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WSSV transmission in *Paratelphusa hydrodomous* fresh water rice field crab concerning with WSSV-419 like protein gene expression studies.

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Abstract-The White Spot Syndrome Virus a major virulent virus against shrimp cultivation was carried out by intramuscular injection wide transmission in the fresh water rice field crab Paratelphusa hydrodomous make use of maintaining live stock for researchers. The transmission was conformed by use of Polymerase chain reaction by monitoring the gene expression level in the host by use of SYBR green Real Time Polymerase chain reaction under the consideration of WSSV-419 like protein gene.
Keywords: WSSV, Paratelphusa hydrodomous, WSSV-419 like protein gene, transmission, RT-PCR expression

I. INTRODUCTION

Viruses are considered to be the most important pathogens in shrimp have caused severe production and economic losses in the past two decades [1]. The WSSV is responsible for the huge economic loss in the shrimp culture industry worldwide [2]. The principal clinical sign of WSSV is the presence of white spots on the exoskeleton and carapace of the diseased shrimp. Other signs include a rapid reduction in food consumption, lethargy and reddening of the appendages; mortality rates are usually very high and cumulative mortality can reach 100% within 3 to 10 days from the onset of visible gross signs [2]. The serious impact of WSSV in the shrimp culture industry worldwide and the broad host range call for novel control strategies against this virus. Previous strategies generally used to control the WSSV including immunostimulation, neutralization, vaccination, quarantining and environmental management [3].

The principal clinical sign of WSSV is the presence of white spots on the exoskeleton and epidermis, ranging from 0.5-3mm in diameter. Affected shrimp present lethargy, anorexia loose cuticle and go off their feed. WSSV also transferred through shrimp live feeds like polychaetes [4]. In shrimp ponds, they congregate in the shallows along the edges of the pond and in culture tanks they sink inactively to the bottom where they are frequently attacked and cannibalized by the healthier shrimp [5, 6, 7, 8, 9]. WSSV is found to infect most tissues originating from both ectoderm and mesoderm. These include the subcuticular epithelium, gills, lymphoid organ, antennal gland, hematopoietic tissues, connetive tissue, ovary and the ventral nerve cord [10, 11, 12, 13].

It has been reported that WSSV continues to be the most serious threat associated with penaeid shrimps in all the shrimp growing countries globally and the Asian countries in particular [14, 15]. WSSV has been reported to affect several commercially important species of penaeid shrimps [16] and also infects a wide range of aquatic crustaceans including marine and brackish water penaeids, crabs, fresh water prawns and cray fish [17]. WSSV is considered to be among the most prevalent viruses in farmed shrimp and is also responsible for a large part of crop failures [15]. Here in our research in this sequence we had confirmed that WSSV are also infecting *Paratelphusa hydrodomous* fresh water rice field crab.

II. MATERIALS AND METHODS

A. Preparation of WSSV inoculum

The WSSV strain from the highly infected shrimp of *Peneaus monodon* was prepared by dissecting the hemetopoitic tissue and homogenizing it with 40 ml of sterilized sea water. Then homogenized tissue was centrifuged for 1000g/10 min at 4^{O}C. The supernatant was filtered with 0.45µm filter and carried out for WSSV transmission.

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B. WSSV transmission to the Rice Crab Paratelphusa hydrodomous

The rice crab *Paratelphusa hydrodomous* was injected with 0.1 ml of 1:100 dilutions from the prepared viral stock in to the abdomen region of the crab *Paratelphusa hydrodomous*. Meanwhile the control animals were maintained separately in another tank. The WSSV infected tissues were dissected and stored in 70% ethanol for PCR, RT-PCR analysis.

C. Viral DNA extraction

Viral DNA was isolated from purified virions by treatment with proteinase K (0.2 mg/ml) and Sarkosyl (1%) at 65ºC for 2 h, followed by phenol and chloroform extraction and dialysis against TE. The purity and concentration of the DNA were determined by agarose gel electrophoresis.

D. Agarose gel Preparation and Electrophoresis

The amplified samples are examined on 1.5% agarose gel using 5µL from the reaction. The results are analysed by examined under UV light in a Transilluminator.

E. PCR & Electrophoresis of WSSV

A 1.0µl of Sample DNA (approximately 100 ng/µl) was added to PCR Mixture containing 100mM Tris HCl (pH 8.3),500mM KCl (pH 8.3),2.0µl $MgCl_2$ (25mM), 2.0µl dNTP's (2.5mM), 1.0µl Primer Forward & Reverse (each of 10pm/µl) and 1u /µl of Taq Polymerase. The PCR was conducted in Gene Amp PCR System 9700 (Applied Biosystem, USA).

F. WSSV Samples detection

The primers for WSSV detection was designed using Primer Express software (Version 2.0) based on the WSSV2 (GenBank KF976716). The primer used for DNA based marine viral detection and genome fragment amplification are shown in Table 4

Table 4: Primers used for WSSV amplification and target genes

Virus Name	Primers	Target Gene	Nucleotide sequences	Annealing Tm (ºC)	Product length
WSSV	WSSV 530 F/530 R	419 like protein	5' TGGCATGACAACGGCAGGAGT 3' 5' CGAGCTGCCTTGCCGGAAATTA 3'	55	419 bp

The amplification condition were one cycle of 94^0C for 5min then 35cycles of 94^0C for 30s , 55 ^{0}C for 30s, 68^0C for 60s and 68^0C for 7min. The PCR Products (6 - 10µl) were separated by electrophoresis in 2% Agarose gels containing ethidium bromide(1µg ml^{-1}).

G. SYBR green RT PCR programme for WSSV.

The primers of WSSV for RT PCR were based on the WSSV-419 gene of WSSV (GenBank KU531723) sequence which was already used for the previous PCR detection (Table-4).The standard for the WSSV were confirmed by the PCR are prepared by diluting the DNA sample ~4-fold, made up as dilution of to 21 µl of cDNA sample with 59 µl H_2O for a final volume of 80µl, which was votexed, spun down and then transferred with equal quantity from each sample into a single tube. The cDNA dilution factors and value is listed in table 2.

The forward and reverse primers are maintained in the concentration of 5 pmol/µL. It could be either 25µL or 50µL RT-PCR reaction mixture may use for SYBR green RT-PCR. Here for WSSV we used. 25 µL SYBR Green Mix (2x), 0.5µL cDNA, 2µLprimer pair mix (5 pmol/µL each primer), 22.5µL deionised water was added in each optical tube. Amplification protocol was of 1 cycle at 94°C for 2 min, then 94°C for 20 sec,62°C for 20 sec, 72°C for 30 secand 20°C for 30 sec at final cycle.

Table 2. WSSV positive standard dilution factor and value

Standard Number	Dilution Factor	Dilutions	Value
1	Pool	360µl Standard 1	25600
2	1:4	90µl Stnd. 1 + 270µl H_2O	6400
3	1:16	90µl Stnd. 2 + 270µl H_2O	1600
4	1:64	90µl Stnd. 3 + 270µl H_2O	400
5	1:256	90µl Stnd. 4 + 270µl H_2O	100

III. RESULT

A. *PCR and SYBR green RT PCR result of WSSV infection in rice crab Paratelphusa hydrodomous*

The WSSV infection was expressed in the PCR amplification exposed notable band at 530bp while comparing with the molecular marker showing 848 bp, 630 bp and 333 bp. The RT PCR 0.000001 copies for the Ct value 22.5. The melting curves were observed under 8.0 to 24.5 for the RT-PCR process under the validated denaturation, annealing and elongation variations. The amplification plots were exposing its graphical expression for 94°C, 60°C and 72°C.

IV. DISCUSSION

WSSV is an comprehensive infective virus in Asia and Americas [18] by affecting most commercially important species of Penaeid shrimp including P. monodon, P. japonicus, P. indicus, P. chinensis, P. merguiensis, P. aztecus, P. stylirostris, P. vannamei, P. duorarum and P. setiferus (11, 19, 20, 22]. Wild marine shrimp such as P. semisulcatus, Metapenaeusdobsoni, M. monoceros, M. elegans, Heterocarpussp, Aristeussp, Parapenaeopsisstylifera, Solenoceraindica, Squilla mantis and freshwater cultured species, Macrobrachiumrosenbergii have also been found to harbour this virus [22, 23, 24]. Here we had successfully transmitted WSSV to rice shrimp which it diagnosed with powerful molecular evidencing PCR and RT-PCR. The PCR was recorded with ~530bp of clear band while matched against the marker engaged with 848 bp, 630 bp and 333 bp bands without any over lappings in the bands in it. The ~530bp of WSSV gene recorded is considered to be a medium range of infection. While the same infected tissue was analyzed under SYBR green RT-PCR for expression study of WSSV-419 gene in the rice crab *Paratelphusa hydrodomous* it engages with 0.000001 copies of expression. This shows that the WSSV is considerd to be an infective agent for rice crab *Paratelphusa hydrodomous*e. This experimentation was made by us by means of transmission of WSSV in the fresh water rice shrimp which is for useful means for live stocking of WSSV for the researches in the mean of further transmission and exploiting the research based on WSSV.

V. ACKNPWLEDGEMENT

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FIGURES

Fig 1: WSSV transmitted rice crab *Paratelphusa hydrodomous*

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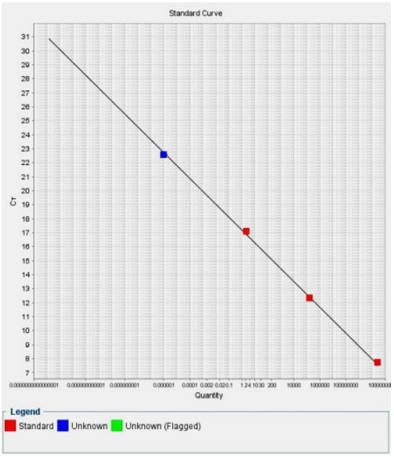
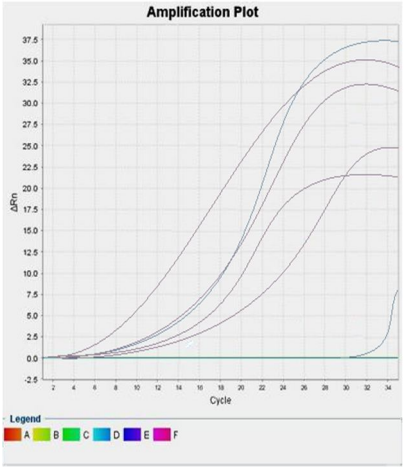
Fig 2: Agarose Gel Electrophorosis exposing ~530bp polymerized WSSV 419 like protein gene of WSSV transmitted to rice crab *Paratelphusa hydrodomous*



A



B

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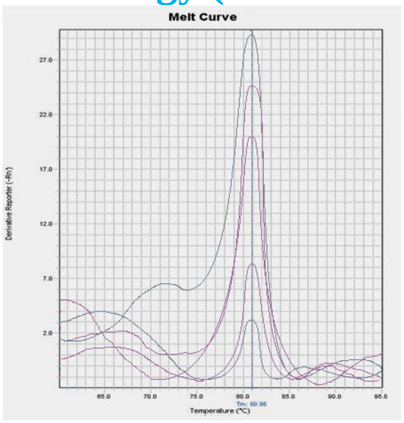
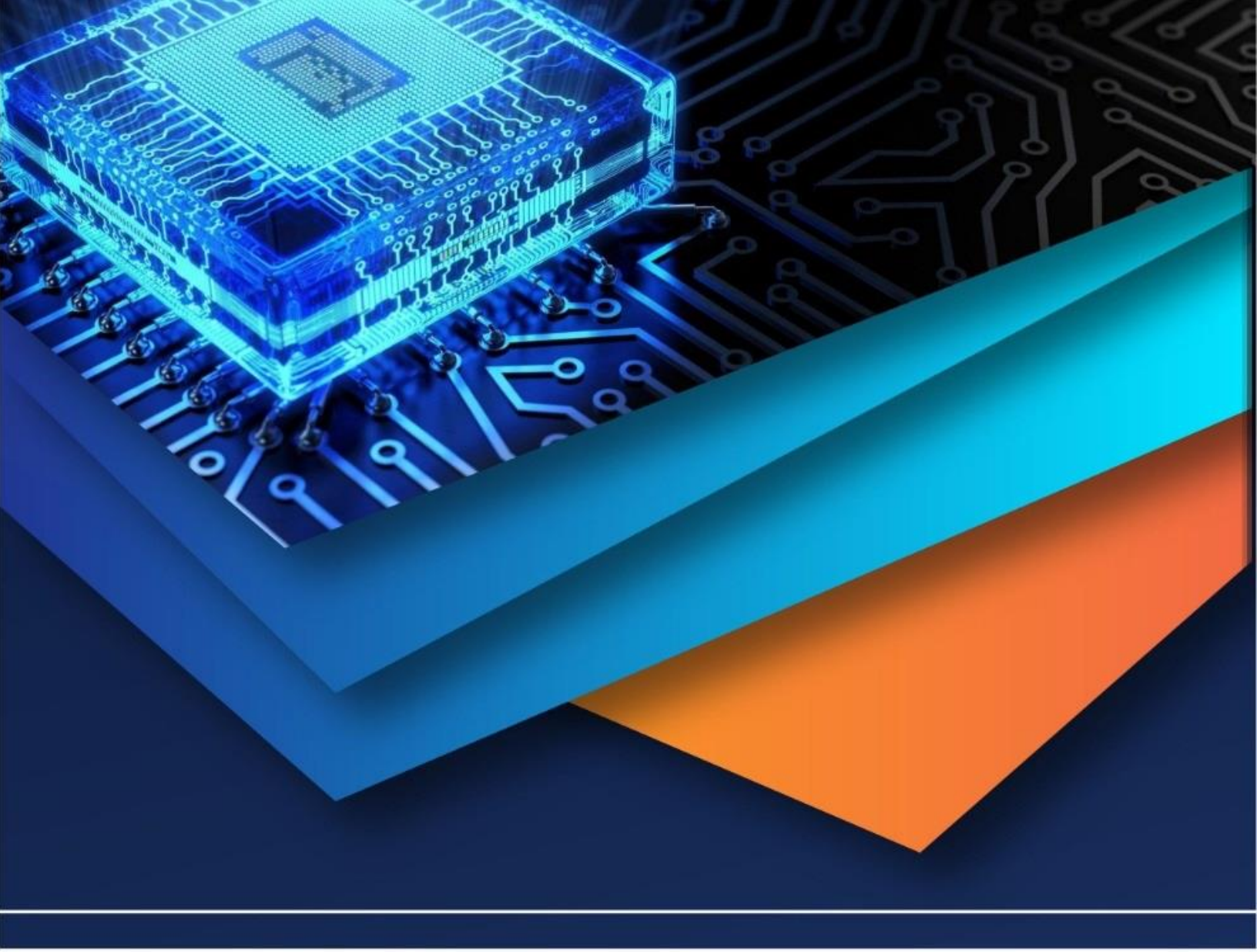


C

Fig 3: WSSV-419 like protein gene expressing 0.000001 copies of gene the rice crab *Paratelphusa hydrodomousIreported* by SYBR green RT-PCR studies: A- Standard curve, B- Amplification curve, C- Melting cruve



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