Diagnostic and Preventive Practices for WSSV in Japan

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ABSTRACT

White spot syndrome (WSS), considered equivalent to PAV (penaeid acute viremia) in Japan, has become the most serious problem not only in the farming industry but also in hatcheries for sea ranching of kuruma prawn, *Penaeus japonicus.* The prevalence of WSSV (white spot syndrome virus), the causative agent of WSS, was examined in wild kuruma prawn broodstocks by nested PCR (polymerase chain reaction). As a result, WSSV was detected at the highest prevalence (10.1%) in the ovary of female prawn. This result indicates that spawners are sources of infection. In 1997, brooders were pre-screened using PCR to detect WSSV before these spawned. WSSV was noted to occur in postlarvae obtained from brooders caught between July and August. In 1998 and 1999, eggs were selected based on WSSV detection by PCR from *receptaculum seminis* of spawned broodstock. Consequently, WSSV did not occur in their offsprings in both years. These results strongly indicate that selection of eggs based on PCR results is a practical way of controlling WSSV in hatcheries.

INTRODUCTION

In 1993, a viral disease caused serious mortalities in the shrimp farming industry of western Japan (Nakano *et al*., 1994). This viral disease, affecting the kuruma prawn *Penaeus japonicus*, was called penaeid acute viremia (PAV), and the causative virus was named penaeid rod-shaped DNA virus (PRDV) (Inouye *et al*., 1996). It is considered that PAV and PRDV are equivalent to WSS (white spot syndrome) and WSSV (white spot syndrome virus), respectively (Lo *et al*., 1996; Takahashi *et al*., 1996). In Japan, WSSV has damaged the farming industry of other shrimp species, as well as the shrimp hatcheries used in sea ranching (Momoyama *et al*., 1997; Satoh *et al*., 1999). In Southeast Asian countries, WSSV was reported in kuruma prawn, black tiger shrimp *P. monodon,* redtail shrimp *P. penicillatus,* and Chinese prawn *P. chinensis* (Chou *et al*., 1995; Peng *et al*., 1995; Wongteerasupaya *et al*., 1995; Lightner, 1996). The present study focused on the epizootiology of this viral disease in both seed production and nursery culture farms from 1996 to 1999. It was found that elimination of eggs from WSSV-positive spawners by polymerase chain reaction (PCR)-based technique was an effective control measure against WSSV in seed production.

MATERIALS AND METHODS

Broodstock and collection of eggs

Adult kuruma prawn, caught in coastal waters of Kyushu and Shikoku islands in Japan, were purchased from dealers and used as spawners between May and September 1996, and between April and August 1997. Transport time from collection areas to the Station of the Japan Sea-Farming Association (JASFA) was 4-10 h.

In 1996, 300-600 spawners were transferred in a 1.6-ton tank filled with sand-filtered seawater. In 1997, 1-7 spawners were placed in a 10 L container filled with UV (30,000 $\mu W/m^3$) - sterilized water. The water temperature in the tank was kept at 20-25°C in 1996 and 15°C in 1997, to suppress breeding of the spawners. Upon arrival at the Station, the spawners were immediately placed in a 35-ton spawning tank and maintained at 23-27°C for 1-3 days to induce spawning. In 1997, the ovaries (0.1 g) of all spawners were sampled using a disposable syringe (3.0 ml; needle, 19G), and WSSV was detected individually by PCR. Only spawners with WSSV-negative ovaries (5-12 individuals) were placed in the spawning tank at 24-28°C to induce spawning.

In 1998 and 1999, 5-18 non-biopsied individuals were stocked separately in 0.5 m^3 spawning tanks at 23-25°C to induce spawning. The *receptaculum seminis* was sampled for WSSV detection by PCR from the spawned broodstocks. Based on the PCR results of the *receptaculum seminis*, only the eggs from WSSV-negative spawners were used in the rearing experiments.

The fertilized eggs in the 1996 spawning were washed with clean filtered seawater, while the eggs during the 1997-1999 spawnings were disinfected with 5mg/l povidone-iodine for 5 min. The eggs were temporarily stocked in 200 L rearing tanks until the end of the process of WSSV detection by PCR. Only the eggs from WSSV-negative spawners were reared in rearing tanks (150-2,500 ton). The water temperature was kept at 24-28°C.

Seed production

Filtered and UV-sterilized seawater was used for seed production in 1996-1999. The hatched nauplii were fed with *Tetraselmis tetrathele*, nauplii of *Artemia salina* and commercial formulated feed. From the hatchery, the shrimp were transferred to nursery facilities and stocked in concrete rearing tanks where water temperature was maintained between 25-28°C. For WSSV detection by PCR, samples were collected at egg, nauplius, zoea, mysis, and postlarval (PL) stages (egg: 0.05 mg, mean body weight; PL1: 1 mg; PL5: 1.5 mg; PL10: 3 mg; PL20: 12 mg; PL30: 30mg; and PL40: 100 mg). In each sampling stage up to PL10, the total amount of the sample was 0.1 g. From PL20-PL40, 30 PL were collected at each sampling time. In nursery facilities, samples were collected at 10-day interval.

Detection of WSSV from wild adult broodstock

Broodstock were captured in five different areas (central Honshu, Shikoku, and Kyushu) of the coastal waters of Japan from July 1996 to April 1998. Samples of hemolymph, stomach, and gonad of wild female (955 individuals) and male (314 individuals) prawn were submitted to WSSV detection by PCR as described below. The mean body weight of females and males were 78.1-105.5 and 44.7-63.9 g, respectively. The hemolymph was collected using a syringe (1 ml; needle, 26G), and 500 μl was mixed with phosphate buffer saline (PBS, pH 8). The stomach and gonad samples were aseptically extracted at a volume of 100 μg, and stocked at -80°C until the PCR analysis.

DNA extraction and detection of WSSV by PCR

The hemolymph, stomach, gonad, *receptaculum seminis*, and whole body of juveniles were individually homogenized and digested with ISOGEN (Japan Gene Co.). The total DNA was amplified using two specific primer sets, P1/P2 and P3/P4 (Kimura *et al*., 1996). After 30 cycles amplification for each primer set at 93°C (60 sec), 57°C (90 sec), and 72°C (60 sec), the amplified products were analyzed by agarose gel electorophoresis. A known WSSV-infected *P. japonicus* was processed as a positive control.

RESULTS

Occurrence of WSSV in seed production

In 1996, WSSV was detected in 4 out of 9 seed production runs, thus the larvae were discarded (Table 1). The developmental stage in which WSSV was first detected by PCR was in eggs, followed by PL5 and PL10, when the water temperature was 22.1-29.4°C. In the other seed production runs, WSSV was detected in PL29 and PL51 at the nursery stage. High mortality rates reaching 50-100% occurred within 10 days in the nursery facilities after detection of WSSV. Moribund juveniles showed red coloration and discoloration of the body, and white spots on the carapace. In 1997 when selection of spawners was based on WSSV detection from ovaries before spawning, WSSV was not detected in a total of 9 seed production trials and 17 nursery cultures (Table 2).

Table 1. Detection and occurrence of white spot syndrome virus (WSSV) in *Penaeus japonicus* in 1996 at Japan Sea Farming Association (JASFA)

Trial No.	Spawners		Detection of WSSV in seed production (eggs to PL20)*2	Occurrence of WSSV	
	Captured date	PCR test*1		In seed production	In nursery culture
1	May 24, 25	-	-	-	- (0/4)*3
2	June 25	-	-	-	- (0/1)
3	July 12	-	-*4 (PL51)*4	-	+ (3/3)
4	July 16, 17	-	-	-	- (0/3)
5	July 24	+	-*4 (PL29)*4	-	+ (5/5)
6	Sept. 3	+	+ (Eggs)	+ (Discarded)	Not done
7	Sept. 4	+	+ (Eggs)	+ (Discarded)	Not done
8	Sept. 12	+	+ (PL10)	+ (Discarded)	Not done
9	Sept. 17	-	+ (PL5)	+ (Discarded)	Not done

*1 Detection of WSSV in the cuticular epidermis of stomach of spawners after spawning by 2-step PCR

*2 Detection of WSSV in larvae and postlarvae sampled during seed production. The stage of prawn in parenthesis represents the developmental stage when WSSV was first detected by 2-step PCR

*3 Number of WSSV cases recorded/conducted

*4 WSSV was detected later in juveniles which were reared in the hatchery after their siblings were transferred to nursery facilities

Table 2. Detection and occurrence of white spot syndrome virus (WSSV) in *Penaeus japonicus* in 1997 at Japan Sea Farming Association (JASFA)

Trial	Spawners	Detection of WSSV from postlarvae*1	Occurrence of WSSV	
	Captured date	PCR test	In seed production	In nursery culture
1	April 11	-	-	- (0/1)*2
2	May 12, 13	-	-	- (0/1)
3	May 16	-	-	- (0/4)
4	May 17	-	-	- (0/1)
5	May 12, 13	-	-	- (0/2)
6	June 11, 12	-	-	- (0/3)
7	July 17	-	-	- (0/3)
8	July 19	-	-	- (0/1)
9	July 22	-	-	- (0/1)

*1 Detection of WSSV in postlarvae just before transport to nursery culture facility
*2 Number of WSSV cases recorded/conducted

Detection of WSSV *from wild adult prawns*

The prevalence rate of WSSV showed high values: [ovary (10.1 %)]>[stomach (7.3 %)]>[hemolymph (5.8 %)] in females as shown in Fig. 1. In males, WSSV was detected in the stomach (6.7 %) and spermatheca (4.8 %), but not in the hemolymph (Fig. 1).

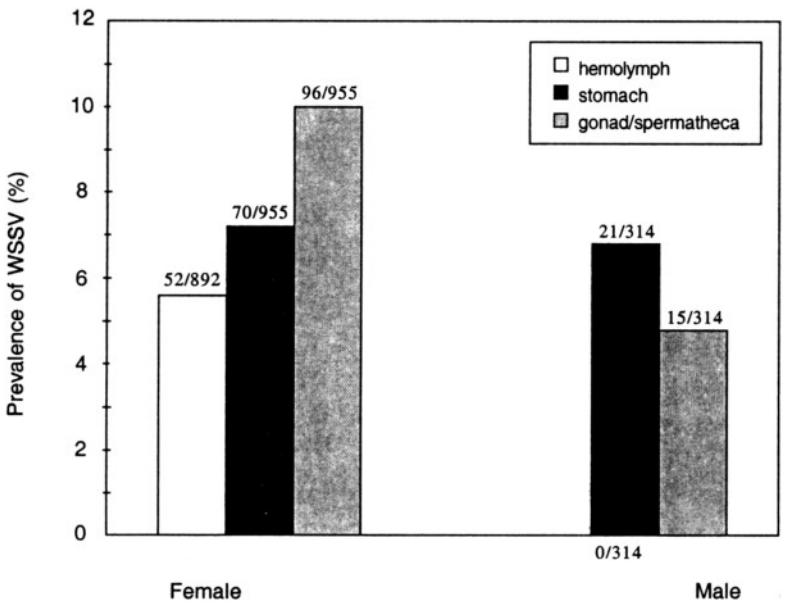

Figure 1. Prevalence of white spot syndrome virus (WSSV) detected by polymarase chain reaction (PCR) in wild adult Kuruma prawn (*Penaeus japonicus*) captured at 5 different coastal waters from 1996 to 1998

Detection of WSSV before and after spawning

The results of WSSV detection in the ovary and *receptaculum seminis* of spawners before and after spawning are shown in Table 3. The prevalence of WSSV in the ovary was 0.9% in 1997, and 0% in 1998 and 1999 before spawning. The values for ovary after spawning were 4.7% in 1997, 0.5% in 1998, and 1.9% in 1999. In the *receptaculum seminis*, the prevalence of WSSV before spawning was 5.6% in 1997, 0% in 1998, and 2.1% in 1999, whereas after spawning the values were 33.5, 6.3 and 10.9%, respectively. Thus, WSSV was detected in the *receptaculum seminis* at a higher rate after spawning than before spawning, and its prevalence increased rapidly from June onwards.

Table 3. Prevalence of WSSV in ovary and *receptaculum seminis* of *Penaeus japonicus* spawners before and after spawning in 1997-1998 at Japan Seafarming Association (JASFA)

Date of purchase		Prevalence of WSSV (%)			
		Ovary		*Receptaculum seminis*	
		Pre-spawning	Post-spawning	Pre-spawning	Post-spawning
1997	April	1.6 (4/248)*1	3.4 (3/87)	NE*2	NE
	May	0 (0/81)	0 (0/40)	NE	NE
	June	0 (0/108)	0 (0/ 37)	0 (0/38)	2.3 (2/86)
	July	0.7 (2/297)	4.0 (4/101)	8.6 (6/70)	39.2 (83/212)
	August	1.3 (3/240)	10.0 (10/100)	5.7 (2/35)	52.6 (41/78)
Total in 1997		0.9 (9/974)	4.7 (17/365)	5.6 (8/143)	33.5 (126/376)
1998	March	0 (0/3)	0 (0/18)	0 (0/3)	0 (0/18)
	April	0 (0/38)	0 (0/587)	0 (0/38)	1.4 (8/587)
	May	0 (0/13)	0.9 (1/111)	0 (0/13)	0.9 (1/111)
	June	0 (0/13)	1.6 (2/122)	0 (0/13)	13.9 (17/122)
	July	0 (0/108)	1.4 (2/148)	0 (0/21)	24.3 (36/148)
Total in 1998		0 (0/175)	0.5 (5/986)	0 (0/88)	6.3 (62/986)
1999	March	0 (0/15)	0 (0/181)	0 (0/15)	0 (0/181)
	April	0 (0/10)	0 (0/262)	0 (0/10)	0 (0/262)
	May	0 (0/5)	0 (0/39)	0 (0/5)	0 (0/39)
	June	0 (0/10)	6.8 (5/74)	0 (0/10)	41.9 (31/74)
	July	0 (0/7)	0 (0/15)	14.3 (1/7)	56.1 (37/66)
Total in 1999		0 (0/47)	1.9 (12/622)	2.1 (1/47)	10.9 (68/622)

*1 PCR positive/examined
*2 Not examined

DISCUSSION

In the 1996 production run, WSSV did not occur until PL20. However, WSSV was detected after the shrimps were transferred to the nursery farms. In 1997 after spawner selection and disinfection of eggs with iodine as WSSV control measures, WSSV was not detected in both seed production and nursery phases of culture. From these results, the major infection route of WSSV in seed production was considered to be a vertical transmission (Satoh *et al*., 1999).

From the results of WSSV detection by PCR in wild adult kuruma prawn, the stomach is not a suitable target organ for WSSV detection. But the ovary or *receptaculum seminis* of spawners is. It was also shown in the present study that the use of eggs from WSSV-negative spawners was an effective way of controlling WSSV in seed production. Moreover, WSSV was detected in the *receptaculum seminis* at a higher rate after spawning than in ovaries before spawning (Mushiake *et al*., 1999). Therefore, the use of *receptaculum seminis* is recommended as the target organ for WSSV detection for broodstock selection. Since 1998 when these procedures were adopted, WSSV has not occurred in the seed production of kuruma prawn at JASFA for the past 3 years (Table 4). These measures are effective.

Table 4. Occurrence of white spot syndrome virus (WSSV) in *Penaeus japonicus* in 1996 to 1999 at Japan Sea Farming Association (JASFA)

Year	Selection of spawners*1	Disinfection treatment of eggs	No. of success/ total conducted
1996	Not done	Not done	6/14
1997	From ovary (before spawning)	Iodine	21/23
1998	From R. S.*2 (after spawning)	Iodine	11/11
1999	From R. S. (after spawning)	Iodine	16/16

*1 Selection based on PCR results
*2 R.S. : *receptaculum seminis*

The increase in the WSSV detection rates from wild-captured kuruma prawn in summer (July to August) corresponds with the result of *P. monodon* (Lo *et al*., 1996). This was probably caused by stress due to multiple spawnings between March and September. The possibility that multiple spawnings could induce viral multiplication in the host has been shown in viral nervous necrosis of the striped jack *Pseudocaranx dentex* (Mushiake *et al*., 1994). There is no data identifying a similar phenomenon in kuruma prawn, however, the possibility might exist because the spawning season of kuruma prawn ranges between April and October and this species repeats copulation and spawning several times in one season.

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