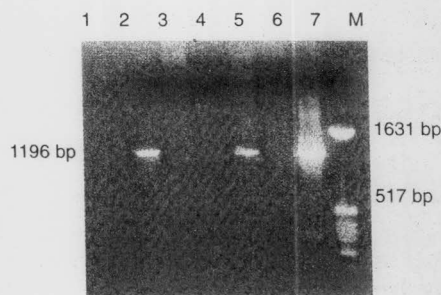


PROMISING TECHNOLOGIES

Improved Semi-nested PCR for Detection of White Spot Syndrome Virus in Shrimp Farms

White Spot Syndrome Virus (WSSV) is the causative agent of a devastating disease that affects penaeid shrimps and hatcheries and has caused severe economic losses in India and several Asian countries. Typical symptoms of the disease are reddish or pinkish discoloration of the body and white spots on the head and shell region of the affected shrimp. The White Spot Disease first appeared in India in late 1995 in the tiger shrimp farms of Andhra Pradesh and subsequently spread to other shrimp farming States like Orissa, West Bengal, Tamil Nadu, Kerala and Karnataka and caused large scale devastation. In 1999, it reappeared on a large scale in the shrimp farms of Ernakulam and Cannanore districts of Kerala. An improved semi-nested Polymerase Chain Reaction was found to be very useful for the detection of WSSV using WSSV specific primers. The first step gave an amplified DNA fragment of 1447 bp length while the



Semi nested PCR amplification of WSSV DNA. Lanes 2, 5 and 7 are positive for WSSV virus. Samples 2 and 5 did not have visible white spot lesions. Sample 7 showed white spot lesions. After about 10 days shrimp from farms 2 and 5 developed white spot disease.

semi-nested method gave a 1196 bp length DNA fragment. The semi-nested PCR was found to be more sensitive than the single step PCR. Samples that were found to be negative for the WSSV by single step method were found positive by the semi-nested PCR. The PCR method was able to detect even latent stages of infection. This method was used to screen WSSV in postlarvae, juveniles and adults of penaeid shrimp (*Penaeus monodon* and *Penaeus indicus*).

WSSV infection in shrimp can be detected as white spots on the

carapace and body shell of the affected shrimp. But white spots become visible only in the last stages of the disease. Within a few days after the appearance of spots the shrimps die *en masse*. Till now, histopathology and electron microscopy were the only methods available for the detection of WSSV virus. Latent Carrier State of WSSV infection in shrimp has been reported but is not discernible by the above methods.

The improved PCR technique has been used for surveillance of shrimp farms and hatcheries with very promising results. In the eight adult shrimp samples tested from farms, while 50% were positive by single step PCR, 100% were positive by the improved semi-nested method. Within ten days after the test was conducted all the samples from farms developed white spot disease. Four of the twenty-two post-larval samples from hatcheries were positive for WSSV by single step method, while 46% of the samples showed to be positive by the improved semi-nested PCR.

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Entry of Foreign Animal Disease to India Prevented

The High Security Animal Disease Laboratory (HSADL) at Bhopal under ICAR is the only biocontainment laboratory of its kind in Asia and has been functional since 1998. One of the prime objectives of the laboratory is to prevent the introduction of foreign animal diseases into the country. The Ministry of Textiles, Government of India recently imported 150 Angora rabbits from Germany for fur development. These rabbits were reported to be free from many diseases including Rabbit Haemorrhagic Disease (RHD) and Aujeszky's. Since the European countries and China are not free from RHD, the HSADL was requested to carry out testing of rabbits

for possible infection with foreign/exotic disease agents. Material from these rabbits was collected by scientists for laboratory testing. As many as 130 serum samples and morbid materials from 6 rabbits were collected for testing. On enzyme linked Immuno-absorbent assay (ELISA), 62 of the 130 rabbits were positive for antibodies against Rabbit Haemorrhagic Disease (RHD). Besides this, the disease was also reproduced by experimental infection of native rabbits. Pathological lesions were observed both in imported and experimentally infected rabbits. The disease was also confirmed by polymerase chain reaction (PCR)

testing using specific oligonucleotide primers of RHD viral genome. The rabbits were negative for Aujeszky's disease (Pseudorabies) virus infection. The test results were communicated to Ministry of Animal Husbandry and Dairying with the recommendation that these rabbits be destroyed. Base line data generated by HSADL indicated that RHD is not prevalent in the country. So far as the Aujeszky's disease is concerned, seroprevalence of the disease has been indicated in Swine and Rabbit population in India.

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