Specific pathogen-free assurance of imported Pacific white shrimp *Litopenaeus vannamei* (Boone, 1931) in the Aquatic Quarantine Facility, Chennai

Viral diseases are considered to be the single most devastating problem in shrimp culture and have seriously impacted the sustainability and economic success of the Indian shrimp aquaculture industry. Shrimp production in India, with main contribution from *Penaeus monodon*, drastically declined to 75,000 mt in 2008 from a moderately high value of 125,000 mt in 2004 (ref. 1). The sharp decline was mainly due to exclusive *P. monodon* culture and disease problems associated with it. The recession caused in the Indian shrimp industry urged the Government of India to allow the import of specific pathogen-free (SPF) Pacific white shrimp, *Litopenaeus vannamei* to India, as this species is reported to be more disease-resistant, has high growth rate and is tolerant to high stocking densities, low salinity and temperature. The protein requirement of *L. vannamei* is also low, which will enable the farmer to cut down production costs[2]. The import of an exotic species necessitated the creation of a quarantine facility essential to avoid the risks of adverse effects arising from the introduction of non-native species[3]. Thus an Aquatic Quarantine Facility (AQF) was set up in Chennai, which started operation in July 2009. The AQF is funded by the National Fisheries Development Board and operates as an extension facility of the Rajiv Gandhi Centre for Aquaculture, a registered society under the Marine Products Export Development Agency. The AQF ensures the SPF status of the *L. vannamei* imported by the shrimp farmers and the hatchery operators with the approval of the Coastal Aquaculture Authority by PCR screening of the seven Office International des Epizooties (OIE)-listed pathogens. After screening, the shrimps are quarantined at the facility and despatched to the concerned hatcheries. The methodology followed at AQF for screening of the pathogens and the PCR results obtained are discussed here.

The list of specific OIE-listed pathogens of *L. vannamei* modified from Lightner[4], is given in Table 1. The AQF screens all these pathogens and quarantines the shrimp consignments before they are dispatched to the hatcheries.

On receipt of a broodstock consignment, usually at midnight or early morning, the pleopods are severed from the exopodite of the 3rd/4th appendage using sterilized scissors and used for DNA and RNA viral diagnosis. OIE-certified kit IQ 2000 Detection and Prevention System (supplied by Farming IntelliGene Tech. Corp., Thailand), is used for screening of WSSV, YHV/GAV, IHHNV, IMNV, TSV and NHPB pathogens at the AQF. Screening of these viruses is done according to the procedure given in the OIE Manual[5]. *Baculovirus penaei* (BP) is detected by PuRe *Taq* Ready-To-Go-PCR Beads (supplied by University of Arizona, USA). For DNA viruses, extracts of DNA are made from the pleopod samples and faecal strands according to the IQ 2000^{TM} detection and prevention system procedure. One half of the extracted DNA is stored for further confirmation test. Screening of RNA viruses is also done in the same manner. All DNA and RNA samples are subjected to PCR using primer designed by the Farming IntelliGene Tech. Corp, USA recognized by OIE. For BP and NHPB, DNA from the faecal matter is extracted according to the standard OIE primer supplier procedure.

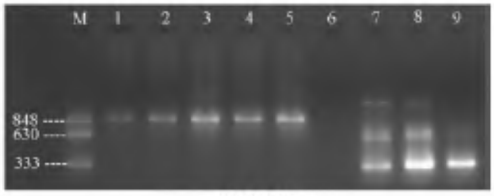
Amplification of WSSV, IHHNV, NHPB, IMNV, TSV, YHV/GAV is performed in a UNI IQ programed thermal cycler (PE Applied Biosystems) with the following protocol: initial heating at 42°C for 30 min and at 94°C for 2 min, followed by 15 cycles of denaturation at 94°C for 20 s, annealing at 62°C for 20 s and extension at 72°C for 30 s with a final extension at 72°C for 30 s and at 20°C for 30 s. The nested PCR reaction starts with 30 cycles of denaturation at 94°C for 20 s, annealing at 62°C for 20 s and extension at 72°C for 30 s with a final extension at 72°C for 30 s and at 20°C for 30 s. For BP, amplification was done in the following conditions: initial heating at 95°C for 5 min followed by 35 cycles of denaturation at 95°C for 30 s, annealing at 60°C for 30 s, initial extension at 72°C for 1 min and final extension at 72°C for 7 min. The PCR products are separated in 2% agarose gel, stained in ethidium bromide and the results are then documented using gel documentation system (BIORAD).

The samples screened at the facility till date since July 2009 (Table 2), were all negative to the listed pathogens and the typical electrophoretic results obtained for each of the seven viruses are shown in Figure 1. No positive samples were recorded in any of the screenings carried out. The positive samples would show in

Table 1. Specific OIE-listed pathogens of *Litopenaeus vannamei* being screened at the Aquatic Quarantine Facility in Chennai

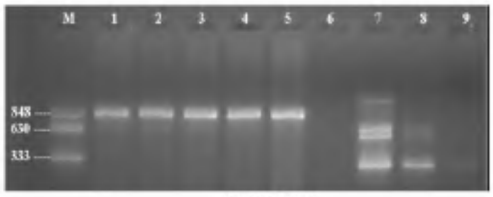
Pathogen type	Pathogen group	Acronym	Pathogen family	Category*
Virus	White Spot Syndrome Virus	WSSV	Nimaviridae	C-1
	Yellow Head/Gill Associated Virus	YHV/GAV	Roniviridae	C-1
	Infectious Hypodermal and Haematopoietic Necrosis Virus	IHHNV	Parvoviridae	C-2
	Infectious Myonecrosis Virus	IMNV	Totiviridae	C-1, 2
	Taura Syndrome Virus	TSV	Dicistroviridae	C-1
	Baculovirus penaei	BP	Baculoviridae	C-2
Prokaryote	Necrotizing hepatopancreatitis α-proteobacterium**	NHBP	Rickettsia	C-2

*C-1 pathogens are defined as excludable pathogens that can potentially cause catastrophic losses of penaeid species; C-2 pathogens cause economically significant disease and are excluded from breeding centres, hatcheries and some types of farms.
**Under study for inclusion in OIE list.



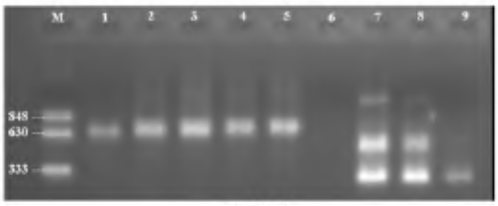
WSSV

Lane M, Molecular weight marker;
lanes 1–5, Absence of WSSV gene amplicon;
lane 6, Negative control (ddH_2O);
lane 7, WSSV P (+) standard, 2000 copies/reaction;
lane 8, WSSV P (+) standard, 200 copies/reaction;
lane 9, WSSV P (+) standard, 20 copies/reaction.



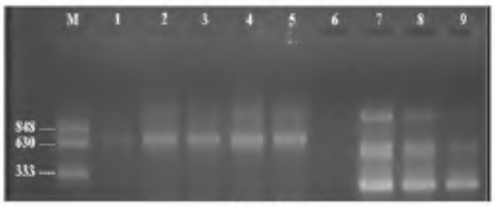
IHHNV

Lane M, Molecular weight marker;
lanes 1–5, Absence of IHHNV gene amplicon;
lane 6, Negative control (ddH_2O);
lane 7, IHHNV P (+) standard, 2000 copies/reaction;
lane 8, IHHNV P (+) standard, 200 copies/reaction;
lane 9, IHHNV P (+) standard, 20 copies/reaction.



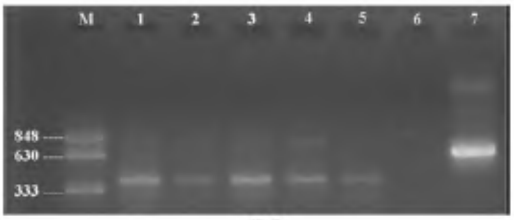
TSV

Lane M, Molecular weight marker;
lanes 1–5, Absence of TSV gene amplicon;
lane 6, Negative control (ddH_2O)
lane 7, TSV P (+) standard, 2000 copies/reaction;
lane 8, TSV P (+) standard, 200 copies/reaction;
lane 9, TSV P (+) standard, 20 copies/reaction.



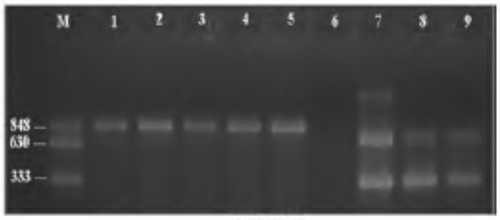
IMNV

Lane M, Molecular weight marker;
lanes 1–5, Absence of IMNV gene amplicon;
lane 6, Negative control (ddH_2O);
lane 7, IMNV P (+) standard, 2000 copies/reaction;
lane 8, IMNV P (+) standard, 200 copies/reaction;
lane 9, IMNV P (+) standard, 20 copies/reaction.



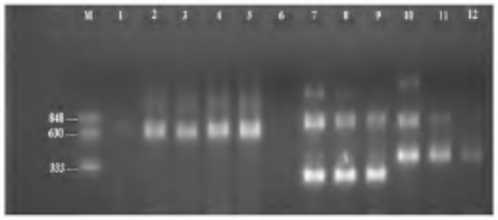
BP

Lane M, Molecular weight marker;
lanes 1–5, Absence of BP gene amplicon;
lane 6, Negative control (ddH_2O);
lane 7, BP positive control plasmid.



NHPB

Lane M, Molecular weight marker;
lanes 1–5, Absence of NHPB gene amplicon;
lane 6, Negative control (ddH_2O);
lane 7, NHPB P (+) standard, 2000 copies/reaction;
lane 8, NHPB P (+) standard, 200 copies/reaction;
lane 9, NHPB P (+) standard, 20 copies/reaction.



YHV/GAV

Lane M, Molecular weight marker;
lanes 1–5, Absence of YHV/GAV gene amplicons;
lane 6, Negative control (ddH_2O);
lane 7, YHV P (+) standard, 2000 copies/reaction;
lane 8, YHV P (+) standard, 200 copies/reaction;
lane 9, YHV P (+) standard, 20 copies/reaction;
lane 10, GAV P (+) standard, 2000 copies/reaction;
lane 11, GAV P (+) standard, 200 copies/reaction;
lane 12: GAV P (+) standard, 20 copies/reaction.

Figure 1. Electrophoretic gel photographs of seven pathogens in pleopod and faecal samples of the screened shrimp, *Litopenaeus vannamei* broodstock.

Table 2. Date-wise number of broodstock imported by the Coastal Aquaculture Authority approved stakeholders

Date of import	Number of broodstock imported	Number of broodstock subjected to analysis*
18.07.09	220	15
20.07.09	500	15
23.07.09	275	15
01.08.09	275	15
04.08.09	404	15
14.08.09	220	15
19.08.09	560	15
28.08.09	550	15
13.09.09	50	15
20.09.09	280	15
04.10.09	230	15
09.10.09	222	15
30.10.09	210	15
05.11.09	600	15
10.11.09	262	15
17.11.09	400	15
20.11.09	550	15
26.11.09	262	15
30.11.09	550	15
06.01.10	560	15
15.01.10	1000	15
24.01.10	556	15
29.01.10	128	15
04.02.10	476	15
08.02.10	447	15
15.02.10	278	15
22.02.10	170	15
01.03.10	280	15
08.03.10	472	15
15.03.10	440	15
22.03.10	660	15
29.03.10	280	15
05.04.10	590	15
09.04.10	280	15

*According to the SOP of AQF, only 15 samples are to be analysed for pathogen screening, irrespective of the number of broodstock imported.

bands at 296 and/or 550 base pairs (bp) and 286 and/or 560 bp for WSSV and IHHNV respectively, and at 325 bp for NHPB. In case of RNA viruses such as YHV, GAV, TSV and for IMNV, the band would appear at 277 and/or 777, 406 and/or 777, 284 and/or 476 and 255 and/or 510 bp respectively. The BP amplicon will be formed at 644 bp. All the samples tested so far were negative and showed only one band at 848 bp for WSSV, IHHNV, NHPB and BP, which was a PCR product of the housekeeping gene (internal control). For YHV/GAV, TSV and IMNV the internal control was at 680 bp. The absence of positive gene amplicons in the samples screened strongly reveals that all the shrimp broodstock imported were free of the specific pathogens and hence can be safely used for production and further culture purpose.

The AQF provides a quarantine clearance certificate only to the broodstock consignments which are negative to the listed pathogens. When screened positive, the entire stock would be incinerated after confirmation of the result by Central Institute for Brackishwater Aquaculture and the referral laboratory at Aquaculture Pathology Laboratory, University of Arizona, USA.

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Digital database on ethno-medicinal plants of Western Ghats

About 70% of the Indian population depends on traditional medicine for primary health care, which includes both codified and non-codified systems[1]. The non-codified system has no written texts as such and is passed verbally from generation to generation. During this process of transfer of knowledge, much of the information has been lost due to various reasons, like lack of interest in the younger generation, non-availability of raw drugs (medicinal plants), absence of official recognition, policy and administrative support by governments at the state and national levels, etc.[2,3]. There is an urgent need to preserve the remaining treasure of traditional medicine, which is also at the verge of extinction. Efforts are on in this direction since the last few decades to document this knowledge for future generations, further exploitation for human welfare and also avoid biopiracy, as in the case of turmeric[4] and basmati[5]. However, it is the need of the hour to preserve data in digitalized form as databases, in this age of electronic communication, which has made the whole earth a global village. We have made an effort to preserve the knowledge in an electronic database on Ethno-Medicinal Plants of Western Ghats (EMPWG), one of the 34 global biodiversity hotspots[6].

A multi-centric survey was conducted all along the Western Ghats by five centres, one each in Goa, Karnataka, Kerala,