International Journal of Sciences: Basic and Applied Research (IJSBAR)

ISSN 2307-4531
(Print & Online)

http://gssrr.org/index.php?journal=JournalOfBasicAndApplied

PCR Based Detection of White Spot Syndrome Virus (WSSV) in Shrimp Post Larvae (PL) of Bangladesh

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Abstract

Shrimp aquaculture is a very promising and rapidly growing industry that contributes about 5% to national GDP in Bangladesh. Shrimp aquaculture is the second highest foreign currency earning source and 97% of the produced shrimp being exported. However, shrimp aquaculture suffered from different diseases particularly viral disease. White Spot Disease (WSD) alone losses tens of billion dollars in every year worldwide as well as in Asia, the industry suggests annual damages is about 4 billion USD. In Bangladesh, White Spot Syndrome Virus (WSSV) infection in shrimp aquaculture alone destroys hundreds of million dollars per year. The detection of WSSV before releasing post larvae (PL) in aqua-farm is immensely important to mitigate this disease from the shrimp aquaculture industry of Bangladesh. In this investigation, a total of 65 PL of shrimp samples were collected from Cox's Bazar (n=40 PL) and Satkhira (n=25 PL) of Bangladesh between 2015 and 2018. Samples were analyzed by conventional PCR using VP664 and VP28 genes specific primers. Among the 65 PL samples, 11 PL samples were found to be positive where six samples in Cox's Bazar and five samples in Satkhira areas were found WSSV positive. The overall prevalence rate was 16.93% in the collected PL samples in Bangladesh. Though the infection rate of WSSV in shrimp PL was 16.93%, it will play a deleterious influence in shrimp aquaculture of Bangladesh. The present work suggests that the segregation of WSSV infected shrimp PL before releasing to the culture ponds/ghers is of immensely important to minimize or prevent the WSD infection in shrimp aquaculture.

Key words: WSSV; Shrimp PL; VP28; VP664; Bangladesh.

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1. Introduction

Shrimp aquaculture is a rapidly magnifying profitable industry which plays vital role in improving community progress, food security, employment opportunities and poverty alleviation [1]. About 97% of the produced shrimp is being exported and these sub-sector is the second highest foreign currency earning source of Bangladesh [2]. Shrimp aquaculture contributes 5% to the national GDP and nearly 8.5 million Bangladeshi peoples, predominantly coastal areas public, unswervingly depend on this industry for their livelihood [3]. The Bangladeshi shrimp industry completely depended on the stocking of wild post larvae (PL) during the initial phases of its development. The prompt expansion of shrimp aquaculture upsurges the demand of PL which escalates to the development of commercial hatcheries. At present, the total production is about 16.4 billion PL per year under operation of 85 shrimp hatcheries [4]. However, a significant constraint to the swift growth and extension of these industry is loss of yield due to the occurrence of a large variety of pathogenic bacteria and viruses particularly White Spot Syndrome Virus (WSSV) which results in huge economic damages every year [5]. This industry has to face damages tens of billion dollars for WSD in every year globally and the projected annual losses in Asia is about 4 billion USD [6]. In addition, WSSV disease in shrimp aquaculture alone harm several million dollars for every year in Bangladesh. WSSV is a devastating viral pathogen initiate the White Spot Disease (WSD) leads to 100% mortality within 3-10 days with or without clinical sign [7-8]. Currently, this viral pathogen surpasses all other disease and lead to the prime reason of production losses in especially Asia [9]. WSSV has a widespread host range such as shrimps, prawn, crabs, cray fish etc. and is capable of spreading both vertically from infected mother to PL and horizontally through water, live feed *Artemia* or other carrier organisms [10]. To shrink the affliction of WSSV, the farmers have to stock WSSV free PL and WSSV free environment. The WSSV negative PL can be obtained from WSSV free brood-stocks. Therefore, early diagnosis for WSSV infection in the brood-stocks and stocking of WSSV free PL is one of the most effectual approaches to monitor the WSSV occurrence in shrimp farming facilities. The knowledge on the prevalence of WSSV infection in shrimp PL is very limited in Bangladesh. Therefore, the present study investigated and reported the prevalence rate of WSSV infection in post larvae (PL) of shrimp in Cox's Bazar and Satkhira areas of Bangladesh.

2. Materials and Methods

2.1 Collection of shrimp post larvae (PL) samples

The shrimp post larvae (PL) samples were collected from the southern part of Bangladesh Cox's Bazar and Satkhira between 2015 and 2018. The collected samples were transported to Microbial Genetics and Bioinformatics Laboratory (www.microbialgen.du.ac.bd), Department of Microbiology, University of Dhaka for further analysis by maintaining cold chain.

2.2 DNA extraction and purification

Total DNA was extracted from whole tissue samples of shrimp PL by automated DNA extractor (MaxWell 16(R) with the help of MaxWell 16(R) Tissue DNA Purification kit (AS 1030, Promega, USA), according to

manufacturer's guidelines. The concentration of extracted DNA was measured and confirmed by Nano-drop2000 (Thermo Scientific, Germany) for PCR amplification.

2.3 Conventional PCR amplification of VP664 and VP28 Gene

The extracted DNA was used to investigate the presence of WSSV by polymerase chain reaction using GoTaq 2× Hot Start Colorless Master Mix (Promega, USA). The primer set 146F1 (5'-ACT ACT AAC TTC AGC CTA TCT AG-3') and 146R1 (5'-TAA TGC GGG TGT AAT GTT CTT ACG A-3') were used to amplify nucleocapsid protein VP664 gene (ORF167) by following the previous methods [11-12] which generated amplicons of 1447bp. VP28F and VP28R primers (amplicons of 643bp) were used to amplify the most abundantly expressed envelope protein gene VP28 conferring previously [13]. The PCR amplified products were analyzed by 1% agarose gels containing ethidium bromide (0.5µg/ml) and visualized under UV illuminator (protein simple α imager, USA) and amplified product was compared with a 1 kb DNA ladder (**Figure 1**).

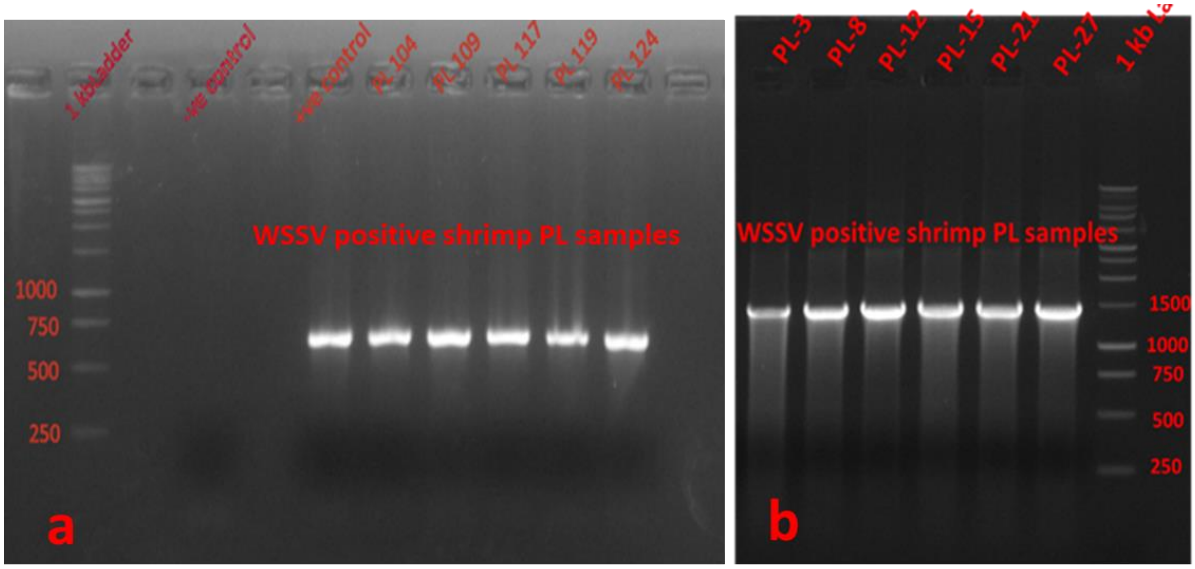

Figure 1: PCR detection of WSSV positive shrimp PL. Here ~643bp amplicon of VP28 gene (a) and ~1447bp amplicon of VP664 gene (b) were amplified and visualized in 1% agarose gel.

3. Results

3.1 Prevalence rate of WSSV in shrimp post larvae (PL)

A total of 65 samples was collected to investigate the prevalence of WSSV in shrimp PL from Cox's Bazar and Satkhira areas of Bangladesh. Six (6) PL samples were found WSSV positive among the 40 samples of Cox's Bazar area and five (5) WSSV infected samples out of 25 samples were detected positive in Satkhira (**Figure 2**). Thirty four (34) and twenty (20) samples were found to be WSSV negative in Cox's Bazar and Satkhira areas, respectively.

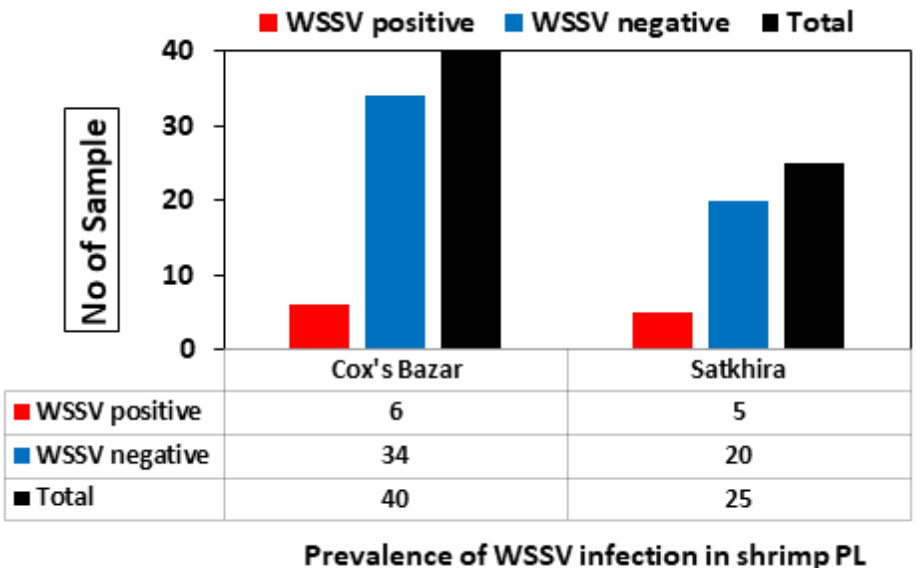

Figure 2: Prevalence of WSSV in shrimp PL of Cox's Bazar and Satkhira areas.

The prevalence rate of WSSV infection in sampled shrimp PL was 15% and 20% in Cox's Bazar and Satkhira areas, respectively. The overall prevalence rate of WSSV infected shrimp PL was 16.93% in these two areas of Bangladesh (**Figure 3**).

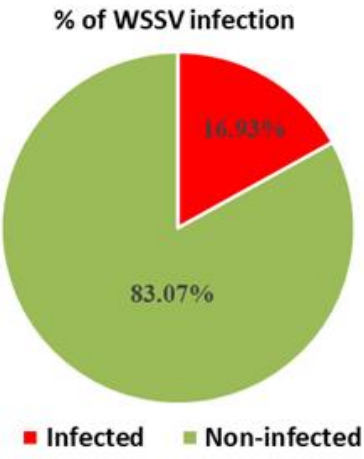

Figure 3: Overall prevalence rate (%) of WSSV infection in shrimp PL.

4. Discussion

The molecular techniques have been found to be convenient for the identification and comparative studies of the viruses [14] and Office for International Epizootics (OIE) suggested PCR as a molecular approach for accurate identification and certification of the WSSV infection status of brood-stock and PL of shrimp [15]. The prevalence rate of WSSV in shrimp PL of Bangladesh was 3-12 % during 2008-2010 [16]. Previous studies found the WSSV prevalence in shrimp PL was 0-12% during 2007-2009 and 4.03-8.89 % during 2009-2014 in Bangladesh [17-18]. A study showed that the average prevalence of WSSV in shrimp PL in Bangladesh was

45.05% during 2014-2016 [19]. The present study revealed that the average prevalence of WSSV (16.93%) in shrimp PL during 2015-2018 was higher than previous report although not as high as reported during 2014-2016 [19]. A study has found high prevalence of WSSV in Satkhira (79%) and Cox's bazar (25%) of Bangladesh during July 2013 to April 2014 [12]. A recent report stated that, the prevalence rate of WSSV was nearly 78% in Satkhira during 2014 to 2017 [13]. The higher viral load found in the previous study indicated an outbreak of WSSV in Bangladesh which may be initiated by the stocking of WSSV infected PL [13]. The infected shrimp PLs transmit the WSSV horizontally to the other non-infected shrimp PLs through water, sediment or other carrier organisms in the shrimp culture ponds/ghers which may the possible reason of the higher WSSV prevalence in cultured shrimp of Bangladesh. Therefore, to reduce the risk of WSSV proliferation, it is necessary to segregate the WSSV infected shrimp PL from the WSSV negative PL in the early stage of their nursery/culture period. The oocytes and follicle cells in the connective tissue of the ovary can be infected by WSSV. A study reported that the batches of eggs derived from WSSV-negative brood-stocks were WSSV free whereas WSSV-infected spawners were WSSV positive which indicated that the virus can be transmitted vertically [20]. Therefore, testing of spawners both before and after spawning and analysis the nauplii/PL for the presence of WSSV might be effective screening and preventive approaches which might be abridged the probabilities of subsequent outbreak of WSSV. In addition, the sources of secondary infection such as entrance of WSSV-contaminated water, presence of weeds and invertebrate animals particularly mollusks and crustaceans should be eliminated in grow-out ponds/ghers to preclude an outbreak of the disease [21]. The screening for WSSV in brood-stock, followed by nauplii and in numerous larval stages of *Penaeus monodon* to separate the WSSV-free status of the PL (due to its higher value and demand) by PCR technology monitored by the shrimp hatcheries in the coastal belt is the focal reason behind the low prevalence found in the present study [15]. The WSSV infection was 75% and 39.4% in the west and east coast of India, respectively, reported by previous studies [22-23] which is higher than found in our present study. A negative correlation exists between WSSV infections with hatching rate [24]. The hatchery owner might avoid the WSSV infected brood. Conventional PCR was used to diagnose the presence of viral pathogen by the hatchery operator which may not detect the low number of viral pathogen. A previous study recommended that the broods with light infection of WSSV, which might not be detected by single-step PCR, should not be used as spawner [25]. Therefore, the development of rapid and sensitive detection tool of WSSV for the screening of carriers in shrimp PL and spawners would be a way out from the devastating disease. A study suggested to stock virus-free PL produced from PCR-negative broods collected from the Bay of Bengal and at the same time maintain a better post-stocking management to diminish risks of contamination from other sources is required to reduce the risks of a WSSV outbreak in the shrimp farms [15]. Therefore, the segregation of WSSV-infected PL of shrimp before stocking to the culture ponds/ghers could be helpful for farming of shrimp in the coastal areas of Bangladesh.

5. Limitations of the study

Shrimp PL samples were collected from Cox's Bazar and Satkhira areas and the numbers of samples were only sixty five (n=65) in the present study. Therefore, it is difficult to reach in a conclusion based on only 65 samples about the prevalence of WSSV infection in these two areas. Thus, more samples need to be tested to make a concreate conclusion about the prevalence of WSSV infection in shrimp PL producing industry in Bangladesh.

6. Conclusions

White Spot Syndrome Virus (WSSV) is the major intimidation of shrimp industry which cannot be eradicated easily. Though the infection rate of WSSV in shrimp PL was 16.93%, it will play a deleterious influence in shrimp aquaculture of Bangladesh. The present work suggests that the segregation of WSSV infected shrimp PL before releasing to the culture ponds/ghers is of immensely important to minimize or prevent the WSD infection in shrimp aquaculture of Bangladesh. Regular disease surveillance and adequate preventive measure will be helpful to get rid of the problem.

Acknowledgements

We would like to acknowledge Microbial Genetics and Bioinformatics Laboratory (www.microbialgen.du.ac.bd), Department of Microbiology, University of Dhaka for giving the support of laboratory facilities. This research work was funded by the UGC, Bangladesh (2014–15) and Ministry of Science and Technology (R & D allocation for 2018–2019), Bangladesh.

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