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WHITE SPOT SYNDROME VIRUS (WSSV) TRANSMISSION RISK THROUGH INFECTED COOKED SHRIMP PRODUCTS ASSESSED BY POLYMERASE CHAIN REACTION (PCR) AND BIO-INOCULATION STUDIES

A. Devivaraprasad Reddy, G. Jeyasekaran, R. Jeya Shakila
Department of Fish Processing Technology, Fisheries College and Research Institute, Tamil Nadu Veterinary and Animal Sciences University, Tuticorin 628 008, India

ABSTRACT
The aim of the study was to evaluate the resistance of white spot syndrome virus (WSSV) in shrimps (*Penaeus monodon*) to the process of cooking. The cooking was carried out at 100^0C six different durations 5, 10, 15, 20, 25 and 30 min. The presence of WSSV was tested by single step and nested polymerase chain reaction (PCR). In the single step PCR, the primers 1s5 & 1a16 and IK1 & IK2 were used. While in the nested PCR, primers IK1 &IK2 – IK3 & IK4 were used for the detection of WSSV. WSSV was detected in the single step PCR with the primers 1s5 & 1a16 and the nested PCR with the primers IK1 &IK2 – IK3 & IK4 from the cooked shrimp samples. The cooked shrimps, which gave positive results for WSSV by PCR, were further confirmed for the viability of WSSV by conducting the bio-inoculation studies. Mortality (100%) was observed within 123 h of intra-muscular post injection (P.I) into the live healthy WSSV-free shrimps (*P. monodon*). These results show that the WSSV survive the cooking process and even infected cooked shrimp products may pose a transmission risk for WSSV to the native shrimp farming systems.

KEYWORDS*:* resistance, white spot syndrome virus, cooking, shrimp, bio-inoculation

INTRODUCTION

The white spot syndrome virus (WSSV), a sole member of the monotypic family Nimaviridae, genus *Whispovirus* (Pradeep, et al, 2009), is known to cause 100% mortality in shrimps within 3–10 days under farming conditions (Lightner, 1996; Pradeep, et al, 2008b). Since its first report from China in 1991-92, the virus has spread rapidly throughout the world (Pradeep, et al, 2008a) causing huge economic loss to the aquaculture industry. During the last decades, this disease has spread worldwide and caused large-scale mortalities and severe damage to shrimp culture, particularly in Asia, leading to massive economic losses to the farmers, processors and the country (Stentiford, et al, 2009). It is estimated that WSSV caused losses of 300,000 metric tons of shrimp, worth more than 1 billion US dollars (Rosenberry, 2001).

Risks of shrimp virus introduction during trading of live shrimp for culture have been described, but other potentially important sources of shrimp viruses such as ship ballast water or frozen seafood products, shrimp reprocessing waste, packaged shrimp diverted for recreational fishing bait and packaged shrimp diverted for shrimp feed in shrimp hatcheries or in shrimp aquaculture ponds have also been suggested (McColl, et al, 2004; Hasson, et al, 2006; Reyes-Lopez, et al, 2009; Flegel, 2009; Reddy, et al, 2010). Whole, fresh/frozen shrimp are overwhelmingly preferred for the last two activities, although it is likely that bait would not be chopped before use, while shrimp feed would most probably contaminate the natural waters/mature animals.

Viral disease diagnostics on imported fresh and frozen stocks have been hardly implemented and have only included stock samples produced in Latin America or Asia (Nunan, et al, 1998; Lightner, 1999; Durand, et al, 2000; Reville, et al, 2005; Hasson, et al, 2006; Reyes-Lopez, et al, 2009). It is listed as a non-exotic disease in EC directive 2006/88 (Stentiford, et al, 2009). Fresh/frozen shrimp products for human consumption imported into Australia have been subjected to mandatory testing since October 2007 using PCR technology for three major shrimp viruses viz., White Spot Syndrome Virus (WSSV), Yellow Head Virus (YHV) and Infectious Hypodermal and Haematpoietic Necrosis Virus (IHHNV) at the level of 5% prevalence with 95% confidence. Batches that tested positive have to be destroyed or cooked in an approved facility (Biosecurity Australia 2007; Sritunyalucksana, et al, 2010). The requirement for IHHNV testing was dropped in September 2008, but testing requirements for YHV and WSSV remain unchanged. Similar testing requirements are not applied for viruses in other fresh/frozen crustaceans, fish or mollusks. Since frozen shrimp products have been considered as one of

the risks for shrimp virus introduction into the native systems, the present study was conducted to find out the effect of cooking on the destruction of WSSV in shrimp products.

MATERIALS AND METHODS

Positive control samples

A clear WSSV infected shrimp (*P. monodon*) sample collected from a shrimp farm located in Nellore, Andhra Pradesh, India, based on the typical clinical symptoms associated with the WSSV, was selected as a positive control. This positive control sample was initially used for the standardization of polymerase chain reaction (PCR) for the detection of WSSV from cultured shrimp products. The highly positive sample was also chosen to investigate the effect of cooking on the survival of the WSSV in shrimps.

Preparation of samples

The highly PCR positive shrimp samples were used in the present study. About 60 shrimps were put in six polythene bags (10 No. each/pack) and sealed. They were cooked in 800 ml of sterile water on a gas stove. One bag containing 10 shrimps was collected at 5 min, 10 min, 15 min, 20 min, 25 min and 30 min after the beginning of boiling. During the cooking process, the water temperature was measured by using the thermocouple (Consort NV, T852, Turnhout, Belgium). The temperature was maintained at 100^0C during the whole process of cooking.

Experimental animals

The live healthy shrimps (*P. monodon)* weighing 6-9 g were used for the bio-inoculation studies. The shrimps were screened for WSSV prior to acclimatization in cement tanks. They were then maintained in 200 L of aged borewell seawater having salinity between 20-25 ppt in cement tanks with air-lift biological aerators at room temperature (24-29^0C). They were fed with artificial pellet feed (CP Feed, Thailand). They were reared in laboratory conditions for 10 days prior to the bio-inoculation studies. The animals were divided into two groups. One group consisting of five animals was injected with WSSV viral inoculum. The other group (also with five animals) was treated as negative control, where the animals were injected with negative shrimp viral inoculum. The inoculated shrimps were continuously observed for WSSV infection and time of mortality.

Preparation of viral inoculum for bio-inoculation studies

Viral inoculum was prepared by the method described by Reddy, et al, (2010). Briefly, pleopods were removed from cooked shrimps separately, homogenized in TN buffer and centrifuged at 1000 g for 10 min at 4^0C in a refrigerated microfuge (Eppendorf AG, Hamburg, Germany). The supernatant fluid was diluted to 1/10 with 2% NaCl and filtered through 0.2 µm membrane filter. About 100 µl of inoculum was injected into the live healthy animals through the dorsal region of the third abdominal segment.

DNA extraction

The WSSV DNA for PCR was extracted by using standard phenol extraction procedure and ethanol precipitation (Jeyasekaran, 2000; Reddy, et al, 2010). Briefly, about 500 mg of homogenized tissue of shrimp was taken in a 2.0 ml sterile microfuge tube. Then, 0.5 ml molecular grade water (Sartorius Stedim Biotech, Gottingen, Germany) was added to each tube and incubated for 15 min at -20^0C. To each tube, 0.5 ml of buffered phenol (which is prepared by mixing 500 ml of phenol with 50 ml of 0.1M Tris (pH 8.0) and 100 µl of β-Mercaptoethanol) was added. The tissue sample was then centrifuged at 10,000 rpm for 15 min at 4^0C in a refrigerated microfuge and the supernatant was transferred to another sterile microfuge tube and 0.5 ml of buffered phenol was added and centrifuged. This process was repeated until the supernatant became clear. The supernatant was then transferred to another sterile microfuge tube and 0.5 ml of diethyl ether was added and centrifuged at 10,000 rpm for 10 min at 4^0C. The supernatant was discarded and 50 µl of 3 M sodium acetate and 1.5 ml of 100% ethanol were added and stored at -20^0C overnight (or) at -80^0C for 2 h. The extract was centrifuged at 10,000 rpm for 15 min at 4^0C and the supernatant was discarded. The pellet was washed with 70% ethanol and dried at room temperature. The pellet was finally dissolved in 20 µl molecular grade water and stored at -20^0C until further use.

Amplification of WSSV DNA

Oligonucleotide primers (1s5-1a16, IK 1-2, IK 3-4) chosen for the amplification of WSSV DNA fragments were based on the earlier reports of East, et al, (2005) and Pradeep, et al, (2009) for single step and nested PCR, respectively (Table 1). The primers produced an amplicon size of 486 bp (for IK 1-2), 310 bp (for IK 3-4) and 198 bp (for 1s5-1a16).

Polymerase Chain Reaction (PCR*)*
First / Single step PCR
The volume of reaction mixture for the first/single step PCR was 25 µl containing 18 µl molecular grade water, 2.50 µl reaction buffer (100mM Tris with 15mM $MgCl_2$), 1 µl each of forward and reverse primer (1s5 and 1a16; IK1 and IK2), 0.25 µl dNTPs (deoxyribonucleotide triphosphates) mix, 0.25 µl Taq DNA polymerase, 2 µl crude DNA extract (template DNA). The PCR reaction was conducted in the Thermalcycler (GeneAmp 9700, ABI Systems, Rotkreuz, Switzerland). The PCR protocol comprised of 35 cycles of 60 sec at 94^0C, 60 sec at 55^0C and 90 sec at 72^0C. The programme included an initial delay of 4 min at 94^0C and final extension of 5 min at 72^0C before and after 35 cycles, respectively.

Second step/nested PCR
In this case, an aliquot of 2 µl from the first step PCR product was used as the DNA template together with the nested primer pair, IK-3 and IK-4. The rest of the PCR mixtures were the same as described above.

Electrophoresis
After completion of PCR, 5 µl of PCR product was taken and mixed with 1 µl of 6X loading buffer and subjected to electrophoresis (GE Healthcare Biosciences, Kowloon, Hong Kong) in 2% agarose gel containing ethidium bromide at a concentration of 0.5 µl /ml in 1X Tris-acetate – EDTA (TAE) buffer and the gel was analysed under UV trans-illumination and photographed using Gel Documentation System (Alpha Innotech Corp. California, USA).

RESULTS
The WSSV infected shrimp samples cooked at 100^0C showed positive results even after 30 min of cooking by the single step PCR for the primer 1s5 and 1a16 (Fig. 1), but not with IK1 & IK2 primers. The product yield was found to be the same in all the samples that were cooked for different durations. However, all these samples were found to be positive by the nested (Fig. 2).

The bio-inoculation studies conducted on the healthy live WSSV-free shrimps (*P. monodon*) produced 100% mortality within 123 h of post injection (P.I.). None of the healthy shrimps died in buffered saline injected negative control group. The WSSV viral inoculum prepared from the cooked shrimps showed a 100% mortality, but only after 123 h of P.I. The mortality appeared only after 95 h of P.I. with pinkish discoloration on the uropods in the first shrimp and after 102 h in the second shrimp. The mortality of third shrimp occurred after 109 h of injection with more intense pink discoloration on the uropods as well as on the pleopods. The fourth and fifth shrimps died after 116 h and 123 h of injection, but had intense pinkish discoloration on the uropods as well as on the pleopods. White spots were not observed in any of the shrimps.

Nested PCR alone yielded the positive results for the first two shrimp samples that died after 95 and 102 h of post injection (Table 2 and Fig.3). The shrimps that died at 109 h of P.I. were found to be positive for WSSV by the single step PCR with the primers 1s5 and 1a16 and not with the primers IK1 and IK2, besides positive by the nested PCR. The shrimps in which mortality occurred after 116 h and 123 h showed positive by the single step and nested PCR with the three sets of primers tested. The present results clearly indicated that the cooking did not destroy the WSSV and found that virus was viable even after 30 min of cooking.

DISCUSSION
The detection of WSSV in the cooked shrimps clearly showed that cooking process at 100^0C even for 30 min did not destroy the WSSV DNA (Figs.1 and 2). Sritunyalucksana, et al, (2010) studied the effect of cooking of whole shrimps at 85^0C on the virus, YHV, before freezing and reported that the cooking temperature given was the core temperature of the processed shrimp. Even though the present findings showed that cooking did not destroy the WSSV DNA, Hasson, et al, (2006) reported that the shipments found to be WSSV-positive should be cooked to inactivate the viral pathogens. Hewitt & Greening, (2006) showed differences in Hepatitis A virus (HAV) and Norovirus (NoV) inactivation in New Zealand greenshell mussels (*Perna canaliculus*) depending on the method of cooking, where boiling for 3 min was more effective than steaming for 3 min to inactivate HAV. In these studies, boiling for 3 min gave an internal temperature of 92^0C, but steaming for the same period gave an internal temperature of only 63^0C. Croci, et al, (2005) studied the resistance of Hepatitis A virus (HAV) in mussels subjected to different domestic cookings and found that the virus was still present even after cooking for 9 min at boiling temperature. It is known that the environment may influence the virus sensitivity to thermal inactivation, particularly in a fat or protein environment, as in shellfish flesh, the virus is more resistant to the

inactivation action of heat (Murphree & Tamplin, 1995; Croci, et al, 1999). However, only a 2-$\log_{10}$ decrease in viable viruses was seen after steaming for 30 min to an internal temperature of 93.7^0C. Virus survivals ranging from 7 to 13% were observed using different cooking methods (Richards, et al, 2010).

The progress of WSSV infection in the bio-inoculated shrimps showed that WSSV first affected the pleopods followed by uropods. The injected shrimps showed gross clinical signs of WSSV infection. The WSSV infected animals, on bio-inoculation, exhibited pinkish red discoloration on the uropods and pleopods. Sudha, et al, (1998) also reported that the shrimps with reddish discoloration without white spots was a preacute form of the disease caused by WSSV. The disease process may be completed in a short time period because of high viral load and small pore size, and therefore, the principal clinical sign of white spot appearance normally associated with WSSV syndrome may not develop at all. The successful WSSV infection on infectivity bioassays in all of the experimental animals caused rapid reduction in feed intake and lethargy (Corbel, et al, 2001), which was also observed in the present study.

The first mortality observed in the shrimps that were injected with viral inoculum obtained from cooked shrimp samples was at 95 h P.I. (Table 2 and Fig. 3). No clinical signs of disease or mortality were observed among negative control shrimps during the experimental period. The action of WSSV was slow due to the cooking process applied, and hence it took more time to develop infection in the healthy shrimps on bio-inoculation. However, all the experimental animals died within 123 h of P.I., which indicated that the WSSV was even viable after cooking and could cause mortality. Lightner, (1996) also reported the appearance of reddish discoloration or small white spots in the WSSV infected shrimp (*P. monodon*). Durand, et al, (2000) observed a 100% mortality in the indicator shrimp [specific pathogen free (SPF) shrimp, *P. vannamei*] on injection of WSSV inoculum from frozen shrimp products. Nunan, et al, (1998) reported that the bioassay using 15 indicator shrimps (*P. stylirostris*) produced 100% mortality within 8 day P.I. of WSSV inoculum from frozen shrimp products. The series of experiments conducted by Nunan, et al, (1998) also showed that frozen shrimp product imported from Asia contains the viral pathogens WSSV and YHV and through the use of bioassay, they demonstrated that the viruses in the frozen imported product were infectious. Durand *et al.* (2000) observed that the challenge bioassay of WSSV by shrimp injection produced the first mortality in indicator shrimp on day 3, but, all the indicator shrimps had died by day 4. Hasson, et al, (2006) observed the clinical signs of disease including lethargy, anorexia and chromatophore expansion resulting in dark body coloration and reddening of both the uropods and antennae, when SPF shrimp (*Litopenaeus vannamei*) juveniles were injected with WSSV PCR-positive shrimp (*Parapenaeopsis* sp.) tissue homogenates resulting in 100% mortality in the experimental shrimps within 48 to 72 h of P.I. Reddy, et al, (2010) observed that the WSSV in frozen shrimp products were infectious and causing a 100% mortality in live healthy WSSV-free shrimps (*P. monodon*) within 45h of intra-muscular post injection (P.I). It is inferred from the present study that the WSSV was virulent in shrimps subjected to cooking process and infectious resulting in 100% mortality of healthy shrimps.

According to Biosecurity Australia, (2007), fresh/frozen shrimp products for human consumption imported into Australia from October 2007 should be subjected to mandatory testing using PCR technology for three major shrimp viruses viz., White Spot Syndrome Virus (WSSV), Yellow Head Virus (YHV) and Infectious Hypodermal and Haematpoietic Necrosis Virus (IHHNV) at the level of 5% prevalence with 95% confidence and the batches that tested positive have to be destroyed or cooked in an approved facility. However, the present study indicated that cooking did not destroy the WSSV DNA in shrimps (*P. monodon*) and the cooked shrimp products may also pose the risk of transmitting the WSSV to the healthy native shrimps.

CONCLUSION

On the basis of the bio-inoculation studies, it can be inferred that the WSSV was not destroyed at 100^0C even after 30 min of cooking process. Results clearly show that a transmission risk of WSSV remains even through the infected cooked shrimp products. Hence, the shrimp farmers, processors and exporters should be more vigilant on WSSV infection in farmed shrimps in order to prevent economic losses.

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REFERENCES

Biosecurity Australia Policy Memorandum 2007/16, 24 July 2007. http://www.baphiq.gov.tw/public/Data/77301011971.pdf

Corbel, V., Zuprizalk., Shi, Z., Huang, C., Sumartono., Arcier, J-M and Bonami J-R. (2001), Experimental infection of European crustaceans with white spot syndrome virus (WSSV). *J. Fish Dis. 24 : 377-382.*

Croci, L., Ciccozzi, M., Medici, D., Di pasquale, S., Fiore, A., Mele, A. and Toti, L (1999), Inactivation of hepatitis A virus in heat treated mussels. *J. Applied Microbiol, 87: 884-888*.

Croci, L., Medici, D. D., Di Pasquale, S. and Toti, L (2005), Resistance of hepatitis A virus in mussel subjected to different domestic cooking. *Int. J. Food Microbiol, 105: 139-144.*

Durand, S.V., Tang, K.F.J. and Lightner, D.V (2000), Frozen commodity shrimp: potential avenue for introduction of white spot syndrome virus and yellow head virus. *J. Aquatic Animal Health, 12: 128-135.*

East, I.J., Black, P.F., Findlay, V.L. and Bernoth, E.-M (2005), A national survey to verify freedom from white spot syndrome virus and yellow head virus in Australian crustaceans. In: *Diseases in Asian Aquaculture V.* Edited by P.J. Walker., R. Lester. and M.G. Bondad-Reantaso), pp. 15-26. Fish Health Section, Asian Fisheries Society, Manila, Philippines,

Flegel, T.W. (2009), Review of disease transmission risks from prawn products exported for human consumption. *Aquaculture 290: 179-189*.

Hasson, K.W., Fan, Y., Reisinger, T., Venuti, J. and Varner, P.W. (2006), White-spot syndrome virus (WSSV) introduction into the Gulf of Mexico and Texas freshwater systems through imported, frozen bait-shrimp. *Dis. Aquat. Org, 71: 91-100*.

Jeyasekaran, G. (2000), A Report on Diagnostics in Fish diseases. Australian Animal Health Laboratory Commonwealth Scientific and Industrial Research Organization, Geelong Australia. 42pp.

Lightner, D. V. (1996), A Handbook of shrimp pathology and diagnostic procedures for diseases of cultured penaeid shrimp. The World Aquaculture Society, Baton Rouge Louisiana, USA, 305pp.

Lightner, D. V. (1999), The penaeid shrimp viruses TSV, IHHNV, WSSV and YHV. *J. Applied Aquaculture, 9: 27-52.*

McColl, K., Slater, J., Jeyasekaran, G., Hyatt, A. and Crane, M.S. (2004), Detection of white spot syndrome virus and yellowhead virus in prawns imported into Australia. *Australian Vet. J, 82: 69-74.*

Murphree, R. L. and Tamplin, M. L. (1995), Uptake and retension of *Vibrio cholerae* 01 in the Eastern oyster, *Crassostrea virginica. Applied Environmental Microbiol, 61: 3656-3660*.

Nunan, L.M., Poulos, B.T. and Lightner, D.V. (1998), The detection of white spot syndrome virus (WSSV) and yellow head virus (YHV) in imported commodity shrimp. *Aquaculture, 160: 19-30.*

Pradeep, B., Shekar, M., Gudkous, N., Karunasagar, I. and Karunasagar, I. (2008a), Genotyping of white spot syndrome virus prevalent in shrimp farms of India. *Dis. Aquat. Org, 78: 189-198*.

Pradeep, B., Shekar, M., Karunasagar, I. and Karunasagar, I. (2008b), Characterization of variable genomic regions of Indian white spot syndrome virus. *Virology, 376: 24-30.*

Pradeep, B., Karunasagar, I. and Karunasagar, I. (2009), Fitness and virulence of different strains of white spot syndrome virus. *J. Fish Dis, 32: 801-805.*

Reddy, AD., Jeyasekaran, G. and Shakila, RJ. (2010), Incidence of white spot syndrome virus (WSSV) in Indian farmed frozen shrimp products and testing for viability through bio-inoculation studies. *Journal of Aquaculture Research & Development 1,102:1-5*.

Reville, C., Al-beik, J., Meehan-meola, D., Xu, Z., Goldsmith, M.I., Rand, W. and Alcivar- Warren, A. (2005), White spot syndrome virus in frozen shrimp sold at Massachusetts supermarkets. *J. Shellfish Research, 24: 285-290*.

Reyes-López, M. A., Salazar-Marroquín, E. L., Oliva-Hernández, A. A., Salinas-López, N. and Narváez-Zapata, J. A. (2009), White-spot syndrome virus diagnostics in frozen shrimp stocks imported to Mexico. *CyTA – Journal of Food, 7: 89-94.*

Rosenberry, B. (2001), World shrimp farming 2001. In: *Shrimp News International, USA* Edited By B.

Rosenberry, pp.324. Shrimp News International, San Diego, CA, USA.

Richards, G.P., McLeod, C., and Le Guyader, F.S. (2010), Processing Strategies to Inactivate Enteric Viruses in Shellfish. *Food and Environmental Virology, 2:183–193*.

Sritunyalucksana, K., Srisala, J., Wangnai, W. and Flegel, T.W. (2010), Yellow head virus (YHV) transmission risk from commodity shrimp is reduced to negligible levels by normal processing. *Aquaculture 300: 32-36*.

Stentiford, G.D., Bonami, J. –R. and Alday-Sanz, V. (2009), A critical review of susceptibility of crustaceans to taura syndrome, yellowhead disease and white spot disease and implications of inclusion of these diseases in European legislation. *Aquaculture 291: 1-17.*

Sudha, P.M., Mohan, C.V., Shankar, K.M. and Hegde, A. (1998), Relationship between white spot syndrome virus infection and clinical manifestation in Indian farmed penaeid shrimp. *Aquaculture 167: 95-101.*

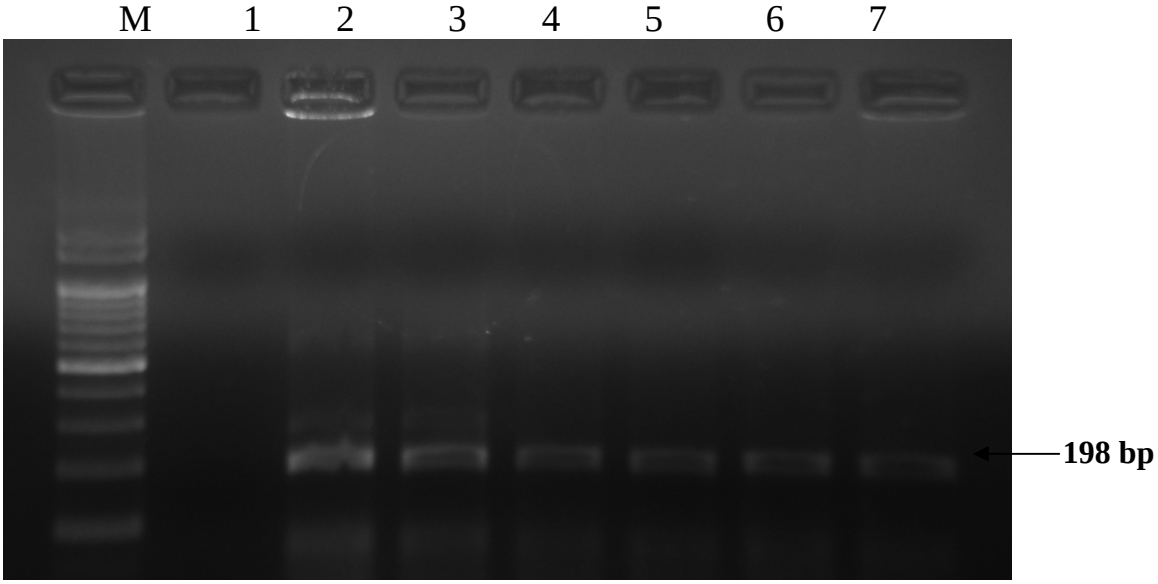

Fig. 1. Detection of WSSV in cooked shrimp samples with the primers 1s5 & 1a16 in a single step PCR. Lane M – 100 bp DNA ladder; Lane 1 – negative control; Lane 2 - 5 min; Lane 3 - 10 min; Lane 4 - 15 min; Lane 5 - 20 min; Lane 6 - 25 min; Lane 7 - 30 min

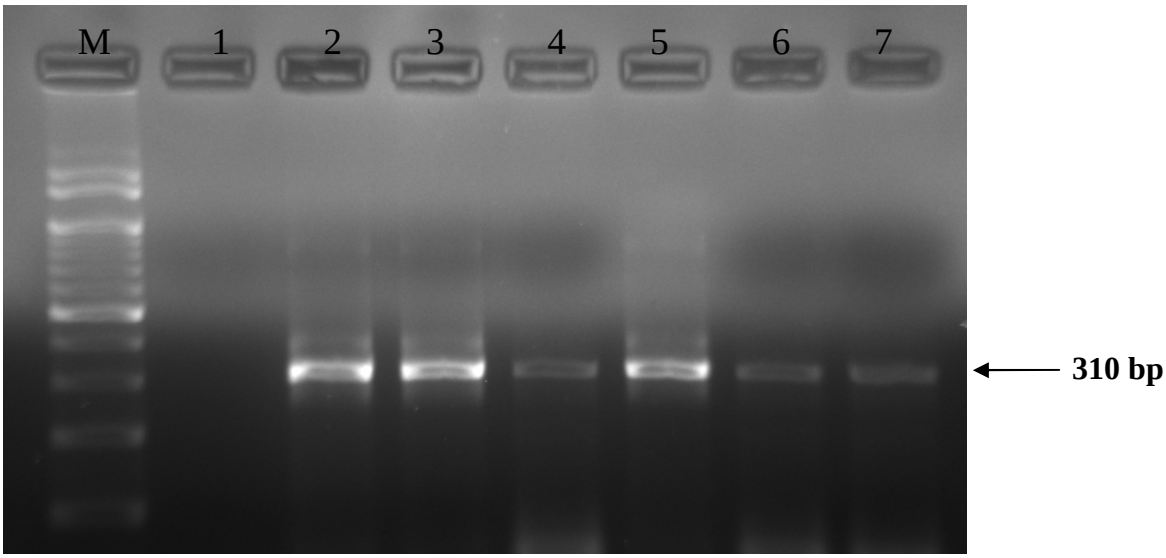

Fig. 2. Detection of WSSV in cooked shrimp samples with the primers IK3 & IK4 in a nested PCR. Lane M – 100 bp DNA ladder; Lane 1 – negative control; Lane 2 - 5 min; Lane 3 - 10 min; Lane 4 - 15 min; Lane 5 - 20 min; Lane 6 - 25 min; Lane 7 - 30 min

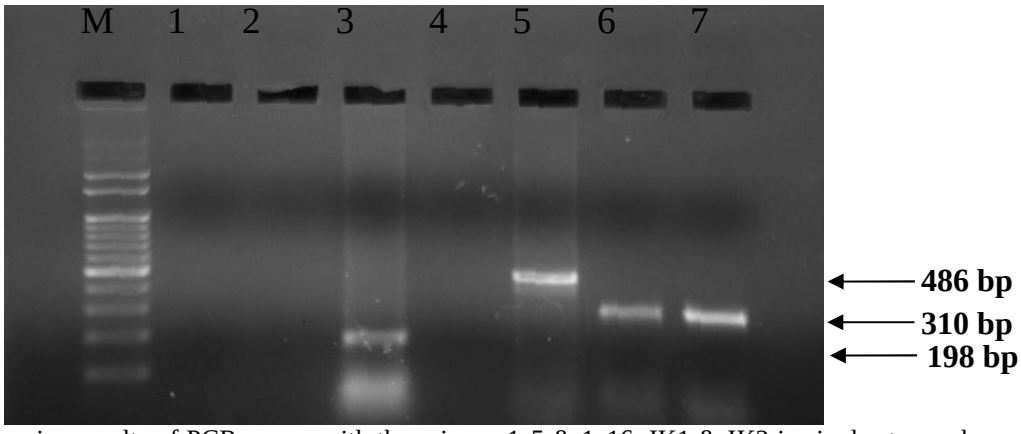

Fig. 3. Agarose gel showing results of PCR assays with the primers 1s5 & 1a16; IK1 & IK2 in single step and IK1 & IK2-IK3 & IK4 in nested PCR from moribund shrimps bio-inoculated with WSSV inoculum from cooked shrimp sample. Lane M – 100 bp DNA ladder; Lane 1 - negative control; Lane 2 – 95 h (for 1s5 and 1a16); Lane 3 – 123 h (for 1s5 and 1a16); Lane 4 - 95 h (for IK1 and IK2); Lane 5 - 123 h (for IK1 and IK2); Lane 6 - 95 h (for IK3 and IK4); Lane 7 - 123 h (for IK3 and IK4)

Table 1:
Primers used for the detection of WSSV from shrimp products

Primer specific for	Primer name	Sequence (5'-3')	Product size
Single step PCR	1s5	CACTCTGGCAGAATCAGACCAGACCCCTGAC	198 bp
Nested PCR	1a16	TTCCAGATATCTGGAGAGGAAATTCC	
1st step			
	IK-1	TGGCATGACAACGGCAGGAG	486 bp
2nd step	IK-2	GGCTTCTGAGATGAGGACGG	
	IK-3	TGTCATCGCCAGCACGTGTGC	310 bp
	IK-4	AGAGGTCGTCAGAGCCTAGTC	

Table 2:
Detection of WSSV by PCR from the tissues of shrimp (*P. monodon*) challenged by intramuscular (I.M) injection of WSSV inoculum from cooked (100^0C for 30 min) shrimp sample

PCR diagnosis	Negative group	Time of mortality (P.I.)				
		95 h	102 h	109 h	116 h	123 h
Single step PCR (1s5 & 1a16 primers)	-	-	-	+	+	+
Single step PCR (IK1 & IK2 primers)	-	-	-	-	+	+
Nested PCR (IK1& IK2 - IK3 & IK4)	-	+	+	+	+	+

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Corresponding author:
G. Jeyasekaran,
Department of Fish Processing Technology, Fisheries College and Research Institute, Tamil Nadu Veterinary and Animal Sciences University, Tuticorin 628 008, India
E-mail address: ttnjerosh99@bsnl.in