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Original Article

DIAGNOSIS OF WHITE SPOT SYNDROME VIRUS USING PCR IN EGGS OF MARSUPENAEUS JAPONICUS

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Abstract
Shrimp culture is affected mostly by various pathogens including virus. White Spot Syndrome Virus (WSSV) is one of the most virulent pathogen causing white spot disease and affecting prawn farming in India and all over the World. Penaeid prawns that are infected by WSSV reach mortality within 5-7 days of infection. Various diagnostic methods are employed to diagnose WSSV infected penaeid prawns in India. Polymerase Chain Reaction (PCR) was employed to confirm the presence of WSSV in the present investigation in the eggs of Marsupenaeus japonicus. The egg samples utilized in the present investigation were raised from spawners collected in Pulicat Lake near Chennai on the southeast coast of India. The present study suggests that the infected eggs of Marsupenaeus japonicus possess WSSV in its genome without any appreciable phenotype before maturation.

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Key words: Marsupenaeus japonicus, Spawning season, WSSV diagnosis, PCR, Pulicat Lake.

Introduction:
White spot disease (WSD) is caused by White Spot Syndrome Virus, resulting in the earliest mortality in shrimp population all over the World. In the 1990s, the disease spread rapidly across Asia and reached India in 1994; the disease had a serious economic impact on the shrimp aquaculture industry in affected countries [1]. The white spot disease virus is believed to have been transmitted through seed brought to India clandestinely from Southeast Asian countries, where the virus has been amplified before [2]. During 1994-95, white spot viral disease caused earlier mortality of cultured shrimp Penaeus monodon and P. indicus along the east coast of India [3]. Many shrimps are seasonal breeders and in tropical countries, generally the breeding season lasts from October to February. Environmental factors particularly temperature, photoperiod and salinity play an important role in enhancing breeding and spawning activity [4]. Marsupenaeus japonicus commonly called kuruma shrimp is a marine prawn. It is mainly captured and cultivated in Japan. So, it is called King prawn in Japan. A typical king prawn has a body shape and cross brands on its carapace and abdomen, looking like that of a tiger [5]. Pathogens affect the growth and survival rate of shrimps.

Material and Method:
Collection of Spawners: Mature spawners of Marsupenaeus japonicus are caught by nets and transported alive in oxygenated polythene bags containing seawater to laboratory from Pulicat Lake near Chennai during the spawning period between October 2008 and April 2009. Caught mature spawners were gradually acclimated to laboratory condition by adjusting the water temperature, salinity, pH, dissolved oxygen to ambient. Mature females weighted 130 gm and males weighted 70 gm were stocked in tanks at a ratio of two females and one male. Light intensity was provided by reduced 100 lux and light quality was maintained blue or green colour. For photoperiod, 12 hours light and 12 hours dark period were maintained. Water tank depth was maintained 80-100 mm and the brooders were fed with clam, mussel and squid @ 15% of the total biomass for four times per day.

Collection of eggs: Mature gravid females maintained in the laboratory were transferred to circular fiber glass spawning tanks with 150L of filter seawater (28-30 ppt) with moderate aeration. Water was microscopically examined for eggs [6]. Eggs from the spawners were gently siphoned from the spawning tank into an egg washer using net. The eggs were always kept immersed in seawater and processed gently to avoid mechanical damage [7].

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Fertilized and unfertilized eggs were determined microscopically and the fertilized eggs were used for the PCR test. A group of 500 eggs were randomly selected for each test of every month from October 2008 to April 2009.

**DNA extraction:** The egg sample was transferred to disposable plastic sachet provided in the PCR kit. 800 μl of digestion buffer was added to sachet and the eggs were crushed and incubated at room temperature for 10 minutes. Then homogenate was transferred to 1.5 ml microcentrifuge tube and centrifuged at 5000 rpm for 5 minutes. 150 μl supernatant was transferred to another centrifuge tube and centrifuged at 14000 rpm for 20 minutes. The precipitate obtained was dried and dissolved in sterile distilled water.

PCR was carried out in 30 μl reaction mixture containing 2.0 μl template DNA, 1X assay buffer (10 mM Tris–HCl, pH 9.0; 1.5 mM MgCl2, 50 mM KCl, 0.01% Gelatin), 200 μM of each the four deoxyribonucleotide triphosphates (dATP, dCTP, dTTP, dGTP), 10 pmol of each primers and 0.9 U of Taq DNA polymerase (Bangalore Genei, Bangalore). The primers used and the cycling conditions were described by [8], consisting of 30 cycles of 30 sec denaturation at 94°C, 30 sec annealing at 55°C and 30 sec extension at 72°C. An initial denaturation for 5 min at 94°C and final extension at 72°C for 5 min were provided.

**Detection method:** The amplified DNA (12 μl) was mixed with 6X gel loading dye (2 μl) and run on 2% agarose gel using 1X TAE running buffer. The ethidium bromide (10 mg/ml) stained gel was visualized under UV transillumination.

### Result and Discussion:

The monthly data of WSSV infection in eggs of *Marsupenaeus japonicus* over period of one year from August 2008 to July 2009 are presented in Table 1 and Figure 1. The results show that the infection of WSSV in the eggs of *M. japonicus* breeds only during northeast monsoon reason samples are made available between October 2008 to April 2009 around Pulicat lake in Chennai. In the month of October, breeding of *M. japonicus* is begun when the monsoon season sets on. From his month onwards the tests give positive result up to April, being the end of breeding season. The detection of WSSV was partially positive in the present study shows that the eggs of *M. japonicus* were infected by WSSV. It is suggested that this infection may not be caused by horizontal transmission. It is inferred that transmission of this virus may be through the genome of the mother to the eggs.

WSSV is detected in different larval stages of *M. japonicus*. Broodstock infection of WSSV is detected by using histopathology [9] and nested PCR [10]. Nauplius stage *M. japonicus* affected by WSSV and is detected by PCR method in Japan [11]. *M. japonicus* affected by WSSV in zoea stage is detected using PCR in France [12]. Mysis stage affected by WSSV is detected by PCR [13]. Postlarval [14] and juvenile stage infection of WSSV is detected by histological studies [15] and PCR [16], respectively. Sub-adult stage infection is diagnosed by histopathology [17]. In the adult stage also WSSV is detected by the method of PCR [18], and Enzyme-Linked Immunosorbent Assay (ELISA) (Hameed et al., 1998) and nanotechnology in *M. japonicus* [19].

Therefore, in the present study of diagnosis WSSV in eggs of *M. japonicus* has been undertaken using PCR. The end products of PCR were analyzed in agarose gel and the bands were identified in 210 base pairs by ultraviolet transillumination. The result shows the values of 60 tests were selected for each test of every month from October 2008 to April 2009.

### Table 1. WSSV infection in eggs of *Marsupenaeus japonicus* of the present study

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Period of test</th>
<th>No. test undertaken</th>
<th>No. eggs used</th>
<th>Result +ve Infection (in number)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>August 2008</td>
<td>-</td>
<td>-</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>September</td>
<td>-</td>
<td>-</td>
<td>0</td>
</tr>
<tr>
<td>3</td>
<td>October</td>
<td>7</td>
<td>500</td>
<td>2</td>
</tr>
<tr>
<td>4</td>
<td>November</td>
<td>10</td>
<td>500</td>
<td>5</td>
</tr>
<tr>
<td>5</td>
<td>December</td>
<td>10</td>
<td>500</td>
<td>7</td>
</tr>
<tr>
<td>6</td>
<td>January 2009</td>
<td>10</td>
<td>500</td>
<td>7</td>
</tr>
<tr>
<td>7</td>
<td>February</td>
<td>10</td>
<td>500</td>
<td>5</td>
</tr>
<tr>
<td>8</td>
<td>March</td>
<td>7</td>
<td>500</td>
<td>3</td>
</tr>
<tr>
<td>9</td>
<td>April</td>
<td>6</td>
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<tr>
<td>10</td>
<td>May</td>
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<td>0</td>
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<tr>
<td>11</td>
<td>June</td>
<td>-</td>
<td>-</td>
<td>0</td>
</tr>
<tr>
<td>12</td>
<td>July 2009</td>
<td>-</td>
<td>-</td>
<td>0</td>
</tr>
</tbody>
</table>

![Figure 1. Bar diagram showing monthly WSSV infection in eggs of *Marsupenaeus japonicus*](image-url)
undertaken in 7 month duration from October 2008 to November 2009. Out of these 30 results show positive and 30 show negative to WSSV disease. As all the egg samples tested are alive and not showing any external symptom for this disease, it is suspected that they might be the carrier of WSSV in their genomes, which could be confirmed by more advanced technologies.

The experimental studies carried in favour of vertical transmission indicate that virus detects in oocytes and connective tissue cells of ovary gives positive result [20]. In turn, brooders could have got the infection through the crab feed or any other live feed [21]. Experimental transmission of WSSV from crab to shrimp broodstock gives positive results as reported by [22]. However, many captured wild shrimp in India have been found positive for WSSV [23]. It is clear that the WSSV present in the genome could not cause mortality to the eggs, because of its inability to replicate in the egg stage as explained by [24]. Hence it is suspected that the transmission of WSSV may occur either vertically from infected broodstock to eggs.

Conclusion:
The present investigation concludes that the transmission of WSSV to eggs is not through the oral or external feeding but may be through hereditary means by vertical transmission from their spawning mothers. If such a transmission of this virus is allowed to be inherited by eggs of subsequent generations of M. japonicus species, it would become a permanent genetic trait as a part of the genome of those generations. It therefore becomes inhospitable for this species to warrant immunity against such virus, which drastically reduces the number of days from the monthly longevity of this species. “Prevention is better than cure” is definitely an appropriate proverb for this species to warrant immunity against such virus, which drastically reduces the number of days from the monthly longevity of this species. “Prevention is better than cure” is definitely an appropriate proverb for such virus, which drastically reduces the number of days from the monthly longevity of this species. “Prevention is better than cure” is definitely an appropriate proverb for

References:


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