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ORIGINAL ARTICLE

Investigation and confirmation of white spot syndrome virus (WSSV) infection in wild caught penaeid shrimps of Andaman and Nicobar Islands, India

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Abstract White spot syndrome virus (WSSV) is one of the most prevalent, widespread and devastating pathogen associated with shrimp population. The present study was aimed at screening the wild caught shrimps from Andaman and Nicobar Islands (ANI) for WSSV infection. Shrimp samples of different penaeid species including *Penaeus monodon, Penaeus indicus, Penaeus merguiensis* and *Metapenaeus monoceros* collected from nine different landing centers across the coast of ANI were screened for WSSV infection. Presence of white spots, a typical clinical sign of white spot disease was observed on the exoskeleton of WSSV infected shrimp samples. Out of 241 shrimp samples, 39 samples of *P. monodon* were found positive for WSSV by nested PCR. Histopathological examination revealed eosinophilic to basophilic intranuclear inclusion bodies in gill tissue which are typical characteristics of WSSV infection. Nucleotide sequence of WSSV isolated from ANI showed 100% identity to the sequences of WSSV reported from Thailand, Taiwan, China, Egypt, Mexico, Korea, France and 99% identity to WSSV reported from India. The detection of WSSV in wild *P. monodon* of ANI further confirms the virus spread and biogeography.

Keywords WSSV · Wild shrimps · *Penaeus monodon* · Andaman and Nicobar Islands

Introduction

Aquaculture in India is a very important economic activity and contributes immensely to the food security of the country. Shrimp aquaculture is the rapidly growing animal food producing sector and at present, around 75% of shrimp production is dominated by two species namely black tiger shrimp (*Penaeus monodon*) and Pacific white shrimp (*Litopenaeus vannamei*). Even though aquaculture production is dominated by shrimps, the sector has been suffering from huge losses due to occurrence of different existing and emerging diseases. Viral diseases and associated mortalities are emerging as the major threat to penaeid shrimp culture [27]. Intensive research on various viral diseases of shrimp has been carried out in mainland India which leads to the detection of White Spot Syndrome Virus (WSSV), Infectious Hypodermal and Haematopoietic Necrosis Virus (IHHNV), Hepatopancreatic Parvovirus (HPV), Monodon Baculovirus (MBV) and Laem-Singh Virus (LSNV) from the culture system [2, 7, 12, 21].

To date, more than 20 viruses have been reported from the cultured and wild shrimps [8]. But none of them has been as devastating as white spot virus that appeared during 1993–1994 [23] and still continues to show its devastating effect throughout the world. WSSV, causative agent of white spot disease (WSD) is a double stranded DNA virus which affects the tissues of ectodermal and mesodermal origin [4]. The clinical signs of WSD include lethargy, anorexia, white spots on the cuticle and generalized reddish to pink discoloration [6]. Histopathological evidence for WSSV includes hypertrophied nuclei with

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eosinophilic to basophilic intranuclear inclusion bodies in ectodermal and mesodermal origin tissues [13].

Andaman and Nicobar Islands (ANI) consist of 572 group of islands situated in the Southeast Bay of Bengal in close proximity with Myanmar and Southeast Asian countries like Thailand, Malaysia and Indonesia. At present, freshwater aquaculture is being carried out with an average production of 194 tons per year while the brackishwater aquaculture mainly shrimp farming and mariculture are the potential sectors for future development in ANI. Around 1680 ha of area has been identified potentially suitable for brackishwater aquaculture in ANI [15]. ANI is well known for its aquatic germplasms mainly shrimps which are used as broodstocks for seed production. Very few aquatic animal diseases have been reported from ANI which may be due to lack of intensified research on this particular area [24]. In case of shrimp, the reported diseases are limited to luminous *Vibrio harveyi* infection in hatchery reared larvae of *P. monodon* [26], 20 and 25% prevalence of LSNV and WSSV, respectively in wild caught *P. monodon* broodstocks of ANI [17, 25]. Globally, the presence of WSSV in wild shrimps has been reported in detail by various groups [9–11, 19, 20]. It is essential to screen the wild shrimps for deadly pathogens since shrimp seed production mainly depends on the wild broodstocks. On the other hand, ANI is located in close proximity with Southeast Asian countries, where the intensified shrimp aquaculture with economic loss due to viral diseases is prominent. However, there is no much information available on WSSV infection from the wild shrimps of ANI except, one report on the detection of WSSV using only polymerase chain reaction (PCR) [25] without other detailed information like the spread of virus, nucleotide sequence and histopathological evidence. The degree of spread of this virus in a comparatively less disease affected area like ANI and the extent of contamination of this water body is not properly known. Therefore, we attempted to make a thorough survey in this area and find out the status with respect to WSSV infection. Here we report the detailed information on the prevalence of WSSV in wild caught shrimps of ANI using PCR, sequencing and histopathology.

Materials and methods

Study area

Shrimp samples were collected from various landing centers across the coast of ANI namely Durgapur (13°16′45.7″N; 93°2′9.1″E), Laxmipur (13°17′33.03″N; 92°57′29.16″E), Mayabunder (12°54′35.3″N; 92°54′29.1″E), Betapur (12°36′1.3″N; 92°57′22.3″E), Yerrata (12°27′36.06″N; 92°53′47.54″E), Junglighat (11°39′25.26″N; 92°43′30.23″E), Lohabarrack (11°37′21.32″N; 92°38′49.03″E), Wandoor (11°35′44.66″N; 92°36′28.81″E) and Campbell Bay (7°0′36.12″N; 93°55′49.38″E) during August, 2015–June, 2016 (Supplementary Fig. 1). A total of 241 shrimp samples of different penaeid species include *P. monodon, P. indicus, P. merguiensis* and *Metapenaeus monoceros* were collected (Table 1). Mean length and mean weight of the collected shrimp samples were 14 cm and 63 g, respectively. Tissues like pleopod and gill were excised aseptically and stored in 90% ethanol until further use. For the histopathological analysis, gill tissues were preserved in Davidson fixative.

Isolation of DNA and PCR detection of WSSV

DNA was extracted using modified CTAB method of Bruce et al. [3]. The extracted DNA was used for PCR reaction. First step and nested PCR reactions were

Table 1 Details of sample collection

Name of the district	Landing center	Number of samples collected				Total number of samples collected
		P. indicus	*P. monodon*	*P. merguiensis*	*M. monoceros*	
North and Middle Andaman	Durgapur		6	5		11
	Laxmipur	5		11		16
	Mayabunder	15				15
	Betapur		19	9		28
	Yerrata	5		15		20
South Andaman	Junglighat		6 (3)	20		26 (3)
	Lohabarrack		95 (31)			95 (31)
	Wandoor				5	5
Nicobar	Campbell Bay		25 (5)			25 (5)
Total		25	151 (39)	60	5	241 (39)

Values within parenthesis indicate the number of samples that were tested positive for WSSV by PCR

performed by following the method of Kimura et al. [14] using WSSV specific primers namely, WSSVF1: ATCATGGCTGCTTCACAGAC; WSSVR1: GGCTGGA GAGGACAAGACAT; WSSVF2: TCTTCATCAGATGC TACTGC; WSSVR2: TAAGGCTATCCAGTATCACG. Known negative and positive controls were included in all the reactions. The PCR amplified products were analyzed in 1.2% agarose gel containing TAE buffer and analyzed using gel documentation system.

Sequencing and sequence analysis

The WSSV positive PCR products were purified and sequenced using ABI 3500 DNA analyzer (Shrimpex Biotech Pvt. Ltd., Chennai) and analyzed using BLAST program in NCBI database for finding homology with other sequences. The multiple sequence alignment of WSSV isolated from ANI with other countries was constructed using MEGA6 software [28].

Histopathology

Tissue samples were cut and processed as per the protocol of Bell and Lightner [1]. Sections (5 μ) from the embedded tissues were processed and stained using hematoxylin and eosin.

Results and discussion

Detection of WSSV in wild shrimps

In this study, 241 samples of different penaeid species were collected from nine landing centres across the ANI coast (Table 1). Out of 241 samples, clear white spots on the exoskeleton were observed only in four number of *P. monodon* shrimp samples (3 samples from Lohabarrack and 1 sample from Campbell Bay landing centres) with mean length of 21 cm and mean weight of 97 g (Fig. 1). It is corroborated that the observation of local lesions and white spots on the carapace is the simplest method to detect

Fig. 1 WSSV infected *P. monodon* showing characteristic white spots (arrow) on the carapace

WSSV infection in shrimp [5]. On the other hand, wild broodstocks of *P. monodon* in Malaysia and wild crustaceans in India were found positive for white spot virus without showing any signs of infection like white spots on the body surface [16, 29]. Out of 241 shrimp samples, WSSV was detected in 39 samples (16%), in which 11 samples detected with WSSV by first step PCR (Fig. 2). Among all the tested penaeid shrimp species in the present study, WSSV was detected only in 39 samples out of 151 *P. monodon* shrimp samples and not detected in other penaeid shrimps including *P. indicus, P. merguiensis* and *Metapenaeus monoceros*. During this study, the frequency of WSSV occurrence in shrimp samples was 31 out of 95 samples from Lohabarrack, 3 out of 26 samples from Junglighat and 5 out of 25 samples from Campbell Bay and not observed in other landing centers. The PCR result was further supported by histopathological investigation. Histopathological analysis of gill sections revealed eosinophilic to basophilic intranuclear inclusion bodies which are typical characteristics of WSSV infection (Fig. 3). This is corroborated with the earlier reports which showed inclusion bodies specific for WSSV infection [13, 22]. Occurrence of high nested positive samples for WSSV in the present study is well supported by the earlier report [25] and a preliminary survey carried out by ICAR-Central Institute of Brackishwater Aquaculture during 2004 (unpublished) indicated the tested samples of *P. monodon* from ANI to be positive for WSSV by nested PCR. Likewise, the incidence of WSSV was recorded as 23% in wild caught crustaceans from Southwest and Southeast coast of India [29].

Sequence analysis of WSSV

Nucleotide sequence of WSSV isolated from *P. monodon* of ANI (GenBank accession number KX980155) showed 100% identity to the sequences of WSSV reported from Thailand (AF295123), Taiwan (AF440570), China (KT995472), Egypt (KR083866), Mexico (KU216744), Korea (JX515788), France (AF343568) and 99% identity to WSSV reported from India (AY374443). Multiple sequence alignment of WSSV isolated from ANI with other countries is shown in supplementary Fig. 2.

The present study indicated that the wild shrimps from South Andaman and Nicobar districts of ANI are infected with WSSV to a lesser extent as compared to the mainland where number of other penaeid shrimps and crustaceans were also found to be positive. However, the present study further confirms the pandemic spread of this virus. Earlier reports mentioned that a major route of potential viral entry into the culture system is post larvae infected via captured broodstock [10, 18, 20]. Though WSSV was found in *P. monodon*, there is possibility that it will spread to other

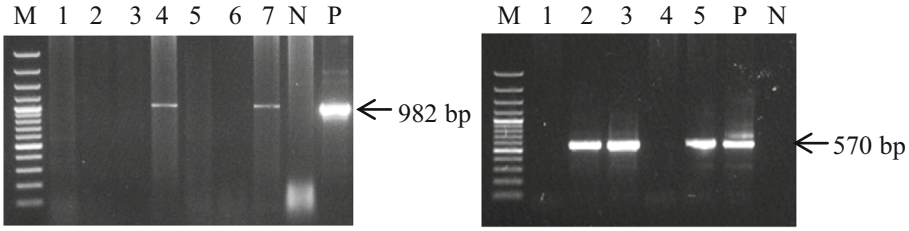

Fig. 2 Detection of WSSV using first step PCR (982 bp product) and nested PCR (570 bp product). M-100 bp marker; 1–7—Samples; P-Positive control; N-Negative control

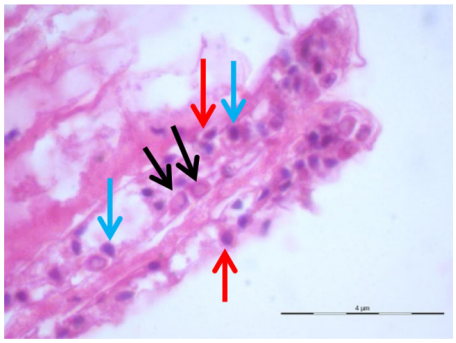

Fig. 3 Gill sections of infected *P. monodon* showing cowdry type A inclusion bodies (red arrows), early eosinophilic inclusions (black arrows) and basophilic inclusions (blue arrows) suggestive of WSSV infection

crustaceans and thereby can contaminate the wild stock. Hence the presence of WSSV in wild caught shrimps of ANI poses a potential threat to the very survival of their population and may affect the shrimp aquaculture. Efforts should be taken to thoroughly screen the broodstocks before seed production there by protect the fragile ecosystem of ANI.

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