

## Mass mortality of *Penaeus monodon* post-larvae due to *Vibrio cholerae* O139 infection

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Vibriosis is one of the most serious threats in the commercial production of larval penaeid shrimp. Large-scale mortalities in larval penaeids associated with *Vibrio harveyi* have been observed globally (Lightner, 1996). Disease outbreaks attributed to other *Vibrio* species such as *V. alginolyticus*, *V. damsela*, *V. parahaemolyticus*, *V. vulnificus* and *V. penaeicida* have been reported in nursery or growout ponds of *Penaeus vannamei*, *P. monodon*, *P. japonicus* and *P. stylirostris* worldwide (Brock and Lightner, 1990; Ishimaru *et al.*, 1995). *V. anguillarum*, *V. campbelli*, *V. nereis*, *V. cholerae* (non O1) and *V. splendidus* have also been reported in association with disease outbreaks in shrimps (Chen 1992; Lavilla *et al.*, 1990; Esteve and Quijada, 1993; Sahul-Hameed *et al.*, 1996). Vibriosis in penaeid shrimp post-larvae might be due to either opportunistic *Vibrio* flora or to pathogenic *Vibrio* spp. specifically infective at one or more larval developmental stages.

In this study, we investigated the cause of mass mortality in *Penaeus monodon* post-larvae in farms located in Ernakulam, Kerala (India). The symptoms of the disease included lethargy and reddish discolouration of the affected post-larvae. The infected *P. monodon* post-larvae died within 48 h after the infection. The infected post-larvae were tested for pathogenic bacteria by plating on to Tryptone Soy Agar (TSA) and Thiosulphate Citrate Bile Salts (TCBS) Agar. Bacterial isolates obtained from the moribund and dead shrimp were confirmed as belonging to *V. cholerae* O139 serogroup, the aetiological agent of cholera in humans, using O139 serogroup-specific antiserum and by a PCR based assay targeting *rfb*-O139 gene (Fig.1). The isolates were found to carry cholera toxin gene *ctx* and genes coding for virulence determinants; *zot* and *tcpA* in PCR assay.

*Vibrio cholerae* is an autochthonous flora of brackish water and estuarine systems. *V. cholerae* O1 and O139 are the major serogroups that cause outbreaks of cholera in human beings. More than 200 serogroups of *V. cholerae* has been identified so far and epidemic cholera has been confined only to isolate within serogroups of O1 and O139. The cholera toxins produced by *V. cholerae* are the causative agents of cholera. There are only limited reports of infections in post-larvae of shrimp due to *V. cholerae*. A *V. cholerae* non-O1, non-O139 isolate from a shrimp farm was reported to be pathogenic to post-larvae and juveniles of *P. monodon* larvae (Halder *et al.*, 2007). In this study, a highly virulent *V. cholerae* O139 strain was isolated from infected shrimp post-larvae.

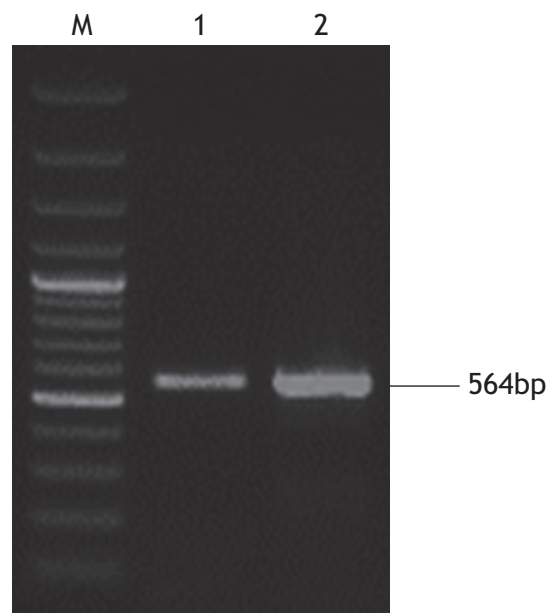


Fig. 1. Detection of *V. cholerae* O139 cholera toxin gene by PCR. Lane 1 - *V. cholerae* MTCC3906; Lane 2 - Isolated strain from infected post-larvae; Lane M - 100bp plus ladder (MBI Fermentas, US)

Experimentally exposed shrimp post-larvae with *V. cholerae* O139 exhibited significant mortalities that increased with increasing doses of bacteria. The LD<sub>50</sub> value of one of the isolates was determined in post-larvae of *P. monodon*, *Fenneropenaeus indicus* and *Litopenaeus vannamei* which ranged from 4.6x10<sup>4</sup> for *L. vannamei* to 7.1x10<sup>6</sup> for *P. monodon*. *V. cholerae* was reisolated from experimentally infected moribund shrimps. Histopathological examination revealed the presence of large numbers of bacteria laden in the hepatopancreas of the infected post-larvae. There was rupture of basal laminae of the hepatopancreatic tubules and severe necrosis, loss of structure, atrophy, vacuolation and rounding into the lumen of tubular epithelial cells, which suggest that tissue integrity was affected in shrimp due to the infection (Fig.2). The bacterial strain isolated from moribund *P. monodon* post-larvae was identified as the causative agent of the mortality by isolation, subculture, reinfection and reisolation according to Koch's Postulates. To our knowledge, this is the first report of *V. cholerae* O139 strain causing high mortalities in shrimp.

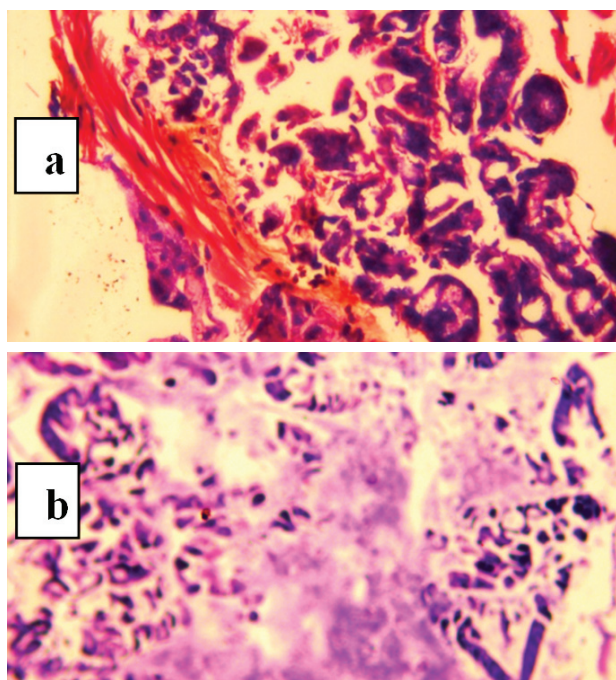


Fig. 2(a) Healthy hepatopancreas in control larvae. (b) Pathology of hepatopancreas in *P. monodon* larvae showing severe necrosis, rounding and sloughing of cells, when infected with *V. cholerae*

This study has wide repercussions since the infected post-larvae can serve as a host for

multiplication and spread of the highly pathogenic *V. cholerae* O139 outside the human host. The involvement of cholera toxin and other regulatory genes in the pathogenesis of shrimp need to be ascertained.

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