SURVEILLANCE OF DISEASES IN FARMED PENAEUS VANNAMEI IN THE INDIAN SUNDERBANS

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ABSTRACT

Abraham, T.J. & Priyadarsani, L. (2019). Surveillance of Diseases in Farmed *Penaeus vannamei* in the Indian Sunderbans. Braz. J. Aquat. Sci. Technol. 23(1). eISSN 1983-9057. DOI: 14094/bjast.v23n1. *Penaeus vannamei* farming has been the major aquaculture practice in the Indian Sundarbans. This study investigated the physicochemical characteristics, management practices, distribution of vibrios, and the prevalence of bacterial, parasitic and viral diseases in 14 *P. vannamei* intensive culture farms in the Indian Sunderbans during 2016. The total heterotrophic counts of the pond water ranged from 3.53 to 6.21 $\log_{10}$ cfu/ml. The presumptive vibrios were in the range of 2.40-4.99 $\log_{10}$ cfu/ml. Mild vibriosis was noted in 3 farms. Twenty out of 21 isolates from the haemolymph samples were confirmed to be *Vibrio parahaemolyticus* by the PCR amplification of the toxR gene. No acute hepatopancreatic necrosis disease (AHPND) causing *V. parahaemolyticus* (Vp_{AHPND}) strain was, however, detected. Of the 14 shrimp farms surveyed, only one farm sample was white spot virus (WSV) positive and all others were negative. All the *P. vannamei* farms were negative for infectious hypodermal and haematopoietic necrosis virus, hepatopancreatic parvo-like virus, and infectious myonecrosis virus. No incidence of *Enterocytozoon hepatopenaei*, white faecal syndrome, running mortality syndrome, protozoan infestation, and luminous vibriosis was observed during the survey period. The survival rate was 90-95% in the normal ponds, while the lowest survival was 60% in an asymptomatic WSV infected farm. The physicochemical characteristics of the farms were well within the optimum, except for the WSV infected farm, which had high ammonia levels. Due to the lack of awareness on the *P. vannamei* farming practices and high operational costs, the biosecurity measures were not strictly followed in the surveyed farms.

Key Words: Indian Sundarbans, *Penaeus vannamei*, viral diseases, AHPND, biosecurity.

INTRODUCTION

Scientific culture of shrimp started in West Bengal, India during the mid-1980s and by 2010 more than 47,588 ha area was brought under shrimp culture. The development of coastal aquaculture in West Bengal was centred on *Peneaus monodon* farming until 2015. By 2015, the area under shrimp culture rose to 53,974 ha (MPEDA, 2015). The current aquaculture production of shrimp in West Bengal increased from 26,800 tons in 2001-2002 to 57369.77 tons in 2014-2015 (MPEDA, 2015). The first setback to the West Bengal shrimp culture industry was in 1996-1997, attributed mainly to the environmental degradation and outbreak of diseases of viral origin (Abraham & Sasmal, 2008). Several viruses caused diseases in *P. monodon*, *P. vannamei*, *P. stylirostris*, *Fenneropenaeus indicus*, *Marsupenaeus japonicus*, etc and the average annual economic losses are in the tune of 1 billion US$ (Claydon et al., 2010; Otta et al., 2014). *Vibrio* species are among the normal bacterial flora of both natural and cultural populations of shrimp and the culture environment, and many of them are pathogenic to shrimp (Yang et al., 2014; Anandaraja et al., 2017). The early mortality syndrome or acute hepatopancreatic necrosis disease (AHPND) is a newly emergent penaeid shrimp disease that caused serious economic losses in shrimp farms in southern China in 2010 and subsequently in Vietnam, Thailand, and Malaysia (FAO, 2013). The AHPND is caused only by some pathogenic strains of *Vibrio parahaemolyticus* (Yang et al., 2014). World farmed shrimp production volumes decreased in 2012 and particularly in 2013, mainly as a result of disease-related problems, such as AHPND (FAO, 2014). Difficulties in captive breeding of *P. monodon* could not make it possible for the development of Specific-Pathogen-Free (SPF) and genetically improved stains with disease resistance (Otta et al., 2014). In this context, the SPF strain of Pacific white leg shrimp (*P. vannamei*) was introduced in India in 2009, which revived the shrimp culture in India. The production of this species surpassed the production of *P. monodon* owing to its faster growth, compatibility to higher stocking rate, less disease risk, euryhaline as well as eurythermic nature, lower dietary protein requirement and lower feed conversion ratio (Raj et al., 2010; Kumaran et al., 2017). The Coastal Aquaculture Authority of India recommended a stocking rate of up to 60 m^2 (Raj et al., 2010). The Sundarbans is a cluster of low-lying islands in the Bay of Bengal, spread across India and Bangladesh,

famous for its unique mangrove forests. The Indian Sundarbans Delta is part of the delta of the Ganga-Brahmaputra-Meghna basin in Asia. This active delta region is among the largest in the world, measuring about 40,000 km^2 (Danda et al., 2011). Since 2014-15, the farming of *P. vannamei* has been the major practice in both brackishwater and freshwater areas of West Bengal including the Indian Sunderbans. This study investigated the management practices, physicochemical characteristics, distribution of vibrios, particularly *V. parahaemolyticus* and the prevalence of bacterial, parasitic and viral diseases in select *P. vannamei* farms of the Indian Sunderbans during 2016.

MATERIALS AND METHODS

Sampling area and sample collection

The disease surveillance in 14 *Penaeus vannamei* farms of the Indian Sunderbans located in Amratala, Gangadharpur, and Krishnanagar of South 24 Parganas district (n=7), and Hasnabad, Abad Mohanpur and Paschim Goberia of North 24 Parganas district (n=7), West Bengal, India (Figure 1) was carried out in 2016 as per the OIE guidelines (OIE, 2013).

Figure 1 - Google map of the Indian Sunderbans.

The water samples from the column region were collected in sterile 250 ml polypropylene bottles for bacteriology. The samples for water quality parameters were also collected from the column region in 300 ml glass bottles. All the samples were placed in an insulated container. Healthy and/or diseased *P. vannamei* samples (n=10) were collected from all the farms, packed separately in oxygen filled polythene bags and brought to the laboratory within 3 h of collection. The farm management practices were collected from the shrimp farmers at the time of sample collection.

Physicochemical analyses

The salinity, pH and dissolved oxygen (DO) were measured by conductivity probe (CDC401), pH probe (PHC281) and luminescent DO probe, respectively using Hach multiparameter kit (Hach, Loveland, CO, USA). The levels of ammonia and alkalinity were determined as per APHA/AWWA/WEF (2012).

Enumeration of bacterial counts in pond water

The spread plate technique was followed for the enumeration of total heterotrophic bacterial counts (THC), luminous bacterial counts (LBC) and presumptive vibrio counts (PVC) from the pond water samples as described earlier (Abraham & Palaniappan, 2004; Priyadarsani & Abraham, 2013). Aliquots (0.1 ml) of appropriately diluted water samples were spread on to the tryptone soya agar supplemented with 1% sodium chloride (NaCl) [TSA], seawater complex (SWC) agar (Ramaiah & Chandramohan, 1993) and thiosulphate citrate bile salt sucrose agar (TCBS) supplemented with 1% NaCl plates in duplicate. The plates were incubated at 30±2 °C for 16-48 h. Luminescence on SWC agar was observed in a dark room after 16-20 h of incubation. The numbers of colonies were counted and expressed as $\log_{10}$ cfu/ml.

Microscopy

The microscopic observations for the ectoparasitic infestation on the gills, exoskeleton and appendages are as described in Lightner (1993).

Vibrio isolation from haemolymph and preparation of bacterial lysate

About 100 µl haemolymph was collected from the ventral sinus of each *P. vannamei* using 26 gauge 1 ml tuberculin syringe, mixed with 10 ml alkaline peptone water (APW) and incubated at 30±2 °C overnight. Inocula from APW were streaked onto the TCBS and incubated 30±2 °C for 24 h. Representative green colour colonies on TCBS with distinct colony morphology were picked, purified by repeated streaking on TSA and then maintained on TSA soft agar tubes. An aliquot of the haemolymph sample was also streaked on to the SWC agar for the isolation of luminous vibrios. Luminescence was observed in dark after 16-20 h of incubation.

For the preparation of bacterial lysate, the bacterial isolates were first streaked onto the TSA plates to get discrete colonies. The isolated colonies were then transferred to tryptone soya broth with 1% NaCl and incubated at 30 °C separately. The culture broth was centrifuged (7,000 rpm, 4 °C, 10 min, Eppendorf, Germany) to obtain the pellet. The pellet was washed with normal saline (0.85% NaCl w/v) twice, and re-suspended in DNA-free sterile distilled water (180 µl). The suspension was boiled for 10 min in a water bath to lyse the cells, and to release the DNA. The lysate was centrifuged (7,000 rpm, 4 °C, 10 min), and the supernatant was stored at -20 °C for further use.

Identification of *Vibrio parahaemolyticus* by species-specific toxR gene and detection of *Vibrio parahaemolyticus* (Vp $_{AHPND}$) strain

The species-specific primers (toxR gene) were used for the identification of *V. parahaemolyticus* strains (Kim et al., 1999). The strains possessing the toxR gene were taken for the detection of Vp $_{AHPND}$ strain (Table 1). The PCR protocol for the amplification of AHPND-AP3 gene was followed for the confirmation of Vp $_{AHPND}$ strain (Sirikharin et al., 2014).

Extraction of viral nucleic acid from the pleopods and muscle tissue

The extraction of viral nucleic acid from the pooled pleopod samples or muscle tissue samples from the 4th and 5th abdominal segments (n=10) of each farm was carried out as per Kiatpathomchai et al. (2001). The primers used, their sequences, amplification sizes and the PCR protocols are as in Table 1.

Statistical analyses

Student 't' test was followed to test the significance of the difference between the various physico-chemical and bacteriological parameters of the pond water samples of two districts. The simple correlation was used to correlate the parameters using the Data Analysis Toolpak in Microsoft Excel.

RESULTS AND DISCUSSION

Management practices

The surveyed farms of the Indian Sunderbans followed the intensive monoculture of *P. vannamei*. The farming was done both in leased and own farms. All the surveyed farms of North 24 Parganas district were of a perennial type. The farms of South 24 Parganas district were of <2 ha; while in North 24 Parganas district, the farms were >7 ha in size. The shape of the pond was almost rectangular with a depth of 1.5–2.0 m. The source water for the farms of South 24 Parganas district was from the Kalnagini River. It was from the Bidyadhari River for the farms of North 24 Parganas district. The ponds of South 24 Parganas district were drained completely, dried for a period of about 7-10 days and scrapped the top layer to remove the sludge before stocking. The farmers used dolomite at 150 kg/ha for the treatment of soil. Biosecurity measures were followed, but not strictly in all the farms surveyed.

The SPF post-larvae (PL-10) of *P. vannamei* from the Oceanic shrimp hatchery, Marakkanam, Tamil Nadu and Rank Aqua, Nellore, Andhra Pradesh were stocked. The stocking densities of the farms varied from 50 to >60 PL/m^2. Artificial feed (CPE Blanca) was given to the shrimp as per the standard feeding schedule. The total duration of the crop was 90-120 days.

Table 1 - Primers used for the detection of toxR and AHPND-AP3 genes of *Vibrio parahaemolyticus*, and the viruses of *Penaeus vannamei.*

Genes	Primers	Size in bp	Reference
Vibrio parahaemolyticus			
toxR gene	toxR - F 5'- GTCTTCTGACGCAATCGTTG -3' toxR -R 5'- ATACGAGTGGTTGCTGTCATG -3'	394	Kim et al., 1999
Vibrio parahaemolyticus (Vp $_{AHPND}$) strain			
AHPND- AP3	CN2- F 5'-ATGAGTAACAATATAAAACATGAAAC -3' CN2-R 5'- GTGGTAATAGATTGTACAGAA -3'	336	Sirikharin et al., 2014
Shrimp viruses			
WSV 1st step PCR	P1- F 5'-ATCATGGCTGCTTCACAGAC -3' P2-R 5'-GGCTGGAGAGGACAAGACAT -3'	982	Kimura et al., 1996
WSV 2nd step PCR	P3- F 5'-TCTTCATCAGATGCTACTGC -3' P4-R 5'-TAACGCTATCCAGTATCACG -3'	570	Kimura et al., 1996
IHHNV	IHHNV392F 5'-GGGCGAACCAGAATCACTTA-3' IHHNV392R 5'-ATCCGGAGGAATCTGATGTG-3'	500	Tang et al., 2000
HPV	H441F 5'- GCATTACAAGAG CCAAGC AG- 3' H441R 5'-ACACTCAGCCTCTACCTT GT-3'	441	Phromjai et al., 2002
IMNV	4587F 5'-CGACGCTGCTAACCATACAA-3' 4914R 5'-ACTCGGCTGTTCGATCAAGT-3'	328	Poulos & Lightner, 2006
	4725 NF 5'-GGCACATGCTCAGAGACA-3' 4863 NR 5'-AGCGCTGAGTCCAGTCTTG-3'	139	

WSV: White spot virus; IHHNV: Infectious hypodermal and haematopoietic necrosis virus; HPV: Hepatopancreatic parvo-like virus; IMNV: Infectious myonecrosis virus; AHPND: Acute hepatopancreatic necrosis disease (*Vibrio parahaemolyticus* (Vp $_{AHPND}$)).

The chemicals and immunostimulants used were geolite (6 kg/1000 m^2), BioSeize (15 ml/kg feed), SuperPS (20 ml/kg feed), and Superbiotic (600 g/1000 m^2). The majority of the farmers used *Centella asiatica* (Indian pennywort, locally called as Thankuni) leaf juice along with feed (400 leaves/10 kg feed) to prevent the white faecal cast. Hydrogen peroxide (2.5 L/1000 m^2) was used for the improvement of water quality. These aquadrugs use patterns corroborate the observations of the earlier reports (Abraham et al., 2007; Anandaraja et al., 2012a). Immunostimulants have been reported to reduce shrimp mortalities associated with vibriosis and WSV (Balasubramanian et al., 2008; Anandaraja et al., 2012a).

Physicochemical characteristics of the pond water

The physicochemical characteristics of the pond water of *P. vannamei* culture systems of the Indian Sunderbans are presented in Table 2. The pH ranged between 7.50 and 9.32. Its level in the farms of South 24 Parganas district was always within the optimum range (6.6-8.5) recommended for shrimp farming (Boyd & Tucker, 1998). While in North 24 Parganas district farms, the pH levels were significantly higher ($P<0.01$) than the recommended level, with a mean of 8.69±0.42. *Penaeus vannamei* is a euryhaline species that can tolerate a wide range of salinities from <0.5 to 45 ppt (Bray et al., 1994; Araneda et al., 2008). The pond water salinity of the present study ranged between 8.52 and 14.20 ppt, with a mean of 10.58±2.46 ppt in North 24 Parganas district farms. On the other hand, its levels were significantly higher ($P<0.01$) in South 24 Parganas district farms (20.45±2.78 ppt). The minimum acceptable concentration of total alkalinity is 75 mg/l and the shrimp may have difficulty in moulting if the total alkalinity is less than 50 mg/l (Boyd & Tucker, 1998). The alkalinity levels were above the requirement except the farm located at Krishnanagar, South 24 Parganas district, which recorded frequent mortalities due to WSV. The oxygen dynamics of brackishwater shrimp culture ponds depend on the balance of the autotrophic and heterotrophic production. The DO values were well above the minimum requirement (3.5 ppm) for shrimp farming (Boyd & Tucker, 1998), with few farms recorded DO values as high as 15.51 ppm. The DO levels of North 24 Parganas district farms (12.74±2.27 ppm) were significantly higher ($P<0.01$), possibly due to the provision of aeration facilities. The results of the water quality parameters corroborate the findings of Priyadarsani & Abraham (2016) recorded in a low saline shrimp pond of West Bengal. The ammonia-N levels varied in different farms with a maximum of 1.54 ppm. The ammonia-N levels in the majority of the farms of the present study exceeded the tolerance limit of 0.1 ppm for *P. vannamei* (Lin & Chen, 2001). The correlation coefficients (r) between the physicochemical and bacteriological quality parameters of the *Penaeus vannamei* pond water are presented in Table 4. The salinity values were significantly but negatively correlated with pH, DO and THC ($P<0.01$). The correlation of pH and DO, pH and THC, DO and THC, and THC and PVC were also significant ($P<0.05$). The WSV infected farm had high ammonia levels possibly because of the wastage of feed due to the reduced feed uptake. In general, the water quality parameters, except ammonia, did not vary substantially during the entire study period, thus, nullifying their influence on the performance of the species.

Bacterial counts in pond water

The results of the THC, LBC and PVC and the proportion of vibrios in the THC of the *P. vannamei* pond water samples of the Indian Sunderbans are presented in Table 3. In shrimp culture ecosystem, certain bacteria play a negative role as they compete with shrimp for food and oxygen, thus, causing stress and disease. The prescribed range of THCs in the shrimp pond water is 3.00–4.00 $\log_{10}$ cfu/ml (Tookwinas, 2000). In the present study, the range of THC in the pond water samples was found to be 3.53-6.21 $\log_{10}$ cfu/ml, well above the prescribed range. Significantly higher bacterial counts ($P<0.05$) were noted in the farms of North 24 Parganas district due to the intensification in post-stocking management practices, particularly the feeding. The PVCs to a maximum of 2.00 $\log_{10}$ cfu/ml are recommended for shrimp

Table 2 - Physicochemical characteristics of the pond water samples of *Penaeus vannamei* culture systems of the Indian Sunderbans.

Parameters	Range (Mean±standard deviation)	Parameters
	South 24 Parganas district farms	North 24 Parganas district farms
pH	7.50-7.85 (7.76±0.12)[a]	8.20-9.32 (8.69±0.42)[a]
Salinity (ppt)	16.33-25.30 (20.45±2.78)[a]	8.52-14.20 (10.58±2.46)[a]
Dissolved oxygen (ppm)	4.20-9.43 (5.61±2.25)[a]	10.00-15.51 (12.74±2.27)[a]
Alkalinity (ppm)	70.00-220.00 (145.71±51.59)	140.00-200.00 (174.29±27.60)
Ammonia (ppm)	0.08-1.54 (0.63±0.59)	0.01-0.69 (0.20±0.29)

The values sharing common alphabet (a) within the row differed significantly ($P<0.01$).

Table 3 - Bacterial counts of the pond water samples of *Penaeus vannamei* culture systems of the Indian Sunderbans.

Parameters	Range (Mean±standard deviation)	
	South 24 Parganas District farms	North 24 Parganas district farms
Log# total heterotrophic bacterial counts (THC)/ml	3.53-4.49 (4.06±0.38)[a]	4.85-6.21 (5.45±0.53)[a]
Log# presumptive vibrio counts on TCBS/ml	2.40-4.09 (3.42±0.72)	3.37-4.99 (3.98±0.63)
Proportion of vibrios on TCBS in THC	5.16-80.39 (35.84±29.95)[a]	0.95-9.65 (4.66±3.34)[a]

#: Log_{10} cfu (colony forming units); None of the water samples exhibited luminous bacterial colonies on SWC agar. The values sharing common alphabet (a) within the row differed significantly (P<0.05).

Table 4 - Correlation coefficient (r) between the physicochemical and bacteriological quality parameters of the *Penaeus vannamei* pond water.

Parameters	pH	Salinity	Alkalinity	Ammonia	DO	THC	PVC
pH	1.000	-0.852*	0.214	-0.407	0.894*	0.838*	0.376
Salinity		1.000	-0.472	0.492	-0.722*	-0.823*	-0.485
Alkalinity			1.000	-0.126	0.239	0.355	0.291
Ammonia				1.000	-0.396	-0.189	0.182
DO					1.000	0.788*	0.174
THC						1.000	0.587**
PVC							1.000

DO: Dissolved oxygen ; THC: Total heterotrophic bacterial counts; PVC: Presumptive vibrio counts; *: P<0.01; **: P<0.05.

culture (Baliao, 2000). In the present study, the PVCs were found to be 2.40-4.99 log_{10} cfu/ml on TCBS agar, which was higher than the recommended level. The proportion of vibrios was significantly high (P<0.05) in South 24 Parganas district farms (35.84± 29.95%), as the mean salinity was 20.45±2.78 ppt. While in North 24 Parganas district farms, it was only 4.66±3.34% of the THC. No luminous bacterial growth was noted in the pond water samples, possibly due to the low and fluctuating salinities. Priyadarsani & Abraham (2013) also recorded comparatively low THC and PVC in a low saline culture system of West Bengal. In contrast, Anandaraja et al. (2017) recorded high levels of THC and PVC in *P. vanammei* farms of Tamil Nadu, India. Yet, the bacterial counts in the brackishwater ponds of West Bengal were reportedly influenced by the salinity and management practices (Abraham & Sasmal, 2009).

PCR confirmation of *Vibrio parahaemolyticus* (Vp_{AHPND}) strain

Mild vibriosis was noted in 3 farms as confirmed by the growth of vibrios from the haemolymph samples on TCBS agar. None of the haemolymph samples exhibited luminescent colonies on SWC agar. A total of 21 *V. parahaemolyticus*-like strains were isolated from 150 haemolymph samples of shrimp. *Vibrio parahaemolyticus* strains possess a regulatory gene, toxR and the PCR method targeting the specific toxR gene was used for their identification (Lin et al., 1993; Kim et al., 1999). Out of 21, 20 strains were confirmed to be *V. parahaemolyticus* by the amplification of the toxR gene (Figure 2a, b).

Nevertheless, none of the 20 *V. parahaemolyticus* strains of the present study was positive for the AHPND-AP3 gene, thus confirming the absence of *V. parahaemolyticus* (Vp_{AHPND}) strain or AHPND in the surveyed farms. The results corroborate the findings of Anandaraja et al. (2017) on AHPND in *P. vannamei* farms of Tamil Nadu, India.

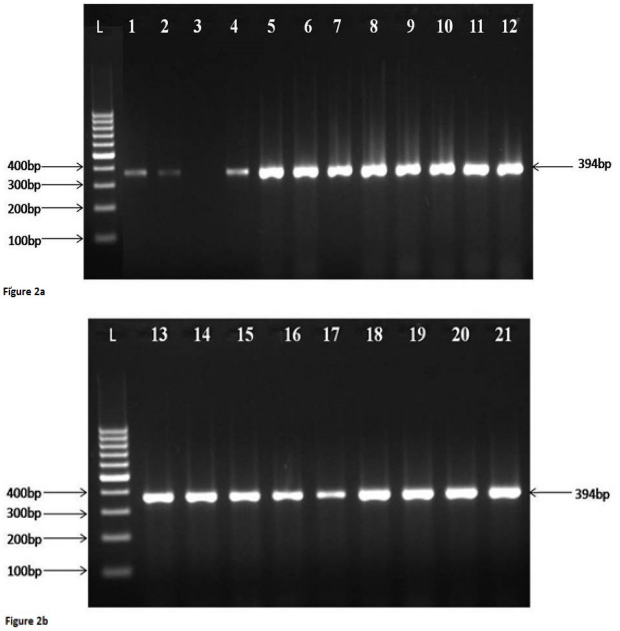

Figure 2 - PCR amplification of toxR gene of *Vibrio parahaemolyticus* strains. Lane L: 100 bp DNA Molecular weight marker; Lanes (a) 1-12, (b) 13-21: *Vibrio parahaemolyticus* strains isolated from shrimp haemolymph.

Detection of shrimp viral diseases and other abnormalities.

Of the 14 shrimp farm samples examined for WSV, only one farm sample from Krishnanagar, South 24 Parganas district was positive in the 1st and 2nd step PCR (Figures 3 and 4), which recorded the lowest survival rate (60%).

All other farm samples were WSV negative. The PCR positive *P. vannamei* samples did not show any typical clinical signs of disease, thus indicating the asymptomatic carrier state. The *P. vannamei* samples from all the farms were negative for IHHNV, IMNV and HPV. These results suggested that WSV is still a problem in shrimp aquaculture in the Indian Sunderbans as was observed in *P. monodon* (Mishra et al., 2005; Abraham & Sasmal, 2008; Anandaraja et al., 2012a,b; Dutta et al., 2013) possibly because of the use of poor quality hatchery-raised seeds and over-dependence of pesudoconsultants for seeds and aquadrugs. Muscle cramp condition was noted in two shrimp farms of South 24 Parganas district. No incidence of *Enterocytozoon hepatopenaei* (EHP), white faecal syndrome, running mortality syndrome, protozoan infestation, and luminous vibriosis were observed during the survey period (Table 5). The survival rate of *P. vannamei* was 90-95% in the normal ponds of both districts. The bodyweight of the shrimp was in the range of 25-35 g at the time of harvest. The production of shrimp was 6-7 tonnes/ha in both South and North 24 Parganas districts and some ponds in North 24 Parganas district achieved production up to 15 tonnes/ha. The epidemiological investigation by Anandaraja et al. (2012a) revealed the prevalence of diseased conditions like white spot viral disease, vibriosis, shell problems, gill problems, stunted and uneven growth, white faecal disease, gas bubble disease and yellow discoloration in brackishwater *P. monodon* culture systems of West Bengal, which was more in the traditional system. According to them, the use of known biosecurity measures had a dramatic impact on disease prevalence. The biosecurity measures were not strictly followed due

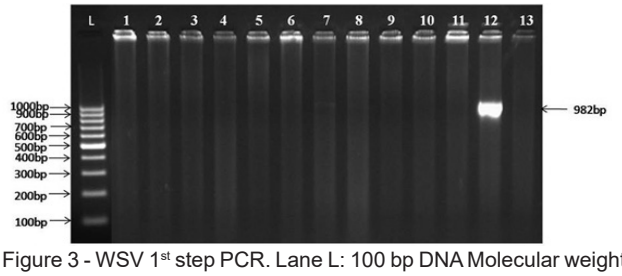

Figure 3 - WSV 1st step PCR. Lane L: 100 bp DNA Molecular weight marker; Lanes 1-13: WSV 1st step PCR products; Lane 7 was weakly positive in 1st step PCR for WSV; Lane 12: WSV positive sample.

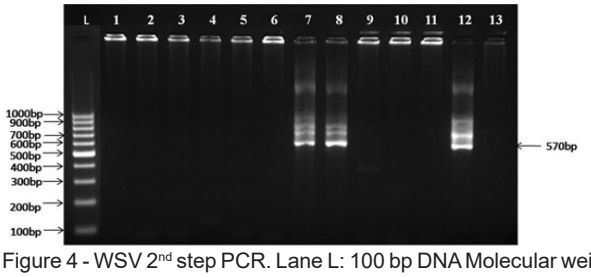

Figure 4 - WSV 2nd step PCR. Lane L: 100 bp DNA Molecular weight marker; Lanes 1-13 WSV 2nd step PCR products; Lanes 7 and 8 were positive in 2nd step PCR for WSV; Lane 12: WSV positive sample.

Table 5 - Diseases of *Penaeus vannamei* cultured in the Indian Sunderbans

	Disease	Status	Diagnosis
Existing diseases of *P. vannamei*			
A.	Viral		
1.	White spot virus	+*(1/14)	Level 3
2.	Infectious hypodermal and haematopoietic necrosis virus	-	Level 3
3.	Hepatopancreatic parvo-like virus	-	Level 3
B.	Bacterial		
1.	Vibriosis	+ (3/14)	Level 2 and 3
2.	Luminous vibriosis	-	Level 2
C.	Parasitic		
1.	Protozoan infestation	-	Level 1
D.	Environmental		
1.	Muscle cramp	+ (2/14)	Level 1
Emerging diseases of *P. vannamei*			
1.	Infectious myonecrosis virus	-	Level 3
2.	Acute hepatopancreatic necrosis disease (*Vibrio parahaemolyticus* (*Vp* AHPND))	-	Level 3
3.	White faecal syndrome	-*	Level 1
4	*Enterocytozoon hepatopenaei*	-	Level 1
5	Running mortality syndrome	-	Level 1

+*: Asymptomatic from South 24 Parganas district; -*: Absent at the time of sampling.

to the high operational costs and lack of awareness of *P. vannamei* farming practices as well as biosecurity measures. The recent study by Kumaran et al. (2017) concluded that the technical efficiency of the *P. vannamei* farms of West Bengal was relatively low compared to other shrimp-producing states of India. According to Anandaraja et al. (2012a), the use of PCR screening, application of immunostimulants, and strict biosecurity measures play major roles in containing disease outbreaks.

CONCLUSION

The main limitation in the shrimp farm activities of the Indian Sunderbans is the WSV disease outbreaks in *P. vannamei*, which is a great concern for the majority of farmers. As the development of brackishwater aquaculture in the Indian Sundarbans has been centred around the shrimp farming and the technical efficiencies of the P. vannamei farms were relatively low, special efforts are, therefore, needed to enhance the skills of the West Bengal shrimp farmers in the selection of disease-free seeds, better management practices, biosecurity measures, and optimize the input usage through the capacity enhancement programmes.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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