Studies on Occurrence and Distribution of Virulent Strains of *Vibrio parahaemolyticus* in Finfishes and Prawns from Different Ecosystem of Gujarat (India)

D.P. Kshirsagar*, M.N. Brahmbhatt, Y.A. Chatur and N. Sinha
Department of Veterinary Public Health, Veterinary College, AAU, Anand
(Received 30.12.2011; accepted 19.07.2013)

**ABSTRACT**

In the present study occurrence and distribution of virulent strains of *Vibrio parahaemolyticus* in finfishes and prawns collected from different ecosystem of Gujarat viz., marine and fresh water were investigated. A total of 155 samples were subjected for isolation of *Vibrio parahaemolyticus*, of which 18 (11.61%) samples were found to be positive. Among 105 marine fish samples comprising 65 finfishes and 40 prawns, 15 (14.28%) were found to be positive for *V. parahaemolyticus* and 50 fresh water fish samples comprising 30 finfishes and 20 prawns yielded 3 positive samples (6.0%). Furthermore, all positive *V. parahaemolyticus* isolates were studied for polymerase chain reaction (PCR) amplification of the toxR, tdh and trh genes. All the biochemically confirmed strains were found to have toxR (368 bp) gene fragment. Only 2 (11.11%) isolates amplified tdh (269 bp) gene. None were found to amplify trh gene.

**Keywords:** Finfishes, prawns, tdh, toxR, trh, *Vibrio parahaemolyticus*

**Introduction**

*Vibrio parahaemolyticus* is a prevalent seafood-borne pathogen in many Asian countries where marine foods are frequently consumed. The organism has been reported as the causative agent of gastroenteritis, wound infections and septicemia because of the consumption of contaminated seafoods (Dileep *et al.*, 2003). Recently with the emergence of pandemic O3:K6 strain of *V. parahaemolyticus* that was reported to cause acute gastroenteritis, this pathogen has acquired greater significance (Matsumoto *et al.*, 2000).

*V. parahaemolyticus* is compatible with a marine aquatic environment adjusting well to the broad range of salinities and this commonly found on prawns and all varieties of finfishes that are traditionally taken from marine and shore areas. Foods, particularly seafoods in which small or limited numbers of organism are often present, may become heavily contaminated by time temperature abuse, a major cause of microbial replication and contamination the short generation time of 12 min for *V. parahaemolyticus* permits the organisms to accumulate in millions in a few hours (Syndman and Gorfach, 1991).

Studies revealed the thermostable direct haemolysin (TDH) and the TDH-related haemolysin (TRH), encoded by the *tdh* and *trh* genes, respectively, as the major virulence factors of this organism (Shirai *et al.*, 1990). The test adopted for Kanagawa phenomenon using Wagatsuma agar consumes time and labor. Moreover, the test may not be determinative as many environmental strains are Kanagawa negative and do not produce TDH (Tada *et al.*, 1992). Presently there is no *in-vitro*
test for the detection of TRH in V. parahaemolyticus. Sensitive and rapid molecular method such as PCR has been applied to identify the presence of tdh and trh genes from V. parahaemolyticus (Wong et al., 1999, Kim et al., 1999). The present study was envisaged to study the occurrence and distribution of virulent strains of V. parahaemolyticus in fishes and prawns of different ecosystems and to compare its enteropathogenicity.

Materials and Methods

Sample collection
A total of 155 samples comprising fin fishes and shellfishes from different ecosystems were collected over a period of one year from various retail fish markets in and around Anand (Gujarat) for microbiological examination. Finfish samples included Mrigal (Cirrhinus cirrhosis), Surmai (Scomberomorus commerson), Mangur (Clarias batrachus), Tarali (Sardinella longiceps), Halwa (Apolectus niger), Jira (Cypselurus comatus).

Isolation of Vibrio parahaemolyticus
The whole fin-fishes and prawns were aseptically cut into small pieces. About 10 g of the fish sample was weighed aseptically and transferred into 100 ml of alkaline peptone water (APW) containing 3% NaCl. A loopful of culture from APW after 18-24 h enrichment was streaked onto thiosulfate citrate bile salt sucrose agar (TCBS) and incubated at 37 °C for 24 h.

The characteristically large colonies (3-4 mm) with light blue or green centers on TCBS were regarded as presumptive V. parahaemolyticus and further subjected to standard biochemical tests as per BAM, USFDA method (Kaysner and DePaola, 2004) for confirmation.

PCR assay
PCR was performed separately for toxR, tdh and trh genes for the biochemically characterized isolates. The DNA of isolates of V. parahaemolyticus was prepared by bacterial lysis by heat application method. Approximately loopful of culture was taken in microcentrifuge in 100 µl of sterilized DNase and RNase-free milliQ water (Millipore, USA). Then vortexed and samples were heated at 95°C for 10 min, cell debris was removed by centrifugation and 3 µl of the supernatant was used as a DNA template in PCR reaction mixture. PCR was performed with three sets of primer pairs specific for the toxR, tdh and trh gene as shown in Table 1.

Results and Discussion
Analysis of result showed that of a total 155 raw fin fish and prawn samples collected from different ecosystem viz., freshwater fishes, freshwater prawns, seafishes and marine prawns resulted in the isolation of 18 isolates (11.61%) of V. parahaemolyticus. Of the total of 105 marine samples comprising 65 finishes and 40 prawns, 15 (14.28%) were found to be positive for V. parahaemolyticus i.e. 9 from finishes and 6 from prawns. Fifty samples comprising 30 finishes and 20 prawns of freshwater origin yielded 3 positive samples (6.0%) as shown in Table 2.

Being a halophilic organism, occurrence of V. parahaemolyticus in coastal areas is not uncommon. But it was interesting to note the occurrence of this halophile from 6.0% of freshwater fishes and 5.0% from freshwater prawns. It could be due to cross contamination due to mixing of fishes from coastal area. Higher incidence (13% - 34%) of V. parahaemolyticus in freshwater fishes has been reported by earlier workers (Sarkar et al., 1985, Nithya Quintiol et al., 2007, Das et al., 2009, Subhashini and Krishnaiah, 2010).

In the present study all the eighteen V. parahaemolyticus isolates were found to amplify the species specific toxR (368 bp) gene and only 2 (11.11%) were found to exhibit positive amplification for the tdh (269 bp) gene. None of the isolates exhibited positive amplification for the trh gene. The findings of the present study seems to be similar with that of Leal et al. (2008) who failed to report trh gene in any of the isolates, while higher prevalence 38.73% reported by Bej et al. (1999) and lower prevalence were reported by Chakraborty et al. (2008), Robert-Pillot et al. (2004) and Dileep et al. (2003).

In conclusion, the present study emphasizes the importance of molecular methods like PCR for identifying both pathogenic and non-pathogenic isolates. As, both pathogenic and non-pathogenic
Table 1. Primer pairs used for virulence characterization of *Vibrio parahaemolyticus*

<table>
<thead>
<tr>
<th>Primer pair target</th>
<th>Primer sequence (5’-3’)</th>
<th>Annealing (°C)</th>
<th>Length (bp)</th>
<th>Reference</th>
</tr>
</thead>
</table>
| toxR               | F: GTC TTC TGA CGC AAT CGT TG  
|                    | R: ATA CGA GTG GTT GCT GTC ATG | 60           | 368         | Kim et al., (1999) |
| tdh                | F: GTA AAG GTC TCT GAC TTT TGG AC  
|                    | R: TGG AAT AGA ACC TTC ATC TTC ACC | 57           | 269         | Bej et al., (1999) |
| thr                | F: TTG GCT TCG ATA TTT TCA GTA TCT  

Table 2. Isolation of *V. parahaemolyticus* from marine and freshwater fishes

<table>
<thead>
<tr>
<th>Nature of Sample</th>
<th>No of samples examined</th>
<th>No of samples positive</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Marine Finfish</td>
<td>65</td>
<td>9</td>
<td>13.84</td>
</tr>
<tr>
<td>Prawn</td>
<td>40</td>
<td>6</td>
<td>15.0</td>
</tr>
<tr>
<td>Freshwater Finfish</td>
<td>30</td>
<td>2</td>
<td>6.66</td>
</tr>
<tr>
<td>Prawn</td>
<td>20</td>
<td>1</td>
<td>5.0</td>
</tr>
<tr>
<td>Total</td>
<td>155</td>
<td>18</td>
<td>11.61</td>
</tr>
</tbody>
</table>

Fig. 1. Agarose gel showing amplification product of *toxR* gene. Lane 1: Positive control; Lane 6: Negative control; Lane 2-5: Amplification product of *toxR* gene (Approximately 368 bp); L: 100bp DNA ladder

Fig. 2. Agarose gel showing amplification product of *tdh* gene; Lane 1: Positive control; Lane 4&5: Negative samples; Lane 2&3: Amplification product of *tdh* gene (Approximately 269 bp); L: 100 bp – 3000 kbp DNA ladder
strains of *V. parahaemolyticus* exist in the seafood, application of PCR specific for the virulence genes (*tdh* and *trh*) will help in detection of pathogenic strains of *V. parahaemolyticus* and consequently reduce the risk of food-borne illness.

**Acknowledgement**

The authors are grateful to Dean, Veterinary College Anand for providing necessary facilities and funds for the work. Authors are thankful to Dr. T. Ramamurthy, Deputy Director, Division of Microbiology, National Institute of Cholera and Enteric Diseases (NICED) in Kolkata for assistance in procuring the reference strains and cooperation on carried out this work.

**References**


