applying 5ppm of iodine for five minutes.

Eradication of crustacea in shrimp farms

First, we applied sodium hypochlorite at a concentration of 50ppm to culture ponds for three days to eradicate crabs living at the bottom of the culture ponds. Next, to eradicate crustacea and crustacean larvae in the breeding seawater, we applied sodium hypochlorite at a concentration of 25ppm for one day to the water in pond A. Then, the disinfected seawater was introduced into ponds B and C (fig. 3). In addition, in Panama, we set up a fence around the culture ponds and storage pond to prevent the invasion of wild crabs. ■

Figure 4: Pink coloured bands on positive and negative results.

A reference list is available on request.

Detection of WSSV by ImmunochromatoKit

We have developed a very easy and rapid detection kit for WSSV in shrimp pond. EnBio Shrimp Virus Detection kit, named Shrimple is a lateral flow, one step and immunoassay for WSSV detection in shrimps. It is fast and easy.

The results are read visually without any instruments. The test system employs unique monoclonal antibodies to selectively identify WSSV in shrimps with a high degree of sensitivity.

This kit is based on the principle of a sandwich immunoassay. The test device consists of a membrane strip and plastic case. A coloured monoclonal rat anti-WSSV colloid gold conjugate (antibody-colloid gold conjugate) pad is placed next to the sample pad region on the membrane strip. The membrane strip is also pre-coated with different monoclonal rat anti-WSSV on the test (T) zone and anti-rat IgG on the control (C) zone.

In the absence of WSSV in the shrimp, the antibody-colloid gold conjugate moves with the sample by capillary action along the membrane through the T zone until it reaches the C zone. At this point, antibody-colloid gold conjugate reacts with the pre-coated anti-rat IgG to produce a visible pink coloured band. When only one pink coloured band appears in the C zone, it means negative.

When the WSSV is present in the shrimp, the antibody-colloid gold conjugate reacts with WSSV to produce antibody-WSSV complex and moves until it reaches the T zone. At this point, the complex reacts with the pre-coated different monoclonal rat ant-WSSV to produce a visible pink colored band. An excess antibody-colloid gold conjugate is still free. It moves through the T zone until the C zone. At this point, an excess antibody-colloid gold conjugate reacts with the pre-coated ant-rat IgG to produce a visible pink colored band. When a pink colored band appears in both T zone and C zone, it means positive (fig 4).

Detection Experience

The sensitivity of this kit is between that of a normal PCR and nested PCR (table 4 and 5). Results with the kit have been confirmed for japonicus, chinensis and monodon shrimps.

When we visited a shrimp farm in Surathani, Thailand in May 2003, we carried out tests on shrimp (PL45) that look healthy without white spot on the carapace. We succeeded in detecting WSSV in these black tiger shrimp using the kit. On the following day, the farmer of the pond was then able to harvest his shrimp before more damage occurred. Thus, we propose that shrimp management now needs rapid methods of disease detection to reduce economic losses. An early finding means that infections and damage can be minimised and output can be.

Table 4: Detection level of WSSV for *Marsupenaeus japonicus.*

	Shrimple	PCR 1st step	PCR 2nd step
Infected live shrimp	57.1% (8/14)	50% (7/14)	100% (14/14)
Infected dead shrimp	100% (60/60)	100% (60/60)	100% (60/60)

Table 5: Detection level of WSSV for *Penaeus monodon.*

	Shrimple	PCR 1st step	PCR 2nd step
Infected live shrimp	96% (24/25)	96% (24/25)	100% (25/25)

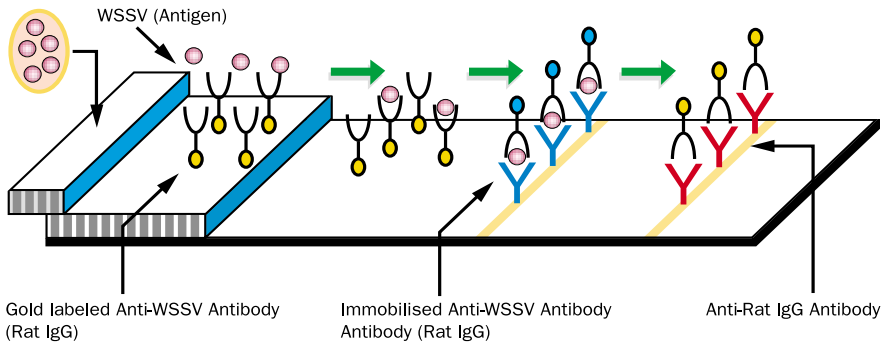

Figure 5: Sample tissue homogenate.