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***Research Article** (Open Access)*

SURVEILLANCE OF VIRAL INFECTIONS IN *LITOPENAEUS VANNAMEI* AND *PENAEUS MONODON* FROM GROW-OUT PONDS AND LANDING SITES

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ABSTARCT
Shrimp is indeed one of the most important crustacean species in aquaculture, primarily because of its high demand in global seafood markets. Infectious disease constitutes a major obstacle to the sustainability of shrimp aquaculture worldwide and a significant threat to shrimp populations and other crustacea. Seven water samples from respective shrimp ponds located in the study areas were collected during the study period for analysis of eight different water quality parameters. By analyzing the samples of *L. vannamei* and *P. monodon* collected from 7 grow-out ponds and 5 landing centers. It was found that one sample was positive for WSSV, another sample for EHP collected from grow-out ponds; whereas the samples from seawater were not. High stocking densities can promote the spread of diseases; limited number of species or strains that reduce genetic diversity; factors such as high ammonia levels, low oxygen levels, and presence of pollutants can stress the shrimp and make them more susceptible to infections; stress due to factors such as handling, transportation, and fluctuating environmental conditions can contribute to infections compared to the wild ones.

***Keywords**: L. vannamei, P. monodon, Water Quality Parameters, Viral Diseases, PCR*

INTRODUCTION

Aquaculture has attained significant growth and momentum in recent decades has been expanding at a remarkable pace, outpacing other sectors of food production particularly in rural areas; and also provides livelihoods to millions of people and helps to augment the production of seafood by to the increasing human population globally.

The age-old practice of capturing fish is showing a declining trend all over the world and to augment fish production, cultured fisheries integrated with agriculture and animal husbandry are practiced all over the world. To achieve this, several species are raised in captivity and there are a variety of farming practices with different intensities of inputs and technological sophistication. Among the crustacean animals, shrimp is an important aquaculture source due to its high demand in the world and marine shrimps account for majority of crustacean production and India, the world's second-largest aquaculture producer, generates 17.5 % of the world's farmed shrimp (FAO, 2020; Patil *et al.*, 2021). The Pacific white shrimp otherwise known as pinkish gold, *Litopenaeus vannamei*, is among the important species cultivated in shrimp farming operations and it continues to dominate the world crustacean production due to its quick adaptation, tolerate salinity levels. fewer outbreaks, accessibility to Specific Pathogen Free (SPF) domesticated strains. Farmers in various regions of India in recent years have begun culturing *L. vannamei* on a large scale in freshwater habitats.

Diseases of Shrimp

Viral and bacterial diseases are very important since they are responsible for huge economic losses in aquaculture systems worldwide. The global loss in shrimp production due to diseases for the past fifteen years has been estimated at US$ 15 billion with maximum loss in Asian countries (Walker and Wong, 2005). To date, 20 viruses belonging to the families of *Baculoviridae, Parvoviridae, Nimaviridae, Roniviridae,*

Totiviridae, and *Dicistroviridae* have been reported in penaeid shrimp (Bonami, 2008; Walker and Winton, 2010). Among these viruses, six viruses have been listed as notifiable diseases by the World Organization for Animal Health (OIE) (OIE, 2018). Economically important eight shrimp viruses are White Spot Syndrome Virus (WSSV), Yellow Head Virus (YHV), Taura Syndrome Virus (TSV), Infectious Hypodermal and Hematopoietic Necrosis Virus (IHHNV), Infectious Myonecrosis Virus (IMNV), Gill-associated Virus (GAV), Laem-Singh Virus (LSNV) and Monodon Baculovirus (MBV) (Flegel, 2010).

Viral Diseases of Shrimp

White Spot Syndrome Virus (WSSV) is one of the important shrimp viral pathogens causing white spot disease responsible for huge economic loss in the shrimp culture industry worldwide, including India. This virus was first reported in 1992 in Taiwan, and from there it spread to all shrimp-growing countries including India (Flegel, 1996). Disease outbreaks can reach a cumulative mortality of up to 100% within 3–7 days of infection (Escobedo-Bonilla *et al.,* 2008).

Infectious Hypodermal and Hematopoietic Necrosis Virus (IHHNV) was first reported in Hawaii in 1981 and was responsible for mass mortalities in farm-reared blue shrimp, *Litopenaeus stylirostris* (Lightner *et al.,* 1983). It is the smallest virus among the known penaeid shrimp viruses measures about 22 nm in diameter and is non-enveloped and icosahedron. It is a widely distributed single-stranded DNA parvovirus and is classified in the family Parvoviridae, genus Brevidensovirus. Two distinct genotypes of IHHNV namely type 1 from the Americas and East Asia and type 2 from South-East Asia have been identified (Tang *et al.,* 2003).

Yellow Head Virus (YHV) is another important RNA viral pathogen of shrimp. This virus was first reported in cultured *P. monodon* in central Thailand by Limsuwan (1991) and subsequent outbreaks have been reported in other Asian countries. Infected penaeid shrimp is characterized by high and rapid mortality, accompanied by gross signs of yellowing of the cephalothorax and general bleaching of body color. Histological examination revealed tissue necrosis containing vacuolated cells with hypertrophied nuclei and basophilic viral inclusions in the cytoplasm of infected cells of gill, lymphoid organs, hepatopancreas, midgut, head muscle, and hemocytes (Chantanachookin *et al.,* 1993).

Taura syndrome (TS) caused by TSV was first described in Ecuador in 1992 (Jimenez, 1992). TS lesions are primarily observed in the cuticular epithelium and subcuticular connective tissues of the gills, mouth, esophagus, stomach, hindgut, appendages, and general body of *L. vannamei* (Lightner *et al.*, 1995). TSV can be transmitted by horizontal transmission or by contaminated water. TSV is not transmitted vertically from broodstock to offspring (Lotz and Ogle, 1997).

Infectious Myonecrosis Virus (IMNV) is an icosahedral-shaped, non-enveloped virus of the *Totiviridae* family (Poulos *et al.,* 2006; Tang *et al.,* 2005). The disease was first described in cultured *L. vannamei* in northeast Brazil (Lightner, 2003) and spread to other shrimp-growing countries including India (Sahul Hameed *et al.,* 2017). IMN causes significant disease and mortality in juvenile and subadult pond-reared stocks of *L. vannamei*. *Enterocytozoon Hepatopenaei* (EHP) an emerging microsporidian shrimp pathogen has become a major threat to shrimp aquaculture around the world in recent years. EHP causes disease namely, Hepatopancreatic microsporidiosis in infected shrimp. Affected shrimp suffer from severe growth retardation without any clinical signs of infection or mortality. The occurrence of EHP infection in *Litopenaeus vannamei* is increasing quite frequently (Chaijarasphong, *et al.,* 2020). Tangprasittipap *et al.,* (2012), reported that EHP can be transmitted directly from shrimp to shrimp by cannibalism. It has been reported in different countries, as well as reported from different regions of India (Rajendran *et al.,* 2016; Santhoshkumar *et al.,* 2016; Biju *et al.,* 2016) and it causes severe economic loss similar to the loss caused by WSSV outbreak.

Survey of Shrimp Diseases

Surveillance of diseases of aquatic animals is becoming an integral component of all Government aquatic animal health programs. Surveillance is necessary to ensure that activities are in place for early warning of diseases of concern, contingency planning and monitoring of disease control measures; and provision of sound aquatic animal health advice to farmers, processors, and other stakeholders (Taukhid *et al.,* 2008).

Surveillance provides a mechanism for collecting and interpreting data on the health status of aquatic animal populations including specific diseases that are of concern to a country (Subasinghe *et al.*, 2004).

MATERIALS & METHODS

Collection of Water Samples

Water samples were collected from grow-out ponds in the sampling areas and landing centers. Water samples were collected in separate fresh plastic containers without any air bubbles. Before sampling, the containers were rinsed and filled with water. After collection, the containers were sealed tightly and labeled. The water samples were brought to the laboratory. Various physicochemical parameters such as water temperature, pH, salinity, and dissolved oxygen were measured at the farm itself. Other parameters such as alkalinity, hardness, ammonia, nitrite and nitrate were measured in the laboratory using standard procedures (Strickland and Parsons, 1968).

Physico-Chemical Analysis of Water Samples

The water temperature was measured in the field by using a mercury thermometer with a 0° to 100°C range and having a least count of 1°C. The transparency of the water was measured by the Secchi disk. Salinity was measured by salinometer (Model No- 211. HANNA instruments). One drop of water sample was placed on the main prism of the meter. Before placing the water sample, the prism of the meter was rinsed with distilled water and the meter scale was adjusted to 0 ppt with distilled water. The pH of water samples was measured using a digital pH meter (Systronics, India) nearest to 0.01. Before using the pH meter, it was calibrated with buffer solutions of pH 7 and 10. The pH electrode was immersed in the water samples to be tested without exceeding the maximum immersion level. Then the sample was stirred gently. After the reading stabilized it was recorded.

Dissolved oxygen in the water samples was measured by Winkler's method (Strickland & Parson 1968). The alkalinity of the water sample was estimated by titrating with standard Sulphuric acid (0.02N) at room temperature using phenolphthalein and methyl orange indicator (APHA, 1992). The total hardness of water samples was measured by a titrimetric method using ethylenediamine-tetra acetic acid (US-EPA, 2012). Nitrate nitrogen (NO3-N) was determined using the phenol disulfonic acid spectral light degree method (US-EPA, 2012). Nitrite nitrogen (NO2-N) was measured using naphthalene ethylenediamine and ammonia nitrogen (NH3-N) was measured by Nessler's reagent colorimetric method (US-EPA, 2012). The below is the result of sea water parameters:

Salinity: Approximately 35 parts per thousand (ppt)
Temperature: Ranges from -2°C to 30°C (28°F to 86°F).
pH:7.5 - 8.4, slightly alkaline.
Dissolved Oxygen: about 4 to 8 milligrams per liter (mg/L).
Nutrient Concentrations: Including NO_3, PO_4, and silicates,
Pressure: 1 atmosphere of pressure / 10-meter depth.

Collection of Animal Samples

Animal samples were collected from grow-out ponds culturing *Litopenaeus vannamei* located at Ponneri, Thiruvallur district and landing center from Chennai, Tamil Nadu on the coast of India for the screening of pathogens of shrimp. The samples include healthy and disease-suspected shrimp. The collected shrimp samples were washed in sterile water, placed in sterile plastic bags, transported to the laboratory on ice and stored at -80°C for further analysis. Conditions and behavior of animals were also observed at the time of sample collection. Both suspected and healthy shrimp samples collected from the grow-out pond and landing center mentioned above were screened by PCR and RT-PCR for WSSV, IHHNV, EHP, and IMNV, respectively.

Preparation of Shrimp Samples

The different organs collected from shrimp were homogenized together in NTE buffer (0.2 M NaCl, 0.02 M Tris–HCl, and 0.02 M EDTA, pH 7.4) using a sterile pestle and mortar. The tissue suspension was prepared in the ratio of 1:10 (w/v) using the NTE buffer. After homogenization, the tissue suspension was centrifuged at 3,000 *rpm* for 15 min at 4°C. After centrifugation, the supernatant was collected and re-centrifuged at 8,000 rpm for 20 min at 4°C. The supernatant was collected and stored at -20°C for PCR and RT-PCR assays to detect WSSV, IHHNV, IMNV, and EHP.

DNA Extraction

The tissue suspension was centrifuged at 3000 rpm for 15 min at 4°C and the supernatant was collected. Then, the tissue suspension prepared from different organs was mixed with an appropriate amount of digestion buffer (100 mM NaCl, 10 mM Tris-HCl, pH 8.0, 50 mM EDTA, pH 8.0, 0.5% sodium dodecyl sulfate and 0.1 mg mL^{-1} proteinase K) and incubated for 2 hours at 65°C to extract the DNA. After incubation, the digests were deproteinized by successive phenol/chloroform/isoamyl alcohol extraction and DNA was recovered by ethanol precipitation, drying, and resuspension in TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 7.5) and quantified by measuring optical absorbance at 260 nm using UV spectrophotometer (Shimadzu, Japan). DNA was recovered by ethanol precipitation and dried. The dried DNA pellet was suspended in the TE buffer and used as a template for PCR amplification to detect WSSV, IHHNV and EHP.

RNA Extraction

The different tissue suspensions prepared from different organs of infected shrimp as mentioned above were mixed with TRIzol separately and the RNA was extracted following the protocol (Invitrogen, USA). Briefly, 1 mL of TRIzol reagent was added to 200 µL of clarified supernatant and mixed thoroughly. After 5 minutes of incubation at room temperature, 0.2 mL of chloroform was added. The sample was vigorously shaken for 2-3 min at room temperature and centrifuged at 12,000 rpm for 15 min at room temperature. RNA was precipitated from the aqueous phase with isopropanol, washed with 75% ethanol and dissolved in 50 µL of TE buffer (10 mM Tris–HCl, 1 mM EDTA, pH 7.5). The RNA was quantified by measuring the absorbance at 260 nm, and the purity was checked by measuring the ratio of OD260 nm/OD280 nm. The RNA was stored at -20ºC for RT-PCR assay.

cDNA *Synthesis and Reverse Transcriptase Polymerase Chain Reaction* (RT-PCR)

Reverse transcriptase-polymerase chain reaction (RT-PCR) was carried out to screen IMNV infection in the shrimp samples collected from the grow-out ponds using standard RT-PCR protocol consisted of reverse transcription (RT) and amplification. To synthesize cDNA, reverse transcription was carried out in a 20 µl reaction containing 2× conversion master mix, 4 µm oligo(dT) primer, and 1 mM dNTP (Invitrogen). The RT reaction was performed at 37°C for 90 min and inactivated at 95°C for 3 min. After cDNA synthesis, PCR was carried out in a 20 µl reaction mixture containing 2 µl of cDNA, and 1 µM of each primer specific to each virus.

The DNA extracted from shrimp samples and cDNA synthesized from the RNA was used to detect the presence of viral pathogens (WSSV, IHHNV, and IMNV) and fungal pathogens (EHP). The primer set used to detect WSSV was based on the nucleotide sequence of WSSV available at GenBank (accession number: AF380842) (Van Hulten *et al.*, 2000; Yoganandhan, *et al.*, 2004).

A primer set designed by Sivakumar et al. (2018) was used to differentiate old and new strains of WSSV. The published primer set was used to detect IHHNV (Hou, Wu, Xu, and Yang, 2009). The primer set designed by Tang et al. (2015) was used to detect EHP (Poulos and Lightner, 2006) designed the primer set to detect IMNV in shrimp samples The details of the primer sequence, annealing temperature, and size of the amplified products are given in Table 1.

Table 1: Details of primer set used to detect viral and fungal pathogens in shrimp samples collected from grow-out farms

Primer set used	Genbank Accession No.	Primer sequence 5′–3′	Annealing temperature (°C)	Product size (bp)	Author's name and Year
WSSV–F WSSV–R	AF380842	CGCGGATCCGATGGATCTTTCTTTCACTCTTTC CCGGAATTCTTACTCGGTCTCAGTGCCAG	58	615	Van Hulten *et al.*, 2000; Yoganandhan, 2004
EHP–F EHP–R	KP825331	GCCTGAGAGATGGCTCCCACGT GCGTACTATCCCCAGAGCCCGA	60	510	Tang *et al.*, 2015
IHHNV–F IHHNV–R	JN616415.1	CGCGAATTCATGAACTTATATCTCTATGGA CCGAAGCTTTTAGTTAGTATGCATAATATA	55	757	Hou, Wu, Xu, and Yang, 2009
IMNV–F IMNV–R	AY570982.3	CGACGCTGCTAACCATACAA ACTCGGCTGTTCGATCAAGT	58	328	Poulos and Lightner, 2006

The mixture of PCR assay consisted of 1 µl of template DNA, 1 mM of each primer specific to WSSV, IHHNV, or EHP, 200 mM of deoxynucleotide triphosphate, and 1.25 U of DNA Taq polymerase in PCR buffer supplied with a commercially available kit. The PCR assay for the detection of WSSV, IHHNV, and EHP was carried out separately with a common PCR program. The PCR-amplified products were analyzed by electrophoresis in 0.8% agarose gels stained with ethidium bromide and visualized by ultraviolet transillumination.

Agarose Gel Electrophoresis

Polymerase chain reaction products were analyzed by electrophoresis in agarose gels stained with ethidium bromide. The reagents required for agarose gel electrophoresis are as follows:

A. Agarose gels ranging from 0.7% - 1.2% were used for the separation of DNA depending on the size of the product or fragments to be separated. Gels were prepared with a TEB (1x) buffer.

B. The PCR amplified product mixed in 1/3 volume of loading dye was loaded along with the DNA marker.

Electrophoresis was performed at 10 V/cm until the dye reached the end of the gel. The gel was then gently removed and stained in an ethidium bromide staining solution for 15 minutes. Destaining was done in distilled water or TEB solution with gentle shaking for 15-30 minutes. The gel was visualized by ultraviolet transillumination and the gel results were documented by the gel documentation system (Bio-Rad, USA).

RESULTS & DISCUSSION

Shrimp is indeed one of the most important crustacean species in aquaculture, primarily because of its high demand in global seafood markets. Viral infections in shrimp can cause significant economic loss in aquaculture operations.

To monitor disease outbreaks in shrimp populations, a survey has been carried out in *L. vannamei* cultured in grow-out farms and landing centers from Chennai. *L. vannamei* along with water samples were collected from the grow-out pond located at Ponneri in Thiruvallur district, and *Penaeus monodon* from the landing center in Chennai, Tamil Nadu. During the surveillance, shrimp samples were collected from 7 different grow-out ponds and 5 samples from the landing center, screened for WSSV, IMNV, IHHNV, and EHP by PCR and RT-PCR using pathogen-specific primers (Table 1). The size of shrimp collected from ponds ranged from 0.1 to 15 g and ranged from 20 to 93 days of culture (DoC). The clinical signs observed in the shrimp collected from some disease outbreaks include anorexia, lethargy, reddening of appendages and abdominal segments, and stunted growth. The mortality of shrimp from these outbreak ponds ranged from 20% to 50%. A total of 7 water samples from 7 shrimp ponds located in the study areas were collected during the study period for analysis of eight different water quality parameters. Water temperature was found to slightly vary in different ponds during the study period between 23 - and 27ºC. The maximum water temperature was recorded at 27ºC. The pH value of pond water reached the highest value of 8.7 and lowest value of 6.4. The salinity ranged from 0 to 48 ppt in the study areas. The dissolved oxygen content of pond water ranged from 2.5 to 8.7 mg/L in the present study. The total alkalinity of pond water ranged from 50 to 300 mg/L. The total hardness of pond water ranged from 900 to 8000 ppm. Ammonia content in pond water samples ranged from 0 to 2 mg/L. Nitrite content observed in the water samples collected from shrimp ponds ranged from 0 to 1.0 mg/L.

All the shrimp samples collected from grow-out ponds and landing centers during the surveillance period were screened for WSSV, IHHNV, and EHP by single-step PCR and IMNV by RT-PCR. A prominent band of 615 bp was observed in shrimp which showed positive to WSSV (Fig. 1), whereas a PCR band of 510 bp was observed in the agarose gel in EHP-positive shrimp samples (Fig. 2). Totally 7 samples collected from ponds located at different places of Ponneri, Thiruvallur district and 5 samples from the landing center from Chennai were screened for WSSV, IHHNV, IMNV, and EHP. Among the samples screened for WSSV, one sample was found to be positive for WSSV, and one sample was positive for EHP in samples collected from grow-out ponds. No sample showed positive for IHHNV or IMNV in the present study. The WSSV detected in the sample was found to be a new strain of WSSV. No multiple infections were observed in the samples collected during the surveillance period. All five samples collected from the landing center were found to be free of screened pathogens.

The present study on disease surveillance carried out revealed the incidence of disease outbreaks caused by WSSV and EHP in the grow-out pond and no pathogens were found to be positive from samples collected from the landing center in Chennai, Tamil Nadu. Shrimp caught from the landing center live in their natural habitat, where they may encounter fewer pathogens compared to those in densely stocked aquaculture ponds. This reduced exposure to pathogens can result in lower infection rates among wild-caught shrimp. Also, they have better immunity due to their exposure to natural environments and predators. This can make them more resilient to infections compared to shrimp raised in aquaculture ponds, where they may be more susceptible to diseases due to high stocking densities and stress.

L. vannamei was introduced legally into India to avoid the WSSV infection and help the shrimp farmers to make use of the facilities developed for *P. monodon* to culture *L. vannamei*. *Penaeus monodon* was gradually replaced by L. vannamei in most of the grow-out ponds and production of *L. vannamei* reached almost 5 lakh tonnes in 2017. As expected, the culture of *L. vannamei* was found to be successful in the beginning but is now facing difficulties because of serious disease problems caused by EHP, IMNV, and WSSV (Rajendran *et al.,* 2016; Sahul Hameed *et al.,* 2017; Santhoshkumar *et al.,* 2016). Recently, a new strain of WSSV with a deletion of 13,170 bp at five positions in the genome of WSSV relative to WSSV-TH was reported after the introduction of *L. vannamei* into Indian waters (Sivakumar *et al.,* 2018). The new strain was found to be more virulent when compared to the old strain of WSSV, and surveillance programs carried out since 2002 revealed the replacement of the old strain of WSSV by the new strain of WSSV in Indian shrimp culture system (Sivakumar *et al.,* 2018). To avoid disease problems and also to make use of the freshwater environment more profitable, regular monitoring, surveillance, and collaboration among

stakeholders are essential for maintaining high standards of shrimp health and safety in seafood supply chains.

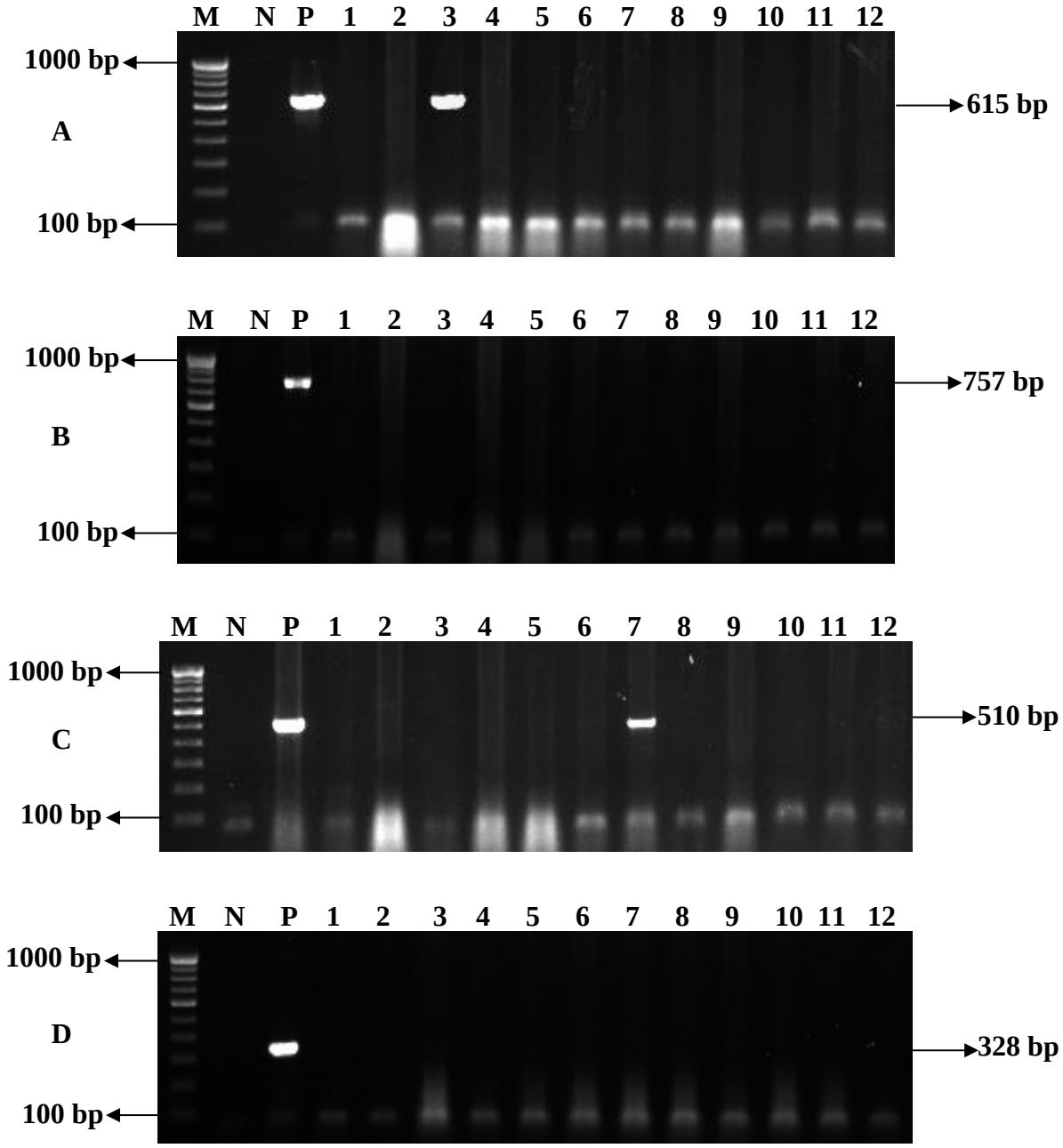

Figure 1: Detection of WSSV (A), IHHNV (B), EHP (C) and IMNV (D) in the shrimp (*Litopenaeus vannamei & Penaeus monodon*) samples collected from grow-out ponds and landing center with disease outbreak. Lane M - 100 bp marker, Lane N - Negative control; Lane P - Positive control; Lane 1 to 7 - Shrimp samples collected from 7 different grow-out shrimp ponds from Ponneri Lane 8 to 12 - Shrimp samples collected from landing center

By analyzing the samples of *L. vannamei* and *P. monodon* collected from 7 grow-out ponds and 5 landing centers. It was found that one sample was positive for WSSV, and one sample was positive for EHP collected from grow-out ponds; whereas the samples from seawater were not. This is due to the high stocking densities, that can promote the spread of diseases; limited number of species or strains, which can reduce genetic diversity; factors such as high ammonia levels, low oxygen levels, and presence of pollutants can stress the shrimp and make them more susceptible to infections; stress due to factors such as handling, transportation, and fluctuating environmental conditions.

Stress weakens the shrimp's immune system, making them more vulnerable to infections, including viral diseases. Shrimp farms often rely on water exchange or movement of live animals, which can introduce pathogens into the system. In contrast, wild or sea shrimp populations have more space, natural predators, and a diverse range of habitats that can help limit the spread of diseases.

Additionally, wild shrimp populations often have stronger immune systems due to natural selection pressures, making them more resilient to viral infections. Thus, farm shrimp are more susceptible to diseases than wild shrimp. To avoid disease problems and also to make use of the freshwater environment profitable regular monitoring, surveillance and collaboration among stakeholders are essential for maintaining high standards of shrimp health and safety in seafood supply chains.

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REFERENCES

APHA. (1992). Standard Methods for the Examination of Water and Wastewater. 18th edition. American Public Health Association (APHA), American Water Works Association (AWWA) and Water Pollution Control Federation (WPCF), Washington, DC.

Biju, N., Sathiyaraj, G., Raj, M., Shanmugam, V., Baskaran, B., Govindan, U., Kumaresan, G., Kasthuri Raja, K.K. and Chellamma, T.S.R.Y., (2016). High prevalence of Enterocytozoon hepatopenaei in shrimps Penaeus monodon and Litopenaeus vannamei sampled from slow-growth ponds in India. Diseases of aquatic organisms, **120**(3), 225-230.

Bonami, J.R. (2008). Shrimp Viruses. In: Mahy, B.W.J., Regenmortel, M.H.V.v. (Eds.), *Encyclopedia of Virology*. Academic Press, Oxford, 567-576.

Chaijarasphong, T., Munkongwongsiri, N., Stentiford, G., Aldama-Cano, D.J., K. Thansa, K., Flegel, T., Sritunyalucksana, K. and Itsathitphaisarn. O. (2020). *Journal of Invertebrate. Pathology 86:107458. doi: 10.1016/j.jip.2020.107458.*

Chantanachookin, C., Boonyaratpalin, S., Kasornchandra, J., Direkbusarakom, S., Ekpanithanpong, U., Supamataya, K., Sriurairatana, S. and Flegel, T.W., (1993). Histology and ultrastructure reveal a new granulosis-like virus in Penaeus monodon affected by yellow-head disease. *Diseases of Aquatic Organisms*, **17**, pp.145-145.

FAO., (2020). *The State of Food and Agriculture 2001* (No. 33). Food & Agriculture Org.

Flegel, T.W., (2010). Importance of host-viral interactions in the control of shrimp disease outbreaks. *The shrimp book*, pp.623-654.

Flegel, T.W., Boonyaratpalin, S., Withyachumnamkul, B., (1996). Current status of research on Yellow-head Virus and White-spot Virus in Thailand. *World Aquaculture Society*. Bangkok, Thailand, 126 Garcia-Ulloa **M. (2004).** Acuacultivos del Real. *Panorama Acuı´cola Magazine.* 10(1):86–87 Green B.W. (2008). Stocking strategies for the production of *Litopenaeus vannamei* (Boone) in amended freshwater in inland ponds. *Aquaculture Research*, **39**(1):10–17. https://doi.org/10.1111/j.1365-2109.2007.01849.x

Jimenez, R., (1992). Síndrome de taura (Resumen). *Acuacultura del Ecuador*.

Lightner, D.V., (2003). The penaeid shrimp viral pandemics due to IHHNV, WSSV, TSV and YHV: history in the Americas and current status. *In Proceedings of the 32nd Joint UJNR Aquaculture Panel Symposium, Davis and Santa Barbara, California*, USA (pp. 17-20).

Lightner, D.V., Redman, R.M., Arce, S. and Moss, S.M., (2006). Specific pathogen-free shrimp stocks in shrimp farming facilities as a novel method for disease control in crustaceans. *In Shellfish safety and quality* (pp. 384-424).

Lightner, D.V., Redman, R.M., Bell, T.A. and Brock, J.A., (1983). Detection of IHHN virus in *Penaeus stylirostris* and *P. vannamei* imported into Hawaii. *Journal of the World Mariculture Society*, **14**(1-4), pp.212-225.

Lightner, D.V., Redman, R.M., Hasson, K.W. and Pantoja, C.R., (1995). Taura syndrome in *Penaeus vannamei* (Crustacea: Decapoda): gross signs, histopathology and ultrastructure. *Diseases of Aquatic Organisms*, **21**(1), 53-59.

Limsuwan, C. (1991) Handbook for cultivation of black tiger prawns Tansetakit Co Ltd, 222 Tansetaketi Building, Wipawadee - Rangsit Road, Ladyaw, Chatujak, Bangkok, 202 p

Lotz, J.M. and Ogle, J., (1997). Taura syndrome virus and reproduction of *Penaeus vannamei.* In "World aquaculture 97", Book of Abstracts. World Aquaculture Society, Baton Rouge, LA, USA, 294.

OIE, 2018. Annual report on antimicrobial agents intended to use in animals. World Organisation for Animal Health (OIE), 2018.

Patil. P.K., Geetha. R., Ravisankar. T., Avunje, S., Gokal S. H., Jawahar. T.A., Pandiyan. V.S.., Poochirian. J.K., Vinayakarao. A.S. and Vijayan. K.k. (2020). Economic loss due to diseases in Indian shrimp farming with special reference to Enterocytozoon hepatopenaei (EHP) and white spot syndrome virus (WSSV). *Aquacult.* Volume **533**: 25 https://doi.org/10.1016/j.aquaculture.2020.736231.

Poulos, B. T., Tang, K. F., Pantoja, C. R., Bonami, J. R., & Lightner, D. V. (2006). Purification and characterization of infectious myonecrosis virus of penaeid shrimp. *Journal of General Virology*, **87**(4), 987–996

Poulos, B.T. and Lightner, D.V., (2006). Detection of infectious myonecrosis virus (IMNV) of penaeid shrimp by reverse-transcriptase polymerase chain reaction (RT-PCR). *Diseases of aquatic organisms*, **73**(1), 69-72.

Rajendran, K.V., Shivam, S., Praveena, P.E., Rajan, J.J.S., Kumar, T.S., Avunje, S., Jagadeesan, V., Babu, **S.P., Pande, A., Krishnan, A.N. and Alavandi, S.V., 2016()**. The emergence of Enterocytozoon hepatopenaei (EHP) in farmed *Penaeus (Litopenaeus) vannamei* in India. *Aquaculture*, 454, pp.272-280.

Sahul Hameed, A.S., Abdul Majeed, S., Vimal, S., Madan, N., Rajkumar, T., Santhoshkumar, S. and Sivakumar, S., (2017). Studies on the occurrence of infectious myonecrosis virus in ponds-reared *Litopenaeus vannamei* (Boone, 1931) in India. *Journal of fish diseases*, **40**(12):1823-1830.

Sahul Hameed, A.S., Abdul Majeed, S., Vimal, S., Madan, N., Rajkumar, T., Santhoshkumar, S. and Sivakumar, S., (2017). Studies on the occurrence of infectious myonecrosis virus in ponds-reared *Litopenaeus vannamei* (Boone, 1931) in India. *Journal of fish diseases*, **40**(12):1823-1830.

Santhoshkumar, S., Sivakumar, S., Vimal, S., Abdul Majeed, S., Taju, G., Haribabu, P., Uma, A. and Sahul Hameed, A.S., (2016). Biochemical changes and tissue distribution of *Enterocytozoon hepatopenaei* (EHP) in naturally and experimentally EHP-infected whiteleg shrimp, *Litopenaeus vannamei* (Boone, 1931), in India. *Journal of fish diseases*, 40(4):529-539.

Sivakumar, S., Vimal, S., Abdul Majeed, S., Santhosh Kumar, S., Taju, G., Madan, N., Sivasubbu, S. 2018(). A new strain of white spot syndrome virus affecting *Litopenaeus vannamei* in Indian shrimp farms. *Journal of Fish Diseases*, **41**, 1129–1146. https://doi.org/10.1111/ jfd.12811.

Strickland, J. D. H. and Parsons, T. R., (1968). A Practical Handbook of Seawater Analysis (2 rid Edition. *Journal of the Fisheries Research Board of Canada* 167), pp 310.

Subasinghe, R.P., McGladdery, S.E. and Hill, B.J. eds., (2004). *Surveillance and zoning for aquatic animal diseases* (No. 451). *Food & Agriculture Org.*

Tang, K.F., Pantoja, C.R., Poulos, B.T., Redman, R.M. and Lightner, D.V., (2005). In situ hybridization demonstrates that *Litopenaeus vannamei*, *L. stylirostris*, and *Penaeus monodon* are susceptible to experimental infection with infectious myonecrosis virus (IMNV). *Diseases of aquatic organisms*, **63**(2-3):261-265.
Tang, K.F., Pantoja, C.R., Redman, R.M., Han, J.E., Tran, L.H. and Lightner, D.V., (2015). Development of in situ hybridization and PCR assays for the detection of *Enterocytozoon hepatorenal* (EHP), a microsporidian parasite infecting penaeid shrimp. *Journal of invertebrate pathology*, **130**:37-41.
Tang, K.F., Poulos, B.T., Wang, J., Redman, R.M., Shih, H.H. and Lightner, D.V., (2003). Geographic variations among infectious hypodermal and hematopoietic necrosis viruses (IHHNV) isolates and characteristics of their infection. *Diseases of Aquatic Organisms*, **53**(2), pp.91-99.
Tangprasittipap, A., Srisala, J., Chouwdee, S., Somboon, M., Chuchird, N., Limsuwan, C., Srisuvan, T., Flegel, T.W. and Sritunyalucksana, K., (2013). The microsporidian *Enterocytozoon hepatopenaei* is not the cause of white feces syndrome in white-leg shrimp *Penaeus (Litopenaeus) vannamei*. *BMC Veterinary Research*, **9**(1):139.
Taukhid, T., Supriyadi, H. and Koesharyani, I., 2008(). SURVEY OF VIRAL DISEASES OF PACIFIC WHITE SHRIMP, *Litopenaeus vannamei* IN INDONESIA. *Indonesian Aquaculture Journal*, **3**(1), 59-68.
U.S. EPA, (2012). Edition of the Drinking water standards and health advisories. Washington, D.C. September 2012.
Van Hulten, M.C.W., Witteveldt, J., Peters, S., Kloosterboer, N., Tarchini, R., Fiers, M., Sandbrink, H., Lankhorst, R.K., Vlak, J.M., (2001). The White Spot syndrome virus DNA genome sequence. *Virology* 286, 7–22.
Walker, A. and Wong, C.K. eds., (2005). East Asian welfare regimes in transition: from *Confucianism to globalization. Policy Press.*
Walker, P.J. and Winton, J.R., (2010). Emerging viral diseases of fish and shrimp. *Veterinary research*, **41**(6), p.51.
Yang, Z.F., Cheng, H.X., Xi, X.H. and Liu, A.H., (2005). Regional ecological geochemical assessment: ideas and prospects. *Geological Bulletin of China*, **24**(8), 689-696.
Yoganandhan, K., Syed Musthaq, S., and Sahul Hameed, A.S. (2004). Production of polyclonal antiserum against recombinant VP28 protein and its application for the white spot syndrome virus in crustaceans. Journal of Fish Diseases, **27**: 517-522.