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Incidence of white spot disease (WSD) in *Penaeus indicus* farms in Bushehr Province, Iran

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Abstract: The incidence of white spot disease in farmed *Penaeus indicus* in Busheher region was evaluated by analyzing 200 shrimp individuals between June and September, 2005. The samples were examined for clinical sign including observation of white spots on the cuticle and tissue. Samples were processed using polymerase chain reaction (Nested-PCR) test. The results showed that 24% of the samples examined were clinically positive having white spots on their cuticles. Also, 92% of the samples examined were positive in PCR examination. Furthermore, 70% of apparently healthy shrimp were positively identified using the by PCR test.

Keywords: Incidence, white spot disease, *Penaeus indicus*, Nested PCR, clinical sign, Iran

Introduction

White spot disease is one of the most important diseases in the shrimp culture (Wang *et al.*, 1999; Mohan *et al.*, 2004; Jiravanichpaisal, 2005). Many of the commercially cultured shrimp species are affected resulting in economically

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significant loses (Inouye *et al.*, 1994; Spann & Lester, 1997; Tokhmafshan *et al.*, 2004) and the spread of the virus has mainly occurred through risky transfer of gravid females and bad management practices without proper quaranteening. The affected specimens usually show clinical signs of white spots on the carapace and along six abdominal segments, edematosed-yellowish hepatopancreas, anorexia, reddish body surface while behaviourally swimming on the water surface is a common feature, particularly on the margins of the ponds during day time. At problems farms the mortality reaches up to 70% to 90% within 3-10 days post-infection (Tokhmafshan *et al.*, 2004; Wang *et al.*, 1995; Lightner, 1996). In histopathological examination, all tissues except hepatopancreas show intranuclear Cowdry type-A inclusion body and under transmission electron microscopy the causative agent is an enveloped, non-occluded, rod shaped DNA virus with average size 300±75nm (Afsharnasab & Akbari, 2004; Lo *et al.*, 1997). The Penaeid shrimp production in Iran has grown rapidly during the past decade. The total production was about 9000 tons in 2004 and estimated to increase further (Iranian Fisheries Report, 2004). The first outbreak of the disease in Iranian farmed shrimp caused a significant loses in Khuzestan province (Tokhmafshan & Tamjidi, 2002; Tokhmafshan *et al.*, 2004). Although attempt was made to eradicate this bankrupting disease from the Iranian farmed shrimp, the second outbreak occurred in June 2005 causing high mortality in Busheher province, the largest shrimp producing region in the country. This work was conducted to investigate some data regarding disease confirmation, distribution and its incidence in farmed shrimp, *Penaeus indicus,* in Busheher province.

Materials and Methods

During June to September 2005, two hundred individuals of *P. indicus* were collected randomly from different shrimp farming sites in Busheher province (Fig. 1 and Table 1).

The samples were first checked for any clinical sign as described by Lightner (1996), and then dipped in ethanol 95% for Nested polymerase chain reaction (Nested-PCR). Tissue samples were taken and stored in 0.5ml tube containing 500µl extraction solutions and then, ground in the tube by a disposal grinder stood in room temperature for five minutes. A volume of 100µl $CHCL_3$ was added, vortexed for 20 seconds, stood for 3 minutes at room temperature, and then centrifuged at 12000g for 5 minutes. A volume of 200µl of upper clear aqueous phase was transferred to clean 0.5ml tube containing 400µl 95-100% ethanol. The supernatant vortex briefly centrifuged at 12000 g for 10 minutes, then decanted and dried the pellet. The pellet was finally dissolved in 200µl DEPC ddH_2O and used for PCR analysis.

About 2µl of extracted DNA of each individual samples was used for PCR amplification using IQ200™ WSV Detection and Prevention System from Farming intelliGene Tech. Corp. The amplified products were electrophoresed in 2% agarose gel containing 1µg/ml ethidium bromide solution. According to the IQ200™ WSV Kit the sample was diagnosed and interpreted. The estimation of incidence was obtained using the equation: Incidence = (number of positive samples/examined samples) × 100 (Natividad & Lightner, 1992).

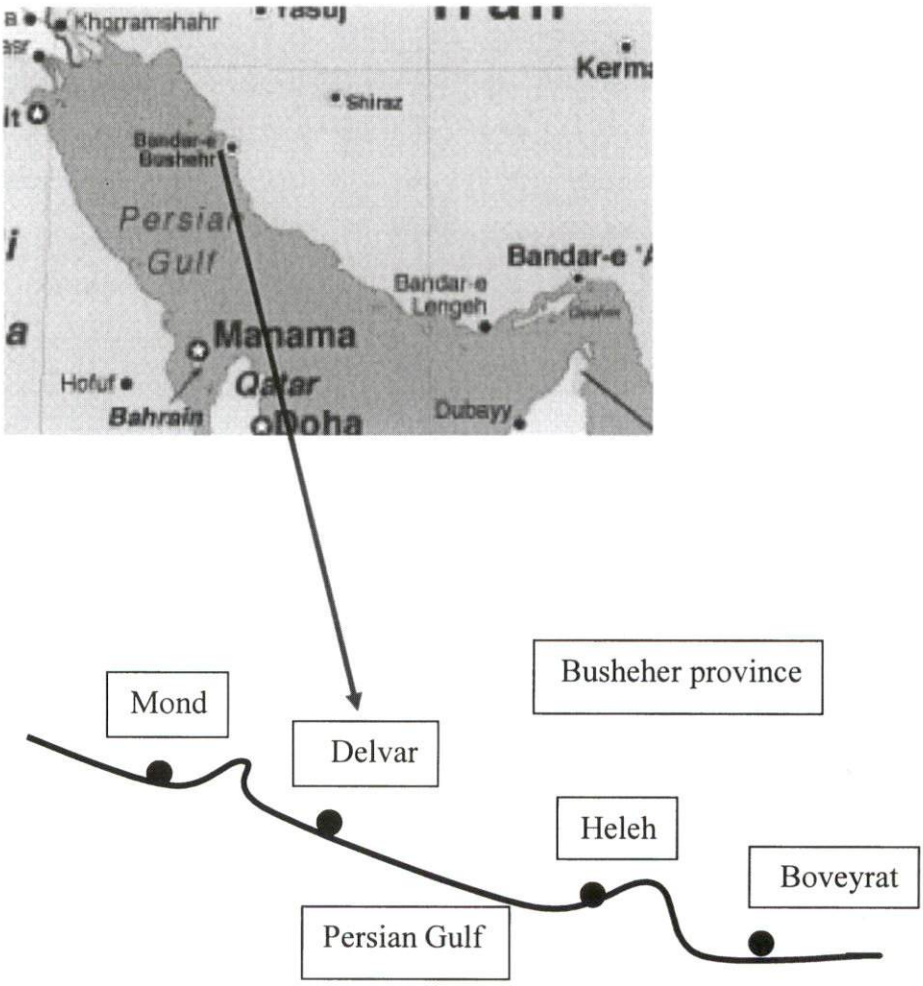

Figure 1: Areas showing shrimp farming sites in Busheher Province from which specimens were sampled for inspection

Table 1: Location, source and number of samples obtained from different farms.

Location	Name of farm	No. of *P. indicus* individuals	Date
Boveyrat	Abpakhsh	11	2/6/05
	Hasanpour	11	5/7/05
	Shakhak Talaey	12	12/8/05
Heleh	Fatehy	12	2/6/05
	Forotan	7	2/6/05
	Hedayat	9	4/6/05
	Kaviani	12	8/6/05
	Rostamyan	11	18/6/05
	Ghodsi	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
	Hajeb	10	16/8/05
	Rafei	11	4/9/05
Total	**19**	**200**	

Results

Based on the total number of shrimp examined for clinical sign (Table 2 and Figure 2), the average incidence of WSD was 24% (48/200).

The Heleh site showed the highest incidence 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 incidence rate 14.7% (5/34). In terms of clinical observation, the remaining samples tested for clinical sign appeared to be healthy and the samples did not show any clinical sign of WSD.

Table 2: Positive WSD samples identified in samples taken during the period June to September 2005 based on observations of clinical signs and Nested – PCR analysis.

Name of Farm	Total number of shrimps examined	Total shrimp with clinical signs (%)	Nested PCR (incidence)
Boveyrat	34	5(14.7)	29(86)
Heleh	84	23(27.3)	84(100)
Delvar	41	9(21.7)	32(87)
Mond	41	11(26.8)	37(95)
Total	**200**	**48(24)**	**84(92)**

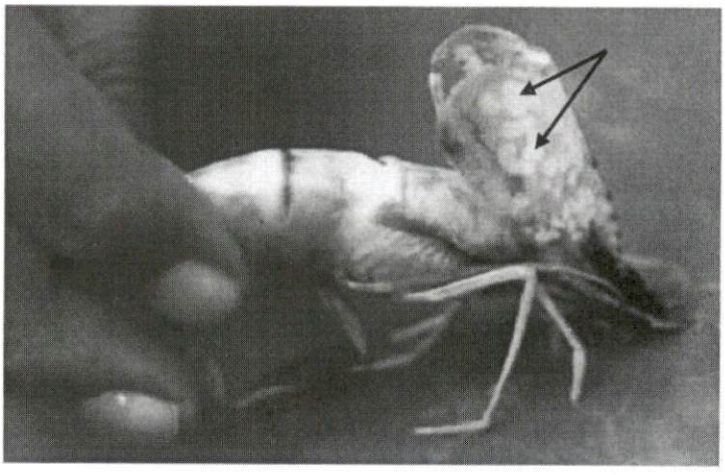

Figure 2: The clinical sign of WSD in shrimp examined showing typical white spots (0.5-2mm) on the carapace (arrows).

The results of Nested polymerase chain reaction showed the average incidence in all samples was 92% (Table 2 and Figure 3). The results presented in Table 2 and Figure 3 show the highest incidence (100%) of WSD in samples (84/84) of Heleh, followed by Mond (95%), Boveyrat (85%) and Delvar (78%), respectively.

The incidence of WSV in shrimp samples in Heleh and Mond farms by Nested PCR was very high and all samples were positive for WSV by Nested PCR and these sites showed heavy infection and mortality (Table 2, Fig. 3), but the results from PCR was not consistence with clinical observation.

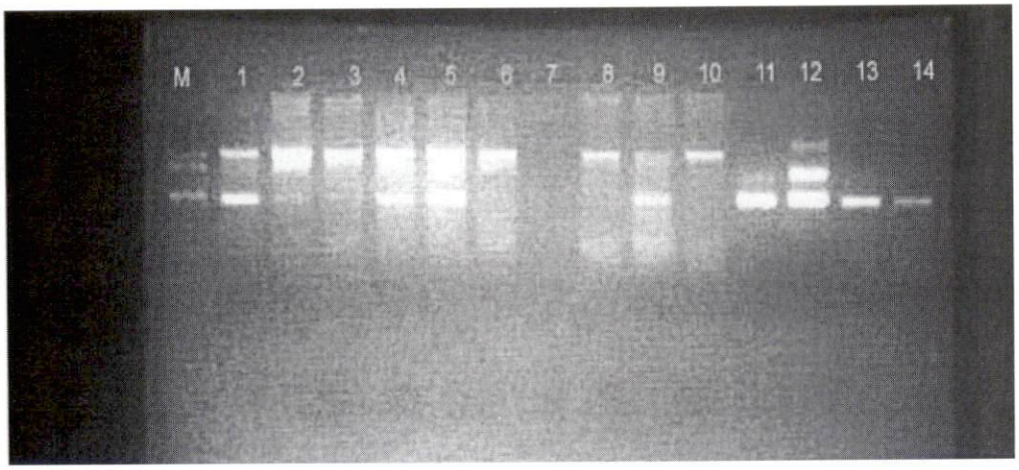

Figure 3: Results of Nested PCR from WSV detection in different farms. (M = marker, 1,2 positive samples of Bovyrat, 3,4,5 and 6 positive sample of Heleh, 7 negative sample (Water for control) , 8,9 positive sample of Delvar and 10,11 positive sample of Mond, 12, 13 and14 control standard in high, moderate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

Discussion

One of the most damaging viral diseases affecting the shrimp aquaculture industry is white spot disease (WSD) caused by white spot virus (WSV), which causes high morbidity and mortality rates in penaeid shrimp. In our study, the incidence of virus was 92% for a total samples size of 200 specimens from four farm sites. These figures are alarming to both hatchery and growout farms. The incidence and distribution of WSV in samples is a function of complex interaction

of several variables, including species, environmental parameter, age and mode of culture and the transfer of gravid females purchased from other areas.

In this study the incidence of WSD by Nested PCR was higher than the clinical observation. The result indicated that the screening WSV in shrimp farming industry should 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 WSV in shrimp and crab and reported the PCR was the best methods for screening the samples.

The results indicated that about 70-80% percent of shrimp samples were in the carrier state or in the transition state (Table 2) while not showing any external sign of the disease. Although the carrier state might persist for a month, the transition state usually lasts for only a few hours and once an individual becomes clinically ill while showing positive results from Nested PCR, it will die within a few days at the most (Lo *et al.*, 1998).

Our finding clearly identify a high proportion of cultured specimens as reservoir for WSV which and would play an important role for transmission of WSV in shrimp farms without necessarily showing clinical signs of WSD. This finding is also consistent with Pitogo and Pena (2004) and Chakraborty *et al.* (2002), who studied the white spot disease in shrimps and mud crab and reported the carrier shrimp and crab did not show any external signs of WSD and molecular techniques were the best methods for diagnostic of WSV.

This study also showed that WSV was widely distributed in specimens from Heleh samples (100%) and Mond samples (95%), both farms being in the vicinity of rivers, where water salinity was lower than the other sites. The lower salinity in these farms might be the reason for a higher prevalence of WSD as compared with the other sites (Boveyrat and Delvar). Several researchers have studied the effect of different salinities on the infectivity of WSV. Chang *et al.* (1998) reported that sodium chloride had no virucidal effect on the infectivity of WSV up to concentrations of 10%. However, lower salinities have been reported to prevent the occurrence of WSV in Taiwan and Thailand (Wang, 2000) where lower salinity (0

and 2.5ppt) seem to have successfully prevented WSV infections or at least reduced the severity of the disease. The difference between the results from Chang *et al.* (1998) and Wang (2000) and those observed in our study might be due to differences in the serotype of the virus, susceptibility of the different shrimp species, and the methods of shrimp culture. Overstreet and Matthews (2002) and Jiravanichpaisal (2005) reported the interaction between environmental parameter such as temperature, salinity and pH which can play an important role in the mechanism to inactivate the WSV.

This study clearly demonstrated that shrimp can act as a reservoir for WSV. Further, the results also suggest that sensitivity of detection can be greatly improved by designing and choosing specific primers for PCR-identification of each species.

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