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Research Article

Prevalence of disease outbreak from cultured whiteleg shrimp *Penaeus vannamei* farms located in Karnataka, India

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Abstract

Penaeus vannamei farming is one of the profitable businesses of the aquaculture sector in India. As a result of excessive farming, shrimps were susceptible to various exotic, transboundary, and emerging pathogens. The purpose of this study is to monitor the prevalence of shrimp pathogens in Karnataka. A total of 91 samples of *P. vannamei* post larvae and juveniles were collected from April 2022 to November 2022 in Karnataka. Samples were screened for pathogens as listed in Office of Internationale des Epizootics (OIE) or World Organisation for Animal Health (WOAH) which include infectious hypodermal and hematopoietic necrosis virus (IHHNV), *Enterocytozoon hepatopenaei* (EHP), acute hepatopancreatic necrosis disease (AHPND), hepatopancreatic parvovirus (HPV), infectious myonecrosis virus (IMNV), yellow head virus (YHV), taura syndrome virus (TSV), white spot syndrome virus (WSSV), and other diseases such as decapod iridescent virus-1(DIV-1), and monodon baculovirus (MBV). Out of 91 samples, 5 (5.5%) samples were positive for WSSV, 26 (28.6%) samples were positive for EHP, and 2 (2.2%) samples had co-infection caused by EHP and WSSV. In this study, we have reported a high prevalence of EHP than WSSV in all three coastal districts of Karnataka. Farmers were using specific pathogen-free (SPF) seeds for culture but still, their cultures are getting infected with the same pathogens which indicates poor pond preparation and bio-security. So we strongly recommend that farmers have to follow good management practices and bio-security to increase the productivity and sustainability of *P. vannamei* farming in India.

Keywords bio-security, polymerase chain reaction, specific pathogen free, whiteleg

Introduction

Shrimp farming is a multibillion-dollar industry in the aquaculture sector with an estimated production of 4.45 million tons in 2018 [1]. In 1990, the culture of tiger shrimp, *Penaeus monodon*, was in steady progress worldwide but later it was wiped out by the massive outbreak of white spot disease [2]. As a result, farmers began seeking alternative species that were resistant and fast-growing when compare to *Penaeus monodon*. Because of several factors, the most important of which was the prospect

of generating an SPF strain, the Pacific whiteleg shrimp (*Penaeus vannamei*) was chosen. Later on, *P. vannamei* became one of the most frequently cultivable shrimp species globally [3]. India is among the top producer of *P. vannamei* with an estimated production of 0.815 million tons in 2021 [4]. An economic boom resulted from the rapid expansion of shrimp aquaculture, but regrettably, disease outbreaks have increased the economic uncertainty and hampered industry growth [5]. Furthermore, special efforts were made to obtain SPF *P. vannamei*, despite its SPF status, is unable to avoid diseases that are already present in the areas where it was introduced.

Globally shrimps are infected by many viral, bacterial, and parasitic pathogens such as acute hepatopancreatic necrosis disease (AHPND), Necrotising hepatopancreatitis bacterium (NHPB), *Enterocytozoon hepatopenaei* (EHP), yellow head virus (YHV), hepatopancreatic parvovirus (HPV), infectious myonecrosis virus (IMNV), monodone baculovirus (MBV), white spot syndrome virus (WSSV), infectious hypodermal and hematopoietic necrosis virus (IHHNV), Taura syndrome virus (TSV) [6, 7]. Moreover, some recently emerging disease such as decapod iridescent virus (DIV1) [8] and covert morality disease (CMD) are also threat to shrimp culture [9]. In India, to date, IMNV, IHHNV, EHP, HPV, MBV, and WSSV have been reported [7, 10, 11, and 12]. Till date, YHV, TSV, NHPB, AHPND have not been reported in India. However, it is believed that transboundary and emerging pathogens that have not yet been reported in India have a high possibility to be present in the culture system. These pathogens were not reported as to farmers the necessary knowledge, there is lack of information regarding new and emerging pathogens and there is lack of diagnostic methods, and thus these pathogens have not yet been reported. In this present study, we did a prevalence study of pathogens from dead and moribund *P. vannamei* from shrimp farms in coastal Karnataka, India.

Methodology

Samples collection

The study was conducted from April 2022 to November 2022. A total of 91 shrimp samples were collected from shrimp farms from Karnataka, India located in Uttara Kannada, Dakshina Kannada, and Udupi districts (Figure 1). Shrimps with clinical signs including pale hepatopancreas, black gills, lethargic behavior, white spots on the carapace, and retarded growth were collected and brought to the lab in cold condition. Under aseptic conditions, moribund or dead shrimp were preserved in 70% ethanol and RNA samples were fixed in RNA fixative.

Figure 1. Location of sample collection area in Karnataka, India

DNA extraction

Penaeus vannamei were dissected aseptically and DNA was extracted from gill, hepatopancreas, and pleopods according to Otta et al. [13] method with a few minor modifications. The tissues were homogenized with 800 µl of digestion buffer (10 mM Tris-HCl, pH 8.0; 0.1 M EDTA, pH 8.0; 0.5% Triton-X100; 6M Guanidine hydrochloride; 0.1 M Sodium acetate) using the sterile homogenizing stick. Following incubation, the homogenate was centrifuged at 6,000 g for 10 min. The supernatant was transferred to a fresh tube and an equal volume of phenol: chloroform: isoamyl alcohol (25:24:1) was added, vortexed, and then centrifuged at 10,000 g for 10 min. The aqueous phase containing DNA was transferred to a fresh tube followed by precipitation by adding 2-3 volumes of 100% ethanol and centrifuged at 14,000 g for 10 min. After ethanol precipitation, the pellet was washed twice with 1 volume of 70% ethanol and then vacuum dried. Finally, the DNA pellet was dissolved in 100 µl of 1X TE buffer pH 8.0 and DNA purity and concentration were checked by using the Nanodrop 1000TM spectrophotometer at 260 nm (Thermo fisher scientific, USA). DNA was either stored at -20°C /-80°C for later use or directly used for PCR analysis.

RNA extraction

For RNA extraction, based on the size, whole post larvae, gill, hepatopancreas, and pleopods of Juvenile shrimp were taken. RNA was extracted by using RNA-XPress™ Reagent (HiMedia, India) by following the manufacturer's instructions. The obtained RNA pellet was resuspended in 50 µl of nuclease-free water. RNA purity was checked at 260 nm similar to that of DNA as mentioned in the above section.

PCR analysis

The extracted shrimp DNA was used to check the presence of WSSV, HPV, MBV, IHHNV, DIV-1, EHP, AHPND, and NHPB. Primers used for the identification of these pathogens were previously reported by several authors as mentioned in Table 1. PCR was carried out in 30 µl reaction mixture containing 3 µl of 10X PCR buffer, 2.5 mM of each dNTPs, 10 pmol of each primer, 1.2 U of Taq polymerase, and 2 µl of nucleic acid. Amplified PCR products were analyzed by electrophoresis in 1.5% agarose gel and visualized by using UV-Transilluminator (Bio Rad, USA).

RT-PCR analysis

The extracted shrimp RNA was used to check the presence of YHV, TSV, and IMNV, by using primers were previously reported (Table 1). For synthesizing cDNA PrimeScriptTM RT reagent kit (Takara, Japan) was used. For each RNA sample 20 µl reaction was prepared which contained 4µl of 5X prime script buffer, 1 µl of oligo dT primer (50 µm), 1 µl of oligo dT primer (50 µm), 1 µl PrimeScript reverse transcriptase (200U/ µl) and 13 µl of normalized RNA (200 ng/ µl). It was incubated for 15 min at 37°C and then the enzyme was inactivated at 85°C for 5 sec. After cDNA synthesis, PCR was performed as previously described using 2 µl of cDNA as a template.

Results and Discussion

Sample collection and clinical symptoms

A total of 91 samples from the shrimp farms were collected from Uttara Kannada (n=43), Dakshina Kannada (n=27), and Udupi (n=21) districts. Infected shrimp samples showing clinical symptoms like white spots on the carapace, black gill, size variation, and reddish discoloration were collected (Figure 2). In Karnataka, the total area under shrimp culture is around 970.39 ha with a production of 2185.84 metric tons in 2021 [4]. Shrimp farming has a significant impact on the economy and livelihood of the farmer residing in the costal districts of the state. The control of disease is important to maintain and increase the level of productivity. In India, WSSV is the only virus that causes huge mortality no other viruses are as destructive as WSSV. Recently, there is an increased occurrence

Table 1. A list of primers for shrimp pathogen detection

Pathogen	Primer Name	Sequence (5′- 3′)	Product base pairs	References
WSSV	IK1	TGGCATGACAACGGCAGGAG	486	[20]
	IK2	GGCTTCTGAGATGAGGACGG		
	146F2	GTAACTGCCCCTTCCATCTCCA	941	[21, 22]
	146R2	TACGGCAGCTGCTGCACCTTGT		
HPV	H441F	GCATTACAAGAGCCAAGCAG	441	[23]
	H441R	ACACTCAGCCTCTACCTTGT		
MBV	MBV 1.4F	CGATTCCATATCGGCCGAATA	533	[24]
	MBV 1.4R	TTGGCATGCACTCCCTGAGAT		
IHHNV	IHHNV309F	TCCAATCGCGTCTGCGATACT	309	[25]
	IHHNV309R	TGTCTGCTACGATGATTATCCA		
TSV	9992F	AAGTAGACAGCCGCGCTT	231	[26]
	9195R	TCAATGAGAGCTTGGTCC		
IMNV	4587F	CGACGCTGCTAACCATACAA	328	[27]
	4914R	ACTCGGCTGTTCGATCAAGT		
YHV	10F	CCGCTAATTTCAAAAACTACG	135	[28]
	144R	AAGGTGTTATGTCGAGGAAGT		
EHP	ENF176F	CAACGCGGGAAAACTTACCA	176	[29]
	ENF176R	ACCTGTTATTGCCTTCTCCCTCC		
AHPND	AP4-F1	ATGAGTAACAATATAAAACATGAAAC	1269	[30]
	AP4-R1	ACGATTTCGACGTTCCCCAA		
	AP4-F2	TTGAGAATACGGGACGTGGG	230	
	AP4-R2	GTTAGTCATGTGAGCACCTTC		
NHP	NHPF2	CGTTGGAGGTTCGTCCTTCAGT	379	[31]
	NHPR2	GCCATGAGGACCTGACATCATCATC		
DIV1	SHIV-F1	GGGCGGGAGATGGTGTTAGAT	457	[8]
	SHIV-R1	TCGTTTCGGTACGAAGATGTA		

Figure 2. Clinical symptoms observed during this study (A) White spots on the carapace (B) Size variation (C) Red discoloration (D) Black gill (E) Dead shrimps

of new virulent strains of WSSV in India [7]. During 2018-19 in India, financial loss due to WSSV was estimated to be US$ 567.62 million [14]. Recently, a newly emerging disease caused by microsporidian, EHP causes a huge economic loss of US$ 238.33 million during 2018-19 in different parts of India [10, 14, 15].

Confirmation of pathogen by PCR assay

Samples were showing clinical symptoms of size variation, discoloration and white spots on the carapace which concurred with reports of Babu et al., [12] and Rajendran et al., [10]. All samples were screened for IHHNV, WSSV, EHP, MBV, HPV, AHPND, DIV-1, and NHPB. Among these 5 (5.5%) samples were infected with WSSV and 26 (28.6%) samples were infected with EHP. WSSV was confirmed by using two primers (Figure 3A, B) and the band at 176 bp confirm samples were infected with EHP (Figure 3C). Among these 2 (2.2%) samples had co-infection caused by EHP and WSSV.

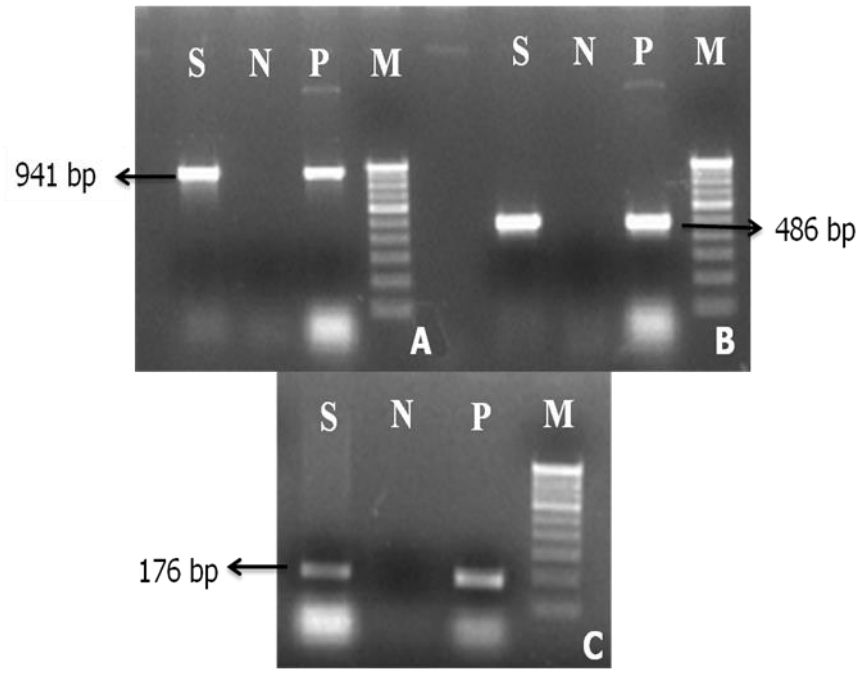

Figure 3. Agarose gel picture showing PCR amplification (A) PCR detection of WSSV using specific primer sets 146F2 and 146R2 (B) PCR detection of WSSV using specific primer sets IK1 and IK2 (C) PCR detection of EHP using specific primer sets ENF176F and ENF176R; M- 100 bp ladder, P- positive control, N- negative control, S- sample

The prevalence of WSSV was high in Dakshina Kannada followed by Udupi and Uttara Kannada, whereas the prevalence of EHP was high in Udupi followed by Uttara Kannada and Dakshina Kannada. Incidence of WSSV and EHP co-infection was only reported in Dakshina Kannada (Table 2). However, Babu et al., [12] collected 112 samples and reported a 25.4% prevalence of WSSV, 32.4% prevalence of EHP, and 7.6% prevalence of WSSV and EHP from the Southeast coast of India. Rathipriya et al., [16] collected 171 samples and reported prevalence of WSSV and EHP was 49.12% and 66.66% from Nagapattinam district in Tamil Nadu. Thamizhvanan et al., [17] collected 240 samples and reported an 11.6% prevalence of WSSV, 12.5% prevalence of EHP, and 1.6% prevalence of WSSV and EHP in Tamil Nadu and Andhra Pradesh. Rajendran et al., [10] collected 137 samples and reported 63.5% EHP prevalence on the 2016 Southeast coast of India. Prathisha et al., [18] collected 73 samples and reported 36.98% EHP prevalence in Tamil Nadu. Rajeish et al., [19] collected 81 samples and reported 50.6% WSSV prevalence in Karnataka.

Table 2. Prevalence of pathogen in shrimp farms located in different districts of Karnataka

District	No. of sample collected	No. of RNA samples screened	Pathogen detected and prevalence percentage (%)	Incidence of multiple infection
Uttara Kannada	43	22	WSSV (4.6%) and EHP (30.2%)	Not reported
Udupi	21	7	WSSV (4.7%) and EHP (33.33%)	Not reported
Dakshina Kannada	27	8	WSSV (7.4%) and EHP (22.2%)	WSSV with EHP (7.4%)

Confirmation of pathogen by RT-PCR assay

Out of 91 samples, 37 samples were screened for RNA viruses *viz*., YHV, TSV, and IMNV. All 37 samples were negative for these RNA viruses but Sahul Hameed et al., [11] reported IMNV from West Bengal. So regular screening of RNA viruses is required to ensure that shrimp industry is free of infections. Major pathogens such as AHPND, TSV, YHV, and NHPB are not reported in India so far.

Conclusion

This study gives the current status of pathogen prevalence in cultured *P. vannamei* shrimp farms in Karnataka. In this study, we reported WSSV and EHP either alone or as co-infection. The occurrence of EHP is high than WSSV in all three districts. Farmers were using SPF seeds in spite of the shrimp getting infected with a pathogen which shows improper pond preparation or transmission of the pathogen from the wild. To overcome this occurrence of pathogen we recommend good management practices and proper biosecurity to be followed for sustainable aquaculture of *P. vannamei*.

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References

[1] Sofia **(2020)**. http://www.fao.org/state-of-fisheries-aquaculture.
[2] T. W. Flegel and V. Alday-Sanz **(1998)**. The crisis in Asian shrimp aquaculture: current status and future needs. J. Appl. Ichthyol., **14:** 269-273.
[3] K. Kapiris **(2017)**. Fishing landings of crustacean decapods their culture and problems associated with diseases. *In* Trends in fisheries and aquatic animal health (Bentham Book), 240-261. ISBN: 978-1-68108-581-4.
[4] MPEDA (Marine Products Exports Development Authority) (2022). State-wise details of Shrimp and Scampi Production. Accessed online November 2022 at: https://mpeda.gov.in/?page_id=651.
[5] T. W. Flegel **(2006)**. Detection of major penaeid shrimp viruses in Asia, a historical perspective with emphasis on Thailand. Aquaculture, **258:** 1-33.
[6] OIE (Office International des Epizooties) **(2021)**. OIE-listed diseases, Office International des Epizooties, World Organisation for Animal Health, Paris, France.
[7] G. M. Tandel, K. R. John, M. R. George and M. P. Jeyaseelan **(2017)**. Current status of viral diseases in Indian shrimp aquaculture. Acta Virol., **61:** 131-137.
[8] L. Qiu, M.-M. Chen, X.-Y. Wan, C. Li, Q.-L. Zhang, R.-Y. Wang and D.-Y. Cheng et al., **(2017)**. Characterization of a new member of Iridoviridae, Shrimp hemocyte iridescent virus (SHIV), found in white leg shrimp (*Litopenaeus vannamei*). Sci. Rep., **7:** 11834. doi: 10.1038/s41598-017-10738-8.

[9] Q. L. Zhang, T. T. Xu, X. Y. Wan, S. Liu, X. H. Wang, X. P. Li and X. Dong et al., **(2017)**. Prevalence and distribution of covert mortality nodavirus (CMNV) in cultured crustacean. Virus Res., **233:** 113-119.
[10] K. V. Rajendran, S. Shivam, P. Ezhil Praveena, J. J. S. Rajan, T. S. Kumar, S. Avunje and V. Jagadeesan et al., **(2016)**. Emergence of *Enterocytozoon hepatopenaei* (EHP) in farmed Penaeus (*Litopenaeus*) *vannamei* in India. Aquaculture, **454:** 272-280.
[11] A. S. Sahul Hameed, S. Abdul Majeed, S. Vimal, N. Madan, T. Rajkumar, S. Santhoshkumar and S. Sivakumar **(2017)**. Studies on the occurrence of infectious myonecrosis virus in pond-reared *Litopenaeus vannamei* (Boone, 1931) in India. J. Fish Dis., 40: 1823-1830.
[12] B. Babu, G. Sathiyaraj, A. Mandal, S. Kandan, N. Biju, S. Palanisamy and S. You et al., **(2021)**. Surveillance of disease incidence in shrimp farms located in the east coastal region of India and in vitro antibacterial efficacy of probiotics against *Vibrio parahaemolyticus*. J. Invertebr. Pathol., **179:** 107536. doi: 10.1016/j.jip.2021.107536.
[13] S. K. Otta, I. Karunasagar and I. Karunasagar **(2003)**. Detection of monodon baculovirus and white spot syndrome virus in apparently healthy Penaeus monodon postlarvae from India by polymerase chain reaction. Aquaculture, **220:** 59-67.
[14] P. K. Patil, R. Geetha, T. Ravisankar, S. Avunje, H. G. Solanki, T. J. Abraham and S. P. Vinoth et al., **(2021)**. Economic loss due to diseases in Indian shrimp farming with special reference to Enterocytozoon hepatopenaei (EHP) and white spot syndrome virus (WSSV). Aquaculture, **533:** 736231. doi: 10.1016/j.aquaculture.2020.736231.
[15] S. Thitamadee, A. Prachumwat, J. Srisala, P. Jaroenlak, P. V. Salachan, K. Sritunyalucksana and T. W. Flegel et al., **(2016)**. Review of current disease threats for cultivated penaeid shrimp in Asia. Aquaculture, **452:** 69-87.
[16] A. Rathipriya, T. Anand, A. Uma and K. K. Marx **(2019)**. Prevalence of diseases caused by white spot virus and *Enterocytozoon Hepatopenaei* in *Penaeus vannamei* Shrimp farms in Nagapattinam District, Tamil Nadu, India. Int. J. Curr. Microbiol. App. Sci., **8:** 2616-2622.
[17] S. Thamizhvanan, S. Sivakumar, S. S. Kumar, D. V. Kumar, S. Suryakodi, K. Balaji and T. Rajkumar et al., **(2019)**. Multiple infections caused by white spot syndrome virus and *Enterocytozoon hepatopenaei* in pond-reared *Penaeus vannamei* in India and multiplex PCR for their simultaneous detection. J. Fish Dis., **42:** 447-454.
[18] R. Prathisha, M. R. George and K. R. John **(2019)**. Assessment of occurrence of *Enterocytozoon hepatopenaei* (EHP) in South Tamil Nadu. J. Entomol. Zool. Stud, **7:** 137-142.
[19] Y. M. Rajeish, G. N. Murthy, H. N. Madhushree, B. R. Pujar, S. K. Girisha, T. Suresh and M. N. Venugopal **(2017)**. Detection of viruses in the exotic shrimp *Penaeus vannamei* Boone, 1931 cultured in India. Indian J. Fish., **64:** 63-68.
[20] M. S. Hossain, A. Chakraborty, B. Joseph, S. K. Otta, I. Karunasagar and I. Karunasagar **(2001)**. Detection of new hosts for white spot syndrome virus of shrimp using nested polymerase chain reaction. Aquaculture, **198:** 1-11.
[21] C. F. Lo, J. H. Lei, C. H. Ho, C. H. Chen, S. E. Peng, Y. T. Chen and C. M. Chou et al., **(1996a)**. Detection of baculovirus associated with white spot syndrome (WSBV) in penaeid shrimps using polymerase chain reaction. Dis. Aquat. Org., **25:** 133-141.
[22] C. F. Lo, C. H. Ho, S. E. Peng, C. H. Chen, H. C. Hsu, Y. L. Chiu and C. F. Chang et al., **(1996b)**. White spot syndrome baculovirus (WSBV) detected in cultured and captured shrimp, crab and other arthropods. Dis. Aquat. Org., **27:** 215-225.
[23] Phromjai, J., Boonsaeng, V., Withyachumnarnkul, B. and Flegel, T.W., (2002). Detection of hepatopancreatic parvovirus in Thai shrimp *Penaeus monodon* by *in situ* hybridization, dot blot hybridization and PCR amplification. Dis. Aquat. Org., **51:** 227-232.
[24] C. R. Belcher and P. R. Young **(1998)**. Colourimetric PCR-based detection of monodon baculovirus in whole *Penaeus monodon* postlarvae. J. Virol. Methods, **74:** 21-29.

[25] K. F. J. Tang, S. A. Navarro and D. V. Lightner **(2007)**. PCR assay for discriminating between infectious hypodermal and hematopoietic necrosis virus (IHHNV) and virus-related sequences in the genome of *Penaeus monodon*. Dis. Aquat. Organ., **74:** 165-170.
[26] L. M. Nunan, B. T. Poulos and D. V. Lightner **(1998)**. Reverse transcription polymerase chain reaction (RT-PCR) used for the detection of Taura syndrome virus (TSV) in experimentally infected shrimp. Dis. Aquat. Organ., **34:** 87-91.
[27] B. T. Poulos and D. V. Lightner **(2006)**. Detection of infectious myonecrosis virus (IMNV) of penaeid shrimp by reverse-transcriptase polymerase chain reaction (RT-PCR). Dis. Aquat. Organ., **73:** pp.69-72.
[28] P. G. Mohr, N. J. G. Moody, J. Hoad, L. M. Williams, R. O. Bowater, D. M. Cummins and J. A. Cowley et al., **(2015)**. New yellow head virus genotype (YHV7) in giant tiger shrimp Penaeus monodon indigenous to northern Australia. Dis. Aquat. Organ., **115:** 263-268.
[29] A. Tangprasittipap, J. Srisala, S. Chouwdee, M. Somboon, N. Chuchird, C. Limsuwan and T. Srisuvan et al., **(2013)**. The microsporidian Enterocytozoon hepatopenaei is not the cause of white feces syndrome in whiteleg shrimp *Penaeus* (*Litopenaeus*) *vannamei*. BMC Vet. Res., **9:** 139. doi: 10.1186/1746-6148-9-139.
[30] K. Sritunyalucksana, S. Dangtip, P. Sanguanrut, R. Sirikharin, S. Thitamadee, S. Taengchaiphum and R. Mavichak et al., **(2015)**. A two-tube, nested PCR detection method for AHPND bacteria. Network of Aquaculture Centres in Asia-Pacific, (http://www.enaca.org/modules/news/article.php?article_id=2030, Bangkok).
[31] L. F. Aranguren, K. F. J. Tang and D. V. Lightner **(2010)**. Quantification of the bacterial agent of necrotizing hepatopancreatitis (NHP-B) by real-time PCR and comparison of survival and NHP load of two shrimp populations. Aquaculture, **307:** 187-192.