Molecular diagnosis of white spot syndrome virus (WSSV), infectious hypodermal and hematopoietic necrosis virus (IHHNV), monodon baculovirus (MBV), and acute hepatopancreatic necrosis disease (AHPND) causative agent in black tiger shrimp hatcheries

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Abstract. The shrimp industry has been experiencing many diseases, particularly those caused by viral and bacterial pathogens that are considered serious intimidation to the commercial tiger shrimp (*Penaeus monodon*) farming industry. The study aimed to investigate the scenario of viral and bacterial contamination in the brood, nauplii, zoea, mysis, post larvae (PL) of shrimp, and other hatchery inputs like artemia, algae, squid, and mussels in commercial shrimp hatcheries in Cox's Bazar. The prevalence of these dreadful viral and bacterial infections was studied in 10 randomly chosen hatcheries in the Cox's Bazar district of Bangladesh. The causative agents were detected using nested polymerase chain reaction (PCR). Out of 118 randomly selected samples 23.45%, 37.93%, 34.48%, and 4.14% of the total samples were found positive for white spot syndrome virus (WSSV), infectious hypodermal and hematopoietic necrosis virus (IHHNV), monodon baculovirus (MBV), and acute hepatopancreatic necrosis disease (AHPND), respectively. The study showed that the diseases affected all stages of the life cycle of shrimp as well as squid, mussels, and other hatchery inputs were also potential hosts for the viruses and bacteria. Hence, the present study suggests focusing on ensuring the production of specific-pathogen-free (SPF) shrimp PL in commercial shrimp hatcheries by maintaining proper biosecurity management. The findings of the study would aid farmers and decision-makers in developing plans to protect the shrimp sector from WSD and AHPND and thereby maintain sustainable shrimp farming in Bangladesh.
Key Words: brood, hatchery, *Penaeus monodon,* polymerase chain reaction, post larvae, prevalence, shrimp virus.

Introduction. Bangladesh is the 5th largest aquaculture producing country in the world next to China, India, Vietnam, and Indonesia. In 2019-2020, Bangladesh produced 43,033 metric tons of fish (DoF 2020). *Penaeus monodon*, usually known as black tiger shrimp, locally called *Bagda*, is the most important fisheries product of Bangladesh. It is a marine crustacean and dispersed nearby the Indo-Pacific territory extending from northern Australia to the eastern coast of Africa, the Arabian Peninsula, Southeast Asia, and the Pacific Ocean (Waqairatu et al 2012). Following the ready-made garments industry, the shrimp and prawn sector holds the position of the second-largest export sector in Bangladesh. However, many diseases pose significant challenges to the global shrimp industry. The prevalence of shrimp diseases is mostly attributed to viral, bacterial, and parasitic pathogens. White spot syndrome virus (WSSV) is one of the viruses responsible for shrimp farming's severe mortality and production losses, which was found for the first time in 1992 In Taiwan (Chou et al 1995). During that period, the

shrimp farming industry in numerous Asian and Latin American countries experienced significant production losses (Wongteerasupaya et al 1995). White spot syndrome virus (WSSV), a member of the Nimaviridae family, is a large enveloped double-stranded DNA virus that infects larvae, post larvae (PL), and broodstock (Kou et al 1998). The Cox's Bazar region noticed an epidemic of WSSV in cultured penaeid shrimp in 1994. The disease disseminated to the Khulna region, situated in the southwestern part of the country, resulting in an impact on about 90% of traditional shrimp farms and leading to a decline of 20% in shrimp production during that period. Another smallest virus among the recognized penaeid shrimp viruses is the infectious hypodermal and hematopoietic necrosis virus (IHHNV), also known as *Penaeus stylirostris penstyldensovirus* 1 (Yu et al 2021). It was first reported in cultured *P. stylirotics* and *Penaeus (Litopenaeus) vannamei* in Hawai, the USA in 1981 which caused up to 90% of mortalities (Xia et al 2015). IHHNV can naturally infect shrimp species in the Palaemonidae family, including penaeid shrimp species as well as *Palaemon macrodactylus*. Monodon baculovirus (MBV) causes significant disease in *P. monodon* larvae, postlarval stages, and early juvenile stages raised in hatcheries. Monodon baculovirus (MBV) a double-stranded DNA virus is a nuclear polyhedrosis virus (NPV) of the family Baculoviridae (Hsieh et al 2006; Martorelli et al 2010). The clinical signs of MBV infection include anorexia and lethargy. In cases of severe infection, the destruction of hepatopancreatic and anterior midgut epithelial cells may occur (Natividad 1991). Acute hepatopancreatic necrosis disease (AHPND) is a highly consequential bacterial disease that is currently causing widespread mortality within the shrimp industry throughout many Asian and American countries (Devadas et al 2019; Hossain et al 2020). In 2009, this bacterial disease was first reported to cause mass mortality in China, then it was found in Vietnam, Malaysia, Thailand, Mexico, Philippines, and South America in 2011, 2011, 2012, 2013, 2015, and 2016 respectively (de la Pena et al 2015; Soto-Rodriguez et al 2015). This bacterium contains a 70-kbp plasmid and the Pir (Photorhabdus insect-related) toxins encoded by the plasmid are mainly responsible for shrimp mortality in AHPND (Tran et al 2013). Whiteleg shrimp (*P. vannamei*) and black tiger shrimp (*P. monodon*) are found to be sensitive species to AHPND (Hong et al 2016). In Bangladesh, the shrimp sector mostly depends on wild PL stock (Debnath et al 2016). A few nurseries for wild tiger shrimp, Penaeus monodon, were established at Cox's Bazar during the late 1980s. The global dissemination of WSSV and AHPND has significant ramifications for both commercial enterprises and ecological systems as well as pose substantial challenges to shrimp production.

Therefore, the present study was conducted to investigate the spread of virus and bacterial agents in various stages of *P. monodon* in the Cox's bazar hatchery using the PCR technique so that the possible sources and transmission of the diseases could be revealed.

Material and Method

Study area and sample collection. Shrimp (*P. monodon*) hatchery samples (shrimp brood, zoea, mysis, postlarvae (PL), nauplii, artemia, algae, squid, mussel) were collected from 10 randomly chosen commercial hatcheries named Balaka, Quality, Satata, Modern, NR, ARC, New Madina, Golden aqua ltd, Platinum, and Sonali located at the hatchery zone of Kolatoli and Sonapara in Cox's Bazar, Bangladesh (Figure 1). A total of 118 samples were collected from 10 hatcheries in December 2020. For screening different viruses (WSSV, IHHNV, MBV, and AHPND), samples were taken from various parts of the healthy brood, including the pleopods, muscles, gut, and hepatopancreas. Additional samples from the hatchery, including Pl, zoea, nauplii, mysis, artemia, squid, and mussels, were also obtained in limited quantities. Primarily, 70% ethanol was used to preserve all of the gathered samples and finally transported to the Molecular and Genetics lab of the Fisheries and Marine Resource technology discipline, at Khulna University for virus detection. DNA was extracted from all samples and stored at -20°C at the Molecular and Genetics lab of Khulna University (Courtesy of World Fish, Khulna).

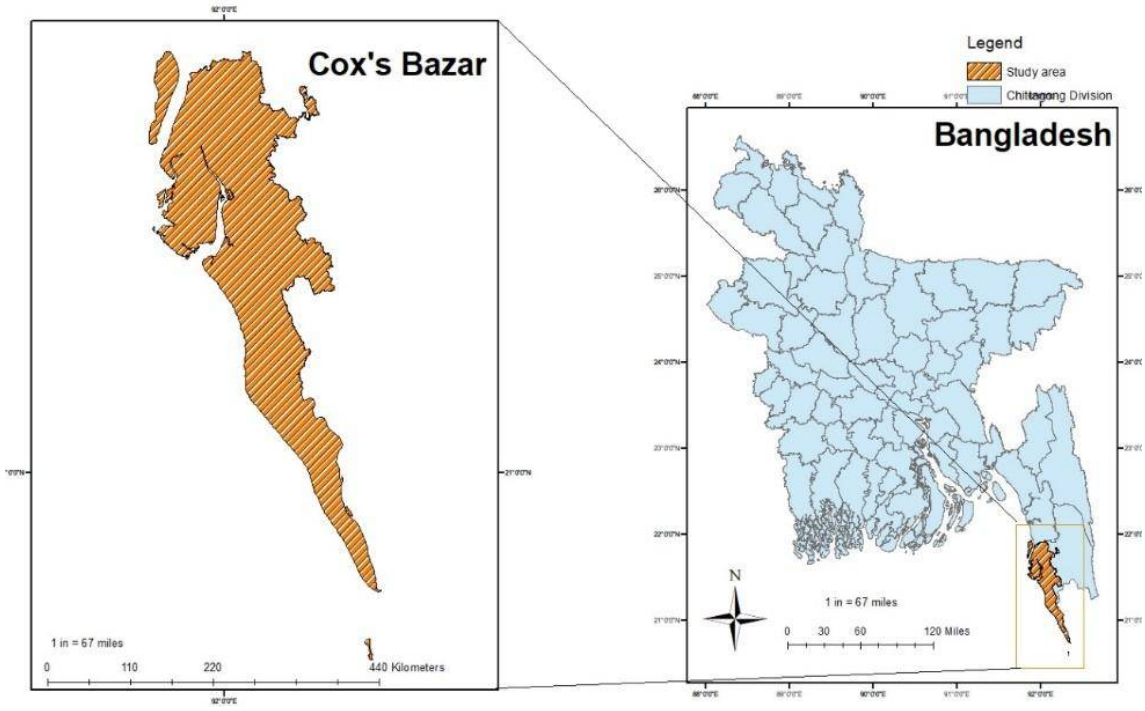

Figure 1. Sampling area in Cox's Bazar district. The navigational sign indicates the area of sampling hatcheries.

DNA extraction for WSSV, IHHNV, MBV, and Vibrio parahaemolyticus detection. The GeneJet DNA purification kit was employed for DNA isolation using the supplied protocol. Initially, 20 mg samples were collected and placed in a 1.5 mL tube for grinding. Next, 180 µL of the digestion solution was carefully ground. After that 20 µL of proteinase K solution was added and mixed thoroughly by vortexing or pipetting to obtain a uniform suspension. The sample was then incubated for 3 hours at 56°C, after which 20 µL of RNase solution was added, mixed, and incubated for 10 minutes at ambient temperature. After incubation, 200 µL of lysis solution was added and vortexed for 15 seconds until a homogenous mixture was obtained. Then, 400 µL of ethanol at a concentration of 50% was pipetted in. After adding 500 µL of wash buffer 1, the sample was centrifuged for one minute at 12000 rpm and the flow-through was discarded before the collection tube was recapped. Next, 500 µL of wash buffer 2 was introduced into the system, followed by centrifugation at a speed of 12000 rpm for 3 minutes. Subsequently, the flow through was once again discarded, and the collecting tube was securely closed. Finally, the sample was centrifuged for 1 minute at 12000 rpm, the column was discarded, the 1.5 mL microcentrifuge tube was adequately sealed, and the isolated DNA was stored at -20°C or -70°C until use.

Primer selection. The primer pairs were used for this study (WSSV 146F1, WSSV 146R1, WSSV 146F2, and WSSV 146R2), (IHHNV389F, IHHNV389R), (MBV 1.4F, MBV 1.4R, MBV 1.4NF, MBV 1.RNR), (AP4-F1, AP4-R1, AP4-F2, and AP4-R2) (Lo et al 1996) (Table 1) from the nucleotide sequences of the genomic DNA of the corresponding virus and bacteria.

Table 1
List of primer sequences used for the amplification of WSSV, IHHNV, MBV, and AHPND

Virus/ disease	*Primer*	*Sequences*	*Size of amplicon (bp)*
WSSV	WSSV 146F1	5′-ACTACTAACTTCAGCCTATCTAG-3′	1447
	WSSV 146R1	5′TAATGCGGGTGTAATGTTCTTACGA3′	
	WSSV 146F2	5′-GTAACTGCCCCTTCCATCTCCA-3′	941
	WSSV 146R2	5′-TACGGCAGCTGCTGCACCTTG T-3′	
IHHNV	IHHNV 389F	5′-CGG-AAC-ACA-ACC-CGA-CTT-TA-3′	389
	IHHNV 389R	5′-GGC-CAA-GAC-CAA-AAT-ACG AA-3′	
MBV	MBV 1.4F	5′-CGA TTC CAT ATC GGC CGA ATA-3′	533
	MBV 1.4R	5′-TTG GCA TGC ACT CCC TGA GAT-3′	
	MBV 1.4NF	5′-TCC AAT CGC GTC TGC GAT ACT-3′	361
	MBV 1.RNR	5′-CGC TAA TGG GGC ACA AGT CTC-3′	
AHPPND	AP4-F1	5′-ATGAGTAACAA TATAAAACATGAAAC-3′	1269
	AP4-R1	5′-ACGATTTCGACGTTCCCCAA-3′	
	AP4-F2	5′-TTGAGAATACGGGACGTGGG-3′	230
	AP4-R2	5′-GTTAGTCATGTGAGCACCTTC-3′	

PCR detection of WSSV, IHHNV, MBV, and Vibrio parahaemolyticus. The protocol of WSSV, IHHNV, MBV, and *Vibrio parahaemolyticus* detection using the PCR approach was performed using the method obtained in the OIE manual (OIE 2003). Total DNA was isolated from the samples using GeneJet DNA purification kit. The solution of 25 µL composed of 9.5 µL RNase-free water, 12.5 µL taq green PCR master mix, 1 µL of the corresponding primer (0.5 µL Forward primer and 0.5 µL Reverse primer), and 2 µL DNA sample. PCR cycle was then carried out. The first cycle was 1 denaturation cycle which was carried out at 94°C for 4 min, 55°C for 1 min, and 72°C for 2 min. Then 39 elongation cycle was carried out at 94°C for 1 min, 55°C for 1 min, and 72°C for 2 min for primary PCR of WSSV detection, and then 1 termination cycle was at 72°C for 5 min. Then the PCR products were held at 4°C. PCR amplification of IHHNV was performed under the following conditions: denaturation at 95°C for 5 min, followed by 35 elongation cycle including heat denaturation at 95°C for 30 sec, primer annealing at 60°C for 30 sec, and DNA elongation at 72°C for 30 sec followed by a termination cycle at 72°C for 5 min. At last, the PCR products were held at 4°C. The DNA template, primers, and PCR ingredients were combined and denatured at 96°C for 5 min. Then 40 elongation cycles were carried out including heat denaturation at 94°C for 30 sec, primer annealing at 65 °C for 30 sec, and DNA extension at 72°C for 60 sec. Then 1 termination cycle was at 72°C for 7 min, and then held at 4°C before continuing with the nested PCR for primary PCR of MBV and denatured at 96°C for 5 min, then 35 elongation cycle was carried out at 94°C for 30 sec, 60°C for 30 sec, and 72°C for 60 sec, then 1 termination cycle was at 72°C for 7 min. After all cycles, it was held at 4°C for nested PCR. For AHPND detection there was 1 denaturation cycle which was carried out at 94°C for 2 min. Then 39 elongation cycle was carried out at 94°C for 30 sec, 55°C for 30 sec, and 72°C for 90 sec. Then 1 termination cycle was at 72°C for 2 min to carry out the primary and nested PCR. After all cycles, PCR products were held at 4°C.

Evaluation of PCR products by gel electrophoresis. To prepare the agarose gel, 2 g agarose was boiled with 100 mL Tae buffer and 10 µL sybr safe dye for 2 min. The boiled agarose was poured into a mold and placed with a comb to load the sample. After preparing the gel 4-5 µL PCR product of each sample was loaded in one of the wells of the comb, then 100 bp DNA ladder was used as a marker. After loading the sample, the gel was exposed to the electric field in the range of 80-100 volts for 45 min. A UV-transilluminator was used to detect the bands, and the image was then taken. The gel electrophoresis image is shown in Figure 2.

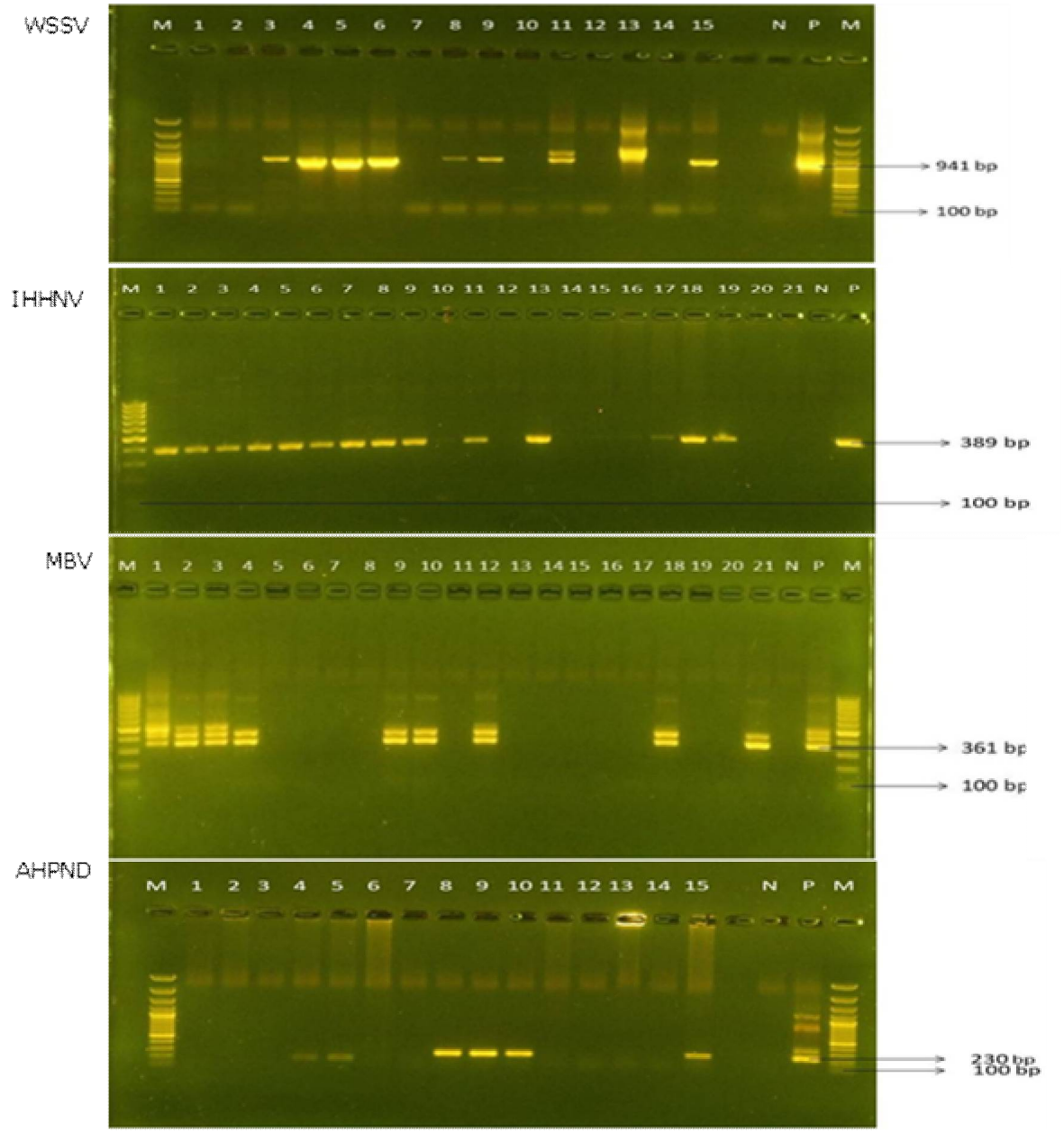

Figure 2. Depiction of the gel electrophoresis analysis of different viral infections in hatchery samples. Lane- N, P, and M indicate negative control, positive control, and marker (100 bp), respectively. WSSV: Lane 1-15 denotes samples (Lane 1-3: PL; Lane 4: zoea; Lane 5-6: brood (male), Lane 7-9: artemia; Lane 10: algae: Lane 11: nauplii; Lane 12-14: brood (female); Lane 15: brood (male)). Amplification of the 941bp fragment was observed only in the positive sample and positive control. IHHNV: Lane 1-21 indicates samples (Lane 1-3: PL; Lane 4-6: zoea; lane 7-9: mysis; Lane 10-12: artemia; lane 13-14: algae; lane 15-19: Brood (female); and Lane 20-21 brood (male)). Samples 1-9, 11, 13, 17, 18, and 19 showed the 389 bp amplification. MBV: Lane 1-21 indicates samples (Lane 1-3: PL; Lane 4-6: zoea; lane 7-9: mysis; Lane 10-12: artemia; lane 13-14: algae; lane 15-19: Brood (female); and Lane 20-21 brood (male)), Amplification of the 361 bp fragment was observed only in the positive control and positive tissues. AHPND: Lane 1-15 denotes samples (Lane 1-3: PL; Lane 4: zoea; Lane 5-6: male, Lane 7-9: artemia; Lane 10: algae: Lane 11: nauplii; Lane 12-14: brood (female); Lane 15: brood (male)). Amplification of the 230bp fragment was detected only in the positive sample and positive control.

Results

Prevalence of the viruses. Based on the results of the PCR test, the maximum prevalence of IHHNV (37.93%) and the lowest prevalence of AHPND (4.14%) were found among the total samples. The prevalence of MBV and WSSV was determined to be 34.48% and 23.45% respectively (Figure 3).

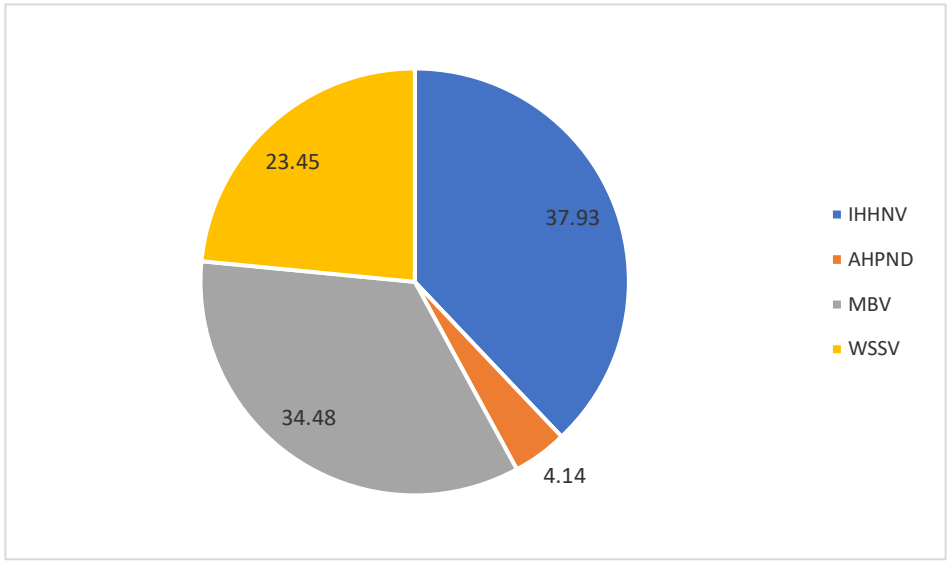

Figure 3. For IHHNV, AHPND, MVB, and WSSV 37.93%, 4.14%, 34.48%, and 23.45% prevalence rates were found respectively.

Prevalence of the pathogens in different types of samples. Among the total (118) samples, 34 samples (28.81%) were WSSV positive in the hatcheries of Cox's Bazar. Based on the findings of the research, it was determined that all (100%) male brood, nauplii, squid, and mussel specimens tested positive for the presence of WSSV. In the case of female brood, PL, zoea, mysis, artemia, and algae, the prevalence of WSSV was seen to be 61.11%, 23.33%, 14.29%, 18.18%, 16.67%, and 0% respectively (Figure 4). The findings of the study indicated that the zoea and mysis samples exhibited the highest incidence of IHHNV, with a prevalence rate of 100% (Figure 4). The prevalence of PL was found to be 90%, whereas male brood, female brood, artemia, and algae exhibited prevalence rates of 40%, 27.28%, 6.67%, and 7.14% respectively (Figure 4). The prevalence of MBV in hatchery samples, as determined through PCR screening, was found to be 34.48%. The male brood exhibited the highest prevalence of MBV, with a rate of 80%. Furthermore, the prevalence of MBV in PL, zoea, mysis, and algae was found to be 63%, 28.57%, 27.27%, and 21.43% respectively (Figure 4). A single specimen of squid was obtained and subsequently yielded a positive result for MBV upon testing. AHPND-positive samples were detected alone at a single hatchery among a total of ten hatcheries. A mere 5.08% of the overall samples exhibited positive results for the AHPND. The samples contained 40% male brood, 14.29% zoea, 6.67% artemia, and 7.14% algae that tested positive for the AHPND while AHPND was not detected in any female brood, PL, mysis, nauplii, squid, or mussel samples (Figure 4).

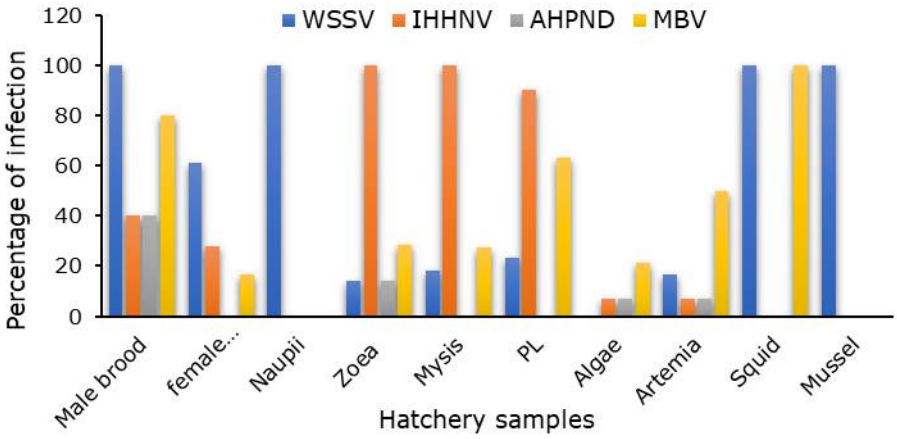

Figure 4. Status of different virus infected hatchery samples.

Prevalence of infected samples in hatcheries. Based on the conducted study, it was determined that Platinum Hatchery exhibited the most substantial proportion (60%) of samples testing positive for WSSV. Among the whole sample of the respective hatcheries, the WSSV percentages for Sonali, Satata, Golden Aqua, Balaka, NR, ARC, Quality, New Madina, and Modern Hatchery were 41.18%, 33.33%, 35.29%, 9.09%, 0%, 8.33%, 42.86%, 0%, and 0%, respectively (Figure 5). The highest prevalence (66.67%) of IHHNV was found in Satata Hatchery. The remaining hatcheries, namely Sonali, Golden Aqua Ltd., Balaka, NR, ARC, Platinum, Quality, New Madina, and Modern Hatchery, exhibited positive sample rates of 47.6%, 35.29%, 45.45%, 50%, 25%, 46.67%, 42.46%, 50%, and 50% respectively (Figure 5). Regarding the detection of MBV, the PCR analysis revealed that NR and Modern hatcheries exhibited a prevalence of 100%, while Sonali, Satata, Golden Aqua, Platinum, Quality, and New Madina hatcheries showed prevalence rates of 11.76%, 42.85%, 35.29%, 73.33%, 26.65%, and 66.67% respectively. No MBV-positive sample has been found in Balaka and ARC hatcheries. Moreover, only 40% of samples from Platinum Hatchery across all hatcheries tested positive for AHPND (Figure 5).

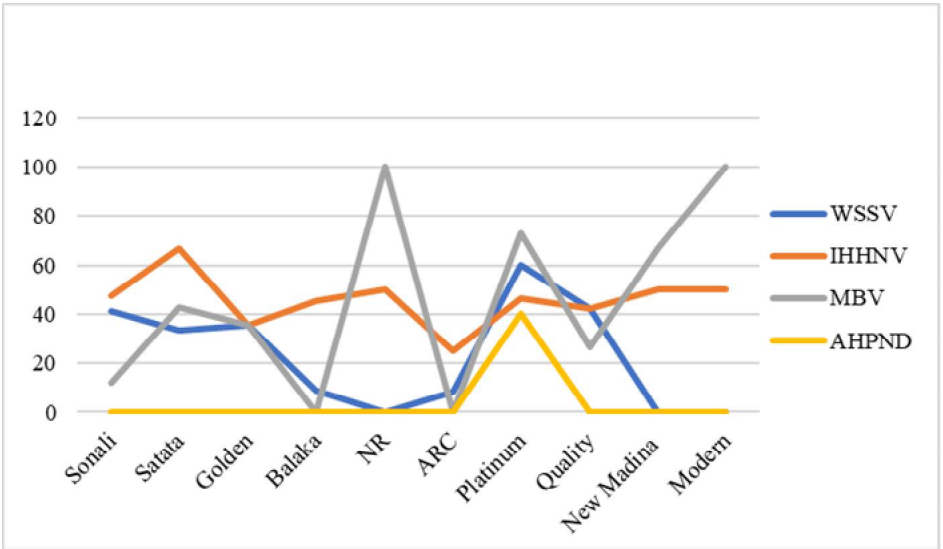

Figure 5. Percentage of viral infected sample in different hatcheries.

Discussion. This investigation has attempted to assess the presence of different infectious viruses of shrimp at a couple of hatcheries in Cox's Bazar District of Bangladesh because they pose a threat to industrial shrimp farming since they can have serious pathogenic consequences on penaeid shrimp. All the studied viruses (WSSV, IHHNV, MBV, and AHPND) have been listed as crustacean diseases by the World

Organization for Animal Health (OIE) because of their significant impact on economic losses in global shrimp farming (Dhar et al 2019).

In this study, the prevalence of IHHNV was the highest (37.93%) among all hatchery samples but lower than the 88% infection rate detected in shrimp brood from several hatcheries in Cox's Bazar reported by Chakrobortty et al (2020). WSSV was detected in 23.45% of the total samples which is lower compared to the 30% infection described by Ayub et al (2008) from five hatcheries of Cox's Bazar. Moreover, it was lower than the prevalence of 75% reported by Otta et al (1999) from India's west coast as well as lower in comparison to the 39.4% prevalence reported by Uma et al (2005) from the south coast of India. The prevalence of MBV (34.48%) was found higher than that of WSSV. A greater incidence of MBV and IHHNV in shrimp brood from hatcheries in Cox's Bazar was observed in comparison to WSSV (Chakrobortty et al 2020). In addition, the hatcheries of Cox's Bazar had the lowest prevalence of AHPND (4.14%) compared to the prevalence of 30.56% reported by Kongrueng et al (2015). Furthermore, the prevalence observed in this study was comparatively lower than the 90% reported by Soto-Rodriguez et al (2015) and the 49.04% positivity rate reported by Dangtip et al (2015). In their study, Eshik et al (2017) reported a prevalence of 14.29% for AHPND-positive samples in the year 2017 collected from the districts of Khulna, Satkhira, and Bagerhat. When it came to brood, all males tested positive for WSSV, 80% tested positive for MBV, and 40% tested positive for AHPND, however female broods had lower prevalences of all of those viruses than males. High prevalence of MBV was reported in both broodstock and larvae along the southeast coast of India (Uma et al 2005). IHHNV was found in 40% of males and 27.28% of females. The positive brood sample may transmit the infection to larvae and post-larvae through vertical transmission (Rajendran et al 2012; Dey et al 2020). There is existing documentation that supports the transmission of MBV to both larvae and postlarvae through vertical and horizontal transmission (Kanjanasopa et al 2015). In the case of horizontal transmission, the broodstock and offspring can be infected via contaminated water by feces or infected broodstock. On the other hand, in vertical transmission, the infected brood directly passes the viral infection to the offspring. Kanjanasopa et al (2015) reported the trans-ovarian transmission of MBV from brood to offspring. Therefore, the high prevalence of MBV infection in the offspring could be due to contaminated water and the PL could be infected vertically by the infected brood. According to Lightner et al (1983) MBV exposure to larval and post-larval stages in shrimp is likely to cause severe infection and mortality compared to exposure to juvenile and adult stages. However, the severity has been shown to diminish with shrimp size or age.

Our study also reported that the early stages of shrimp are more sensitive to all those viruses. The highest WSSV prevalence (100%) was found in nauplii whereas a high prevalence of IHHNV was found in zoea (100%), mysis (100%), and PL (90%). Some previous studies reported the same result that IHHNV can infect any life stage of a penaeid shrimp species however the juvenile stages are the most severely impacted (Lightner 1999; Rai et al 2012; Chakrobortty et al 2020). In case of MBV, 63% of PL were found to be positive for this virus whereas zoea and mysis showed 28.5% and 27.2% prevalence respectively. AHPND showed a very low prevalence (14%) in zoea whereas in nauplii, mysis, and PL it was nil. Therefore, this study suggests that all of the above viruses affect more or less all stages of the shrimp life cycle. DNA virus is generally transmitted vertically from brood stock to the offspring through eggs via nauplii.

This result also reported that squid, mussels, and other hatchery samples can also be potential hosts for the studied viruses, and horizontal transmission from these samples can infect the PL, because all squid and mussels were found to be positive for WSSV. In this study, we found an MBV-positive squid though there is no available report on the infection of MBV on squid whereas Johnson (1984) reported reoviral infection in squid. In artemia, the MBV prevalence was highest followed by WSSV, IHHNV, and AHPND. Besides, this low prevalence of IHHNV, MBV, and AHPND was detected in algae. Hsieh et al (2006) reported in their study that *Artemesia longinaris* is one of a host of IHHNV infections. Another study by Muhammed (2018) informed that horizontal

transmission of IHHNV infection can be caused by infected water which can further infect artemia, algae or other nutrition supplementary feed in a shrimp hatchery and the contaminated artemia and algae could bring the disease to the hatchery. Moreover, viral inferences were found in almost all types of samples in this study.

The present study aimed to investigate the presence of WSSV, IHHNV, MBV, and AHPND in different commercial hatcheries in Cox's Bazar, and the result showed that all 10 hatcheries (Sonali, Satata, Golden Aqua ltd., Balaka, NR, ARC, Platinum, Quality, New Madina and Modern) that were selected for viral screening were found to be positive for those viruses. Debnath et al (2012) investigated the shrimp hatchery sectors in Bangladesh and revealed that the broodstock quality and performance declined sharply from 2007 to 2013 and also reported high rates of WSSV infection. Chakrobortty et al (2020) also reported the prevalence of WSSV, IHHNV, MBV, HPV, and YHV in broodstock in five commercial hatcheries in Cox's Bazar. However, the result of this study revealed poor bio-security management practices in commercial shrimp hatcheries in Bangladesh. The viral infection might reduce the immunity of the brood and PL and facilitated the viral outbreak at the farm level and may cause huge mortality hampering the economic feasibility of commercial shrimp farms.

This study suggested further research on the management of viral disease in shrimp hatcheries and the development of PCR-based management practices to produce specific pathogen-free (SPF) PL that can significantly increase shrimp production in Bangladesh.

Conclusions. Shrimp culture makes an important contribution to the aquaculture industry in Bangladesh. Hence, an infected brood could be a source of contagious viruses in hatcheries and shrimp farms. The highest prevalence of IHHNV among the four viruses was reported. IHHNV is a new hazard to shrimp farming, even though WSSV is still the main concern in Bangladeshi shrimp aquaculture, where it leads to significant mortalities in cultured shrimp. However, their combined infection might reduce the immunity of the brood and cause huge economic loss at the farm level. Consequently, the current investigation recommends that it is crucial to differentiate virus-infected shrimp PL before releasing them into the grow-out pond or gher to reduce the viral infection in Bangladesh's shrimp culture. To minimize the problem, regular disease monitoring and effective preventative measures should be taken. The hatchery managers should follow the biosecurity aspects to conduct hatchery operations. Accordingly, the production of WSSV as well as other harmful virus and bacteria free PL could emergently increase shrimp production in Bangladesh. This study suggests conducting further research for integrated management of these dreadful viral and bacterial diseases in shrimp aquaculture by controlling disease intrusion into brood stock, PL, and other hatchery components.

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Conflict of interest. The authors declare that there is no conflict of interest.

References

Ayub F., Sarker M. Y., Alam M. S., 2008 Prevalence of white spot syndrome virus infection detected by one-step and nested PCR in selected tiger shrimp (*Penaeus monodon*) hatcheries. Aquaculture International 16:405-415.

Chakrobortty D., Ali M. R., Dey B. K., Gupta N., Islam S. S., Sui L., 2020 Viral contamination of tiger shrimp *Penaeus monodon* broodstock in Bangladesh. Aquaculture International 28:2161-2172.

Chou H. Y., Huang C. Y., Wang C. H., Chiang H. C., Lo C. F., 1995 Pathogenicity of a baculovirus infection causing white spot syndrome in cultured penaeid shrimp in Taiwan. Diseases of Aquatic Organisms 23:165-173.

Dangtip S., Sirikharin R., Sanguanrut P., Thitamadee S., Sritunyalucksana K., Taengchaiyaphum S., Mavichak R., Proespraiwong P., Flegel T. W., 2015 AP4 method for two-tube nested PCR detection of AHPND isolates of *Vibrio parahaemolyticus*. Aquaculture Reports 2:158-162.

de la Pena L. D., Cabillon N. A. R., Catedral D. D., Amar E. C., Usero R. C., Monotilla W. D., Calpe A. T., Fernandez D. D., Saloma C. P., 2015 Acute hepatopancreatic necrosis disease (AHPND) outbreaks in *Penaeus vannamei* and *P. monodon* cultured in the Philippines. Diseases of Aquatic Organisms 116(3):251-254.

Debnath P. P., Karim E., Haque M. A., Uddin M. S., Karim M., 2012 Prevalence of white spot syndrome virus in broodstock, nauplii and post-larvae of tiger shrimp (*Penaeus monodon* Fabricius, 1798) in Bangladesh. Journal of Advanced Scientific Research 3(3):58-63.

Debnath P., Khan S. H., Karim M., Belton B., Mohan C. V., Phillips M., 2016 Review of the history, status, and prospects of the black tiger shrimp (*Penaeus monodon*) hatchery sector in Bangladesh. Reviews in Aquaculture 8(3):301-313.

Devadas S., Banerjee S., Yusoff F. M., Bhassu S., Shariff M., 2019 Experimental methodologies and diagnostic procedures for acute hepatopancreatic necrosis disease (AHPND). Aquaculture 499:389-400.

Dey B. K., Dugassa G. H., Hinzano S. M., Bossier P., 2020 Causative agent, diagnosis and management of white spot disease in shrimp: a review. Aquaculture 12(2):822-865.

Dhar A. K., Cruz-Flores R., Caro L. F. A., Siewiora H. M., Jory D., 2019 Diversity of single-stranded DNA containing viruses in shrimp. Virus Disease 30(1):43-57.

DoF, 2020 Yearbook of fisheries statistics of Bangladesh, 2019-20. Fisheries Resources Survey System (FRSS), Department of Fisheries. Bangladesh: Ministry of Fisheries and Livestock, 37:141.

Eshik M. M. E., Abedin M. M., Punom N. J., Begum M. K., Rahman M. S., 2017 Molecular identification of AHPND positive *Vibrio parahaemolyticus* causing an outbreak in south-west shrimp farming regions of Bangladesh. Journal of Bangladesh Academy of Sciences 41(2):127-135.

Hong X., Lu L., Xu D., 2016 Progress in research on acute hepatopancreatic necrosis disease (AHPND). Aquaculture International 24:577-593.

Hossain M. M. M., Uddin M. I., Islam H., Fardoush J., Rupom M. A. H., Hossain M. M., Farjana N., Afroz R., Hasan-Uj-Jaman, Roy H. S., Shehab M. A. S., 2020 Diagnosis, genetic variations, virulence, and toxicity of AHPND-positive *Vibrio parahaemolyticus* in *Penaeus monodon*. Aquaculture International 28(6):2531-2546.

Hsieh C. Y., Chuang P. C., Chen L. C., Tu C., Chien M. S., Huang K. C., Kao H. F., Tung M. C., Tsai S. S., 2006 Infectious hypodermal and hematopoietic necrosis virus (IHHNV) infections in giant freshwater prawn, *Macrobrachium rosenbergii*. Aquaculture 258:73-79.

Johnson P. T., 1984 Viral diseases of marine invertebrates. Helgoländer Meeresuntersuchungen 37:65-98.

Kanjanasopa D., Pongtippatee P., Vanichviriyakit R., Wongtripop S., Pradeep P. J., Withyachumnarnkul B., 2015 An evidence on trans-ovarian transmission of *Monodon baculovirus* (MBV) infection in *Penaeus monodon*. Aquaculture 443:5-10.

Kongrueng J., Yingkajorn M., Bunpa S., Sermwittayawong N., Singkhamanan K., Vuddhakul V., 2015 Characterization of Vibrio parahaemolyticus causing acute hepatopancreatic necrosis disease in southern Thailand. Journal of Fish Diseases 38(11):957-966.

Kou G. H., Peng S. E., Chiu Y. L., Lo C. F. 1998 Tissue distribution of white spot syndrome virus (WSSV) in shrimp and crabs. In: Advances in Shrimp Biotechnology, Flegel T. W. (ed.), National Centre for Genetic Engineering and Biotechnology, Bangkok, Thailand pp. 267-271.

Lightner D. V., 1999 The penaeid shrimp viruses TSV, IHHNV, WSSV, and YHV: current status in the Americas, available diagnostic methods, and management strategies. Journal of Applied Aquaculture 9(2):27-52.

Lightner D. V., Redman R. M., Bell T. A., 1983 Observations on the geographic distribution, pathogenesis, and morphology of the baculovirus from *Penaeus monodon* Fabricius. Aquaculture 32(3-4):209-233.
Lo C. F., Leu J. H., Ho C. H., Chen C. H., Peng S. E., Chen Y. T., Chou C. M., Yeh P. Y., Huang C. J., Chou H. Y., Wang C. H., Kou G. H., 1996 Detection of baculovirus associated with white spot syndrome (WSBV) in penaeid shrimps using polymerase chain reaction. Diseases of Aquatic Organisms 25(1-2):133-141.
Martorelli S. R., Overstreet R. M., Jovonovich J. A., 2010 First report of viral pathogens WSSV and IHHNV in argentine crustaceans. Bulletin of Marine Science 86(1):117-131.
Muhammed S. T., 2018 Surveillance and animal health monitoring±early detection of disease. Asian Fisheries Science 31:194-209.
Natividad J. M., 1991 The *Penaeus monodon* baculovirus (MBV): its epizootiology, prevention, and control in penaeid shrimp hatcheries and grow-out ponds in the Philippines. PhD thesis, The University of Arizona, 203 pp.
OIE, 2003 Manual of diagnostic tests for aquatic animals. Office international des epizooties (OIE), Paris, 366 pp.
Otta S. K., Shubha G., Joseph B., Chakraborty A., Karunasagar I., Karunasagar I., 1999 Polymerase chain reaction (PCR) detection of white spot syndrome virus (WSSV) in cultured and wild crustaceans in India. Diseases of Aquatic Organisms 38:67-70.
Rajendran K. V., Makesh M., Karunasagar I., 2012 *Monodon baculovirus* of shrimp. Indian Journal of Virology 23(2):149-160.
Rai P., Safeena M. P., Krabsetsve K., La Fauce K., Owens L., Karunasagar I., 2012 Genomics, molecular epidemiology, and diagnostics of infectious hypodermal and hematopoietic necrosis virus. Indian Journal of Virology 23(2):203-214.
Soto-Rodriguez S. A., Gomez-Gil B., Lozano-Olvera R., Betancourt-Lozano M., Morales-Covarrubias M. S., 2015 Field and experimental evidence of *Vibrio parahaemolyticus* as the causative agent of acute hepatopancreatic necrosis disease of cultured shrimp (*Litopenaeus vannamei*) in Northwestern Mexico. Applied and Environmental Microbiology 81(5):1689-1699.
Tran L., Nunan L., Redman R. M., Mohney L. L., Pantoja C. R., Fitzsimmons K., Lightner D. V., 2013 Determination of the infectious nature of the agent of acute hepatopancreatic necrosis syndrome affecting penaeid shrimp. Diseases of Aquatic Organisms 105(1):45-55.
Uma A., Koteeswaran A., Karunasagar I., Karunasagar I., 2005 Prevalence of white spot syndrome virus and monodon baculovirus in *Penaeus monodon* broodstock and postlarvae from hatcheries in southeast coast of India. Current Science: 89(9):1619-1622.
Waqairatu S. S., Dierens L., Cowley J. A., Dixon T. J., Johnson K. N., Barnes A. C., Li Y., 2012 Genetic analysis of black tiger shrimp (*Penaeus monodon*) across its natural distribution range reveals more recent colonization of Fiji and other South Pacific islands. Ecology and Evolutionary Biology 2(8):2057-2071.
Wongteerasupaya C., Vickers J. E., Sriurairatana S., Nash G. L., Akarajamorn A., Boonsaeng V., Panyim S., Tassanakajon A, Withyachumnarnkul B., Flegel T. W., 1995 A non-occluded, systemic baculovirus that occurs in cells of ectodermal and mesodermal origin and causes high mortality in the black tiger prawn *Penaeus monodon*. Diseases of Aquatic Organisms 21:69-77.
Xia X., Yu Y., Hu L., Weidmann M., Pan Y., Yan S., Wang Y., 2015 Rapid detection of infectious hypodermal and hematopoietic necrosis virus (IHHNV) by real-time, isothermal recombinase polymerase amplification assay. Archives of Virology 160(4):987-994.
Yu Y. B., Choi J. H., Kang J. C., Kim H. J., Kim J. H., 2021 Shrimp bacterial and parasitic disease listed in the OIE: a review. Microbial Pathogenesis 166(7):105545.

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