Prevalence of white spot syndrome virus (WSSV) in the cultured shrimp *Penaeus indicus* along the coast of Bushehr Province

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Abstract:

During the June until September 2005 two hundred shrimp ***Penaeus indicus*** were collected from different culture site (Bovyerat, Heleh, Delvar and Mond) along the coast of Bushehr province. The Nested polymerase chain reaction (PCR) and clinical sign of white spot disease (WSD) which consist the white spot in the cuticle and reddish discoloration of the body was employed for screening the samples for white spot syndrome virus. The two hundred shrimp *penaeus indicus* samples were check for WSSV by two methods. The results indicate the 2% of shrimp *penaeus indicus* showed the clinical sign of white spot disease (WSD) and 92% of them showed the PCR positive for WSSV and carry the virus. The results showed the prevalence of this virus was 92% in the Busheher province. The virus was also found to be present in the 70% apparently healthy shrimp by PCR and they can carry the WSSV.

Key words: Prevalence, White spot syndrome virus, *Penaeus indicus*, Nested PCR, Clinical sign.

Introduction

White spot disease is one of the most important shrimp diseases in the world. It is affect most of the commercially cultured shrimp species (Inouye *et al*., 1994; Spann and Lester, 1997; Tokhmafshan *et al*., 2004).

The clinical signs of this disease include white spots in the carapace and six abdominal segments, the hepatopancreas was swollen and yellow, the intestine and abdomen was empty and the body color of infected shrimp become reddish. The gathering of affected shrimp around the edge of the pond throughout the day and during three to ten days 70 to 90 percents of shrimp died (Tokhmafshan *et al*., 2004; Wang *et al*., 1995; Lightner, 1996).

The moribund shrimp under histopathology from all tissues except hepatopancreas showed intranuclear Cowdry type-A inclusion body and under transmission electron microscopy the causative agent of WSD is an enveloped, non-occluded, rod shaped DNA virus and the average size is 300±75 nm (Afsharnasab and Akbari, 2004; Lo *et al*., 1997).

The penaeid shrimp production in Iran has grown rapidly during past decade. The total production of Iranian shrimp farming was about 9000 tons in 2004 and estimated the production will be reach 14000 tons in 2005 (Iranian Fisheries Report, 2004).

The presence of WSSV has been described in a wide range of captured and cultured crustaceans and other arthropods, including wild crab (*Calappa lophos*, *portunus sanguinolentus*, *Charybidis sp. Helice tridens*), wild lobster (*Panulirus* sp.), palamonidae pest shrimp, (*Exopalamon orientalis*), copepods, pupae of Ephydried insects, cray fish (*Orconectes punctimans* and *Procambus clarkia*) pest crab (*Sesarma pictum*), mud crab (*Scylla serrata*), krill (*Acetes* sp.) and many other

marine crustaceans (Lo *et al*., 1996; Chang *et al*., 1998;Supamattaya *et al*., 1998; Hossain *et al*., 2001).

Until the middle of the year 2005, the occurrence of WSD in Iran has been limited to Khuzestan province (Tokhmafshan and Tamjidi,2002; Tokhmafshan *et al*., 2004). However, in the June 2005 a sudden and high mortality occurred in the Busheher province, which is the largest shrimp producing province of Iran. The used of cultured broodstock for the hatchery for producing the post larvae for next season is one of the most important part of cultured industry. The cultured broodstock serving as hosts for WSSV may act as reservoirs in the environment. The wild crustaceans such as crab also play an important role as a carrier of WSSV in natural environment. The present study was conducted to investigate and identify the information regarding the presence, distribution and the prevalence of WSSV in shrimp ***Penaeus indicus*** in the coast of Busheher province.

Material and Methods

During June until September 2005 two hundred shrimp ***Penaeus indicus*** was collected from different shrimp farming site in Busheher province (Figure 1 and Table 1).

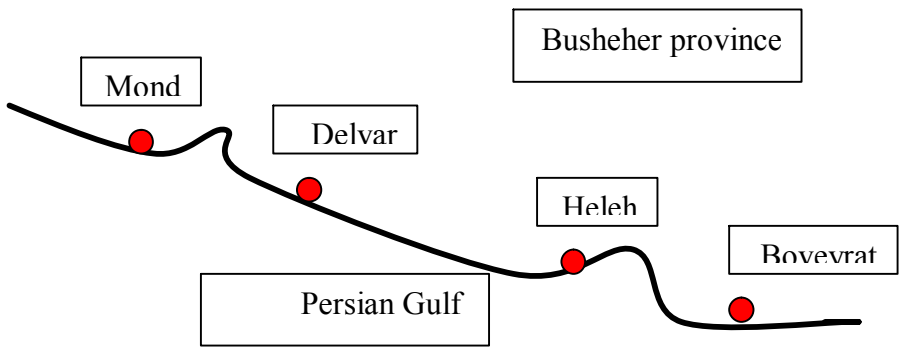

Figure 1. Map showing shrimp farming site in Busheher province

Table 1. Location, source and number of sample in different farm site.

Location	Name of farm	Shrimp (*P.indicus*)	Date
Boveyrat	Abpakhsh	11	2/6/05
	Hasanpoor	11	5/7/05
	Shakhak telaey	12	12/8/05
Heleh	Fathey	12	2/6/05
	Forotan	7	2/6/05
	Hdayat	9	4/6/05
	Kaviani	12	8/6/05
	Rostemyan	11	18/6/05
	Ghodcy	12	24/7/05
	Shirshekan	10	10/8/05
	Boushehr	11	14/8/05
Delvar	Ghnadyan	11	5/6/05
	Shrammygo	10	13/7/05
	Mysagh	12	6/8/05
	Delvar2	8	3/9/05
Mond	Dashtmond	11	8/6/05
	Abzymhatab	9	22/7/05
	Hageb	10	16/8/05
	Rafei	11	4/9/05
Total		200	

The samples were first check for clinical sign as described by Lightner (1996), Tokhmafshan and Tamjidi (2002) and Tokhmafshn *et al*.(2004) and then dipped in ethanol 95% for Nested Polymerase Chain Reaction (PCR). Tissue of shrimp (5 to 10 pieces) put into a 1.5 ml tube with 500 µl Extraction solution. The samples ground in the tube with a disposal grinder and stand in room temperature for five minute. Add 100 µl of $CHCL_3$ then vortex 20 seconds. Site in room temperature for three minute, then centrifuged it at 12000 g (12000 rpm) for 5 minutes. Two hundred µl of upper clear aqueous phase transferred to a fresh 0.5 ml tube with 400 µl 95% or absolute ethanol. The supernatant vortex briefly,

centrifuged at 12000 g for 10 minutes, then decanted and dried the pellet. The pellet was finally dissolved in 200 µl DEPC ddH_2O and used for future analysis. The primer used in this study was employed from IQ200 ™ WSSV Detection and Prevention System from Farming intelliGene Tech. Corp. About 2 µl of extracted samples were added to 15 µl of Nested PCR reaction mixture for DNA amplification. Thermo cycling profile for Nested PCR at the first reaction profile were: 94^0C for 30 seconds, 62^0C for 30 seconds, 72^0C for 30 seconds, repeat 5 cycles, then add 94^0C for 15 seconds, 62^0C for 15 seconds 72^0C for 20 seconds, repeat 15 cycles and add 72^0C for 30 seconds, 20^0C for 30 seconds at the end of the first PCR reaction. The reaction condition for Nested PCR Profile were 94^0C for 20 seconds, 62^0C for 20 seconds, 72^0C for 30 seconds, repeat 25 cycles, then add 72^0C for 30 seconds at the end of final cycle. The amplified products were electrophorsed in 2% agarose gel containing 1 µg/ml ethidium bromide solution. According to the IQ200 ™ WSSV Kit the sample was diagnosed and interpreted.

The estimated of prevalence was computed using the formula below as described by Natividad and Lightner (1992):

$$\text{Prevalence} = \frac{\text{Number of virus positive sample}}{\text{Total number of samples}} \times 100\%$$

Results

Based on the total number of shrimp examined for clinical sign (Table 2 and Figure 2), the average prevalence of WSD was 22/7% (48/200).The Heleh site showed the highest prevalence of WSD 27/3% (23/84), followed by Mond site and Delvar site where 26/8% (11/41) and 21/9% (9/41) of the total number of shrimp examined had WSD. The Boveyrat

site had the lowest prevalence rate 14/7% (5/34).In terms of clinical observation, remained samples tested for clinical sign appeared to be healthy and the samples did not show any clinical sign of WSD.

Table 2: The observed clinical sign (white spot in cuticle and reddiscoloration) of the total shrimp samples collected for WSSV detection in different farm site.

Name of Farm	Total shrimp examined	Total shrimp with clinical sign	Prevalence (%)
Boveyrat	34	5	14/7
Heleh	84	23	27/3
Delvar	41	9	21/9
Mond	41	11	26/8
Total	**200**	**48**	**22/7**

Figure 2: The clinical sign of WSD in shrimp examined.

The results from Nested polymerase chain reaction showed the average prevalence in all samples is 92% (Table 3 and Figure 3 and 4). The results presented in table 3 and figure 3 and 4 showed the highest prevalence 100% of WSSV in samples (84/84) was in Heleh, the second prevalence 95% of WSSV in samples (39/41) was in Mond, the third prevalence 85% in samples (29/34) and fourth prevalence 78% in samples (32/41) was in Boveyrat and Delvar respectively.

The table 3 and figure 3 and 4 showed the prevalence of WSSV in shrimp samples in Heleh and Mond farms by Nested PCR is very high and all samples were positive for WSSV by Nested PCR and these sites showed the heavily infected and peracute mortality but the results from PCR is not consistence with clinical observation (Table 2). The prevalence of WSSV in shrimp samples examined with Nested PCR in different farm site was 85% for Bovyrat, 100% for Heleh, 78% for Delvar and 95% for Mond respectively (Table 3 and Figure 5).

Table 3. Results of WSSV diagnostic Nested PCR with shrimp *penaeus indicus* in different farm site in Busheher province.

Location	**Total No. tested**	**WSSV Positive**	**Prevalence**
Boveyrat	34	29	86
Heleh	84	84	100
Delvar	41	32	78
Mond	41	39	95
Total	**200**	**184**	**92**

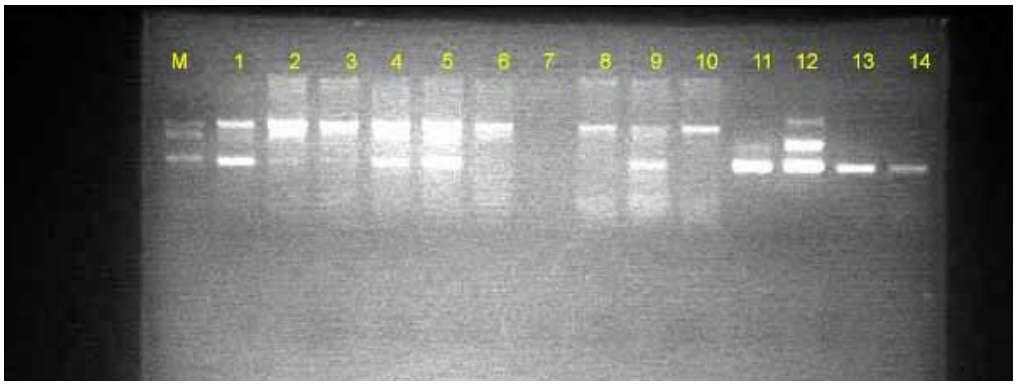

Figure 3: Results from Nested PCR of WSSV detection in different farm site (M = marker, 1,2 positive samples of Bovyrat, 3,4,5 and 6 positive sample of Heleh, 7 negative sample (Water for control) , 8,9 positive sample of Delvar and 10,11 positive sample of Mond, 12, 13 and14 control standard in high, moderate and chronic level).

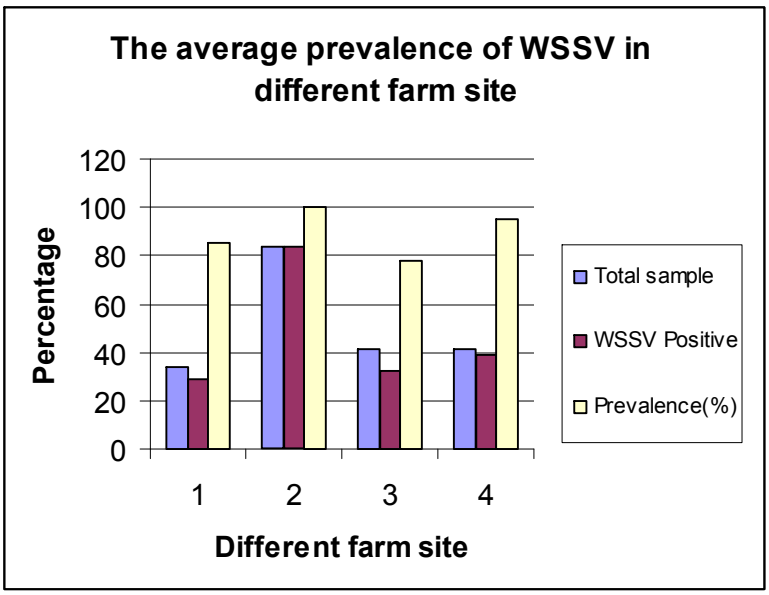

Figure 4: The prevalence of WSSV in different farm sites in Busheher provinc (1= Boveyrat, 2= Helah, 3= Delvar, 4 = Monds).

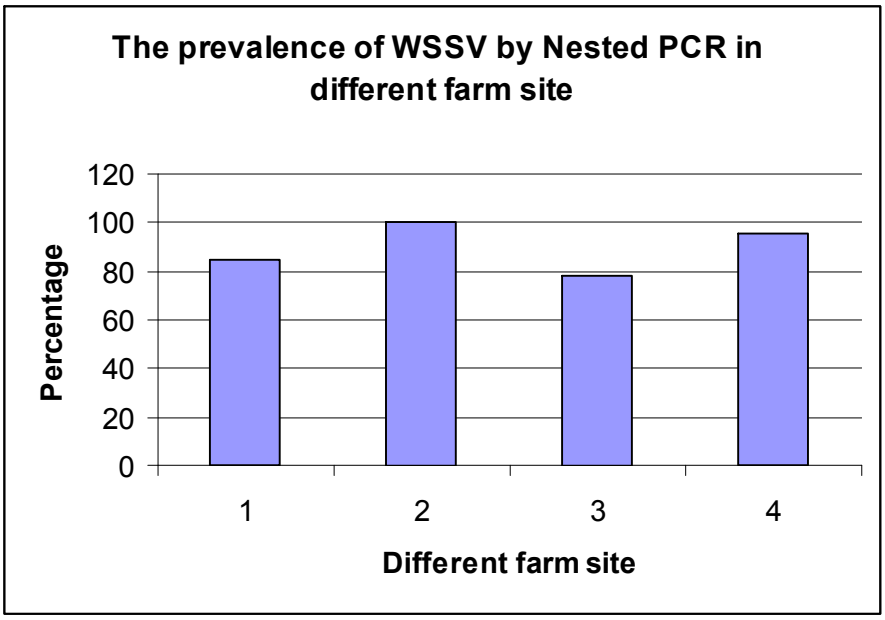

Figure 5: The prevalence of WSSV in shrimp samples in different farm site (1= Boveyrat, 2= Helah, 3= Delvar, 4 = Monds).

Discussion

White spot syndrome virus was the most prevalence virus in shrimp *penaeus indicus* (92%) in accounting for 200 cases. These figures are alarming to both hatchery and growout farmers. The prevalence and distribution of WSSV in samples is a function of complex interaction of several variables, including species, environmental parameter, age and mode of culture.

In this study the prevalence of WSSV by Nested PCR was higher than the clinical observation for shrimp. These result indicated that the screening WSSV in shrimp farming industry will be done by PCR methods and the sensitivity of Nested PCR is about 99% in this case. This finding is consistent with Kou *et al*, (1998) who studied the tissue distribution of white spot syndrome virus (WSSV) in shrimp and crab and reported the PCR is the best methods for screening the samples.

The results from table 2 and table 3 indicated that about 70% and 8% percent of shrimp samples were in the carrier state or in the transition state and did not show any sign of disease. It is important to note that they were not in the patent state (i.e., they had no gross sign of WSSV infection). Although the carrier state might persist for month, the transition state usually lasts for only a few hours and once a sample becomes Nested PCR positive, it will be die within a few days at the most (Lo *et al*., 1998).

Our finding in this study showed some shrimp samples are a reservoir for WSSV and play an important role to transmission of WSSV in shrimp farm without clinical sign of WSD. This finding is also is consistent with Pitogo and Pena (2004) and Chakraborty *et al* (2002), they studied the diagnosis, prevention and control of white spot disease in farmed shrimps and Mud crab species and reported the carrier shrimp and crab do not show any external signs of WSD and molecular techniques is the best methods for diagnostic of WSSV.

This study showed that WSSV is widely distributed in Heleh samples (100%) and Mond samples (95%) and both of these sites are closely near the Heleh river and Mond river and water salinity in both site is lower than from the other sites. The lower salinity in these farms, may be the reason for high prevalence of WSSD with comparing by the other site (Boveyrat and Delvar). Several researchers have studied the effect of different salinity on the infectivity of WSSV. Chang *et al*, (1998) reported that sodium chloride in concentration from 0% to 10% had no virucidal effect on the infectivity of WSSV. However, lower salinity has been reported to prevent the occurrence of WSSV in Taiwan and Thailand (Chang *et al*., 1998).Wang (2000) reported that lower salinity (0 and 2,5 ppt) could successfully prevent WSSV infection or at least reduced severity of the disease. The different between the results from

Chang *et al*, (1998) and Wang (2000) and those observed in our study may be due to different of serotype virus, susceptibility of the different shrimp species, methods of shrimp culture. Overstreet and Matthws (2002) and Jiravanichpaisal (2005) reported the interaction between environmental parameter such as temperature, salinity and pH play an important role to inactivated the WSSV.

This study shows that the crustacean such as shrimp could act as reservoir of WSSV. They could be a source of virus to aquaculture system. Further the results suggest that sensitivity of detection can be improved by designing and choosing specific primer for each species.

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