Uttar Pradesh J. Zool. 20(3) : 243 - 247, 2000 ISSN 0256 - 971X

VIBRIOSIS OUTBREAK IN PRAWN FARMS AND THE GROWTH THRESHOLD OF ENDEMIC PATHOGENS POPULATION

D.R. SHYAMALA AND K. RAMALINGAM
PG & RES DEPTT. OF ZOOLOGY, GOVT. ARTS COLLEGE,
NANDANAM, CHENNAI - 600035, INDIA

Mass mortality among cultured tiger praws (*Penaeus monodon*) due to vibriosis was a common occurrence in summer months in the south-east coast of India. The *Vibrio* species were isolated and identified through culture from the diseased shrimps. A one-year field survey on the presence of *Vibrio* species in *P. monodon* was carried out in about 15 commercial farms around Madras by a conventional culture method. The results revealed that the pathogen is widely distributed in the prawn culture environment. Its population, which increases with the rise in temperature during summer months, is implicated for the mass mortality of the prawns. The *Vibrio* strains isolated from the tissues of diseased prawns were cultured in TCBS agar. The strains identified were *V. parahaemolyticus* and *V. alginolyticus*.

INTRODUCTION

Vibriosis an enzootic disease of shrimps recorded from all over the world, is caused by haplophilic vibrios which exhibit ubiquitous distribution in marine and estuarine environments. Significant mortality occur in larval and postlarval *Panaeus monodon* due to luminescent vibrios caused by *Vibrio harveyi* and *V. splendidus* (Tison & Seidler, 1983; Baticados *et al.*, 1990). Infections may be chronic, subacute or acute and mortality may reach even 100 percent in some cultured populations. Vibriosis in prawns from the early postlarval stages in the hatcheries can cause considerable loss upto the age of 3 months. it may be transmitted vertically and may cause disastrous infection at the next spawning (Lightner, 1996).

Vibrio infections of the cuticle, appendages or gills apparent as black or brown spots (due to melanin produced by the host haemocytes). The clinical signs includes increasing opaqueness of abdominal muscle, anorexia, expansion of chromatophores, anoxia and the appearance of small white speckles on the cuticle. The typical symptoms are light browning of the gills and swelling of the lymphoid organs.

A number of bacteria have been implicated to cause disease and mortality in cultured penaeids especially in the larval, postlarval and juvenile stages (Johnson, 1990; Takahashi *et al.*, 1990). In every reported case of bacterial infections in penaeid shrimp, motile, gram -ive, oxidase positive, fermentive rods have been isolated. Most isolates contained the *Vibrio* species *namely V. alginolyticus, V. parahaemolyticus* and *V. anguillarum. Vibrios* are natural inhabitants of the aquatic environment, especially the marine habitat and transmitted to human beings by the ingestion of raw or inadequately cooked seafood (Curtis *et al.*, 1994). *Vibrio* species have been documented to cause large scale of mortality in cultured penaeids. Vibrios, also act as causative agent of seafood poisoning and as a kind of opportunistic pathogen to the marine animals. Some vibrio species are also pathogenic to human beings (Blake *et al.*, 1980). The endemicity of *Vibrio* in the coastal areas of United States have been revealed by recent investigations (Kaneko *et al.*, 1974; Kaysner *et al.*, 1990; Honda *et al.*, 1992). The endemicity of vibrio-related diseases has been attributed to the persistence of pathogen in their natural aquatic reservoir rather than to the maintenance of the disease in the human community (Kaper *et al.*, 1982). However, such prevalence of *Vibrio* population in the tropical waters to attribute to the outbreak of disease in penaeid species are yet to be established.

In the present study, the identification and distribution of vibrios in the prawn farms around Madras were studied periodically to enumerate and their endemicity in the tropical waters and also to evaluate the potential for food poisoning hazards and risks to public health.

MATERIALS AND METHODS

Juveniles of *P. monodon* weighing about 12 - 20 gm procured from about 15 commercial farms located around Madras were used in this study. The diseased prawns exhibiting clinical signs such as anorexia, anoxia, loss of apetite and lethargy were selected for this study. The samples were transported in aseptic condition to the laboratory and analyzed immediately. The infected portions like gills and hepatopancreas were cut with a sterile blade and homogenized with sterile seawater. Ten fold serial dilutions were done to avoid outgrowth of bacteria as described by Bullock (1971). The diluted samples were inoculated, cultured and raised on the *Vibrio* selective growth medium TCBS agar (Thiosulphate Bile Salts) by spread plate technique. The culture plates were incubated at 37^0C for 24 hours. Morphologically similar and dominant bacterial colonies were selected and streaked on Nutrient Agar plates to obtain pure culture. The biochemical tests such as glucose fermentation, Voges proskauer test, fermentation of carbohydrates to acid and carbohydrates as sole source of carbon were used to assign the isolates to a taxonomic group.

RESULTS

The strains isolated from the diseased prawns by the conventional bacterial culture methods were *V. parahaemolyticus* that appeared as green colonies on TCBS agar and *V. alginolyticus* grown as yellow colonies. The strains exhibited all the properties of the genus *Vibrio*. in the biochemical tests, the *V. parahaemolyticus* do not ferment sucrose whereas *V. alginolyticus* fermented sucrose. In the salt tolerance using Voges proskauer, *V. alginolyticus*, the yellow colour colonies formed red colour within 10 -15 mins exhibiting the growth expressing 90 - 100% strains postive, but *V. parakaemolyticus*, the green colonies forming isolates do not grow. In urea hydrolysis test, the yellow colonies exhibited no growth whereas the green colonies exhibited the positive growth with 11 - 89% strains positive.

In case of tests involving carbohydrates as sole carbon source, using D-gluconate, the yellow colony forming isolates do not exhibit growth but the green colony forming isolates exhibited growth of about 11 - 89% strains positive. In glucose fermentation test, the green colony forming isolates exhibited no colour change *i.e.* negative with initial alkaline green colour, whereas the yellow colonies were positive exhibiting acid-yellow growth. In the fermentation of carbohydrates to acid test, the yellow colonies exhibited negative growth for L-arabinose and growth for sucrose

Table I : Biochemical tests for the identification of *Vibrio* species.

Test	*V. alginolyticus*	*V. parahaemolyticus*
Salt tolerence		
Voges-Proskauer	+	-
Urea hydrolysis	-	v
Ferments		
L-arabinose	-	v
Sucrose	+	-
Sole Carbon Source		
D-glucose	-	v

+ = Growth (90 - 100%); - = No growth; v = Growth (11 - 89%).

whereas green colonies exhibited positive growth for L-arabinose with 11 - 89% strains positive and no growth for sucrose (Table I).

The results involving both the culture and biochemical tests infer that the yellow colonies were *V. alginolyticus* and the green colonies were *V. parahaemolyticus*. Table II indicates the population density of *V. parahaemolyticus* and *V. alginolyticus* and their monthly variations throughout the year 1998, as determined by the bacterial counts.

Table II : Intensity of *V. parahaemolyticus* and *V. alginolyticus* in the year, 1998.

JAN	FEB	MAR	API	MAY	JUN	JUL	AUG	SEP	OCT	NOV	DEC
ND	+	++	++	+++	+++	+++	+++	+++	++	+	+

ND = Not detected; + = Bacterial counts ranging 10^3 - 10^{4CFU}; ++ = Bacterial counts ranging 10^5 - 10^{6CFU}; +++ = Bacterial counts ranging 10^6 - 10^{7CFU}.

DISCUSSION

The prevelance of *Vibrio* species is seafood corroborates earlier reports from other countries revealing their world wide distribution (Bechteler & Holler, 1996). Prawn cultivation has developed from extensive to semi-intensive and then to insentive farming systems, characterized by increasing the stock densities, development of stages preceding and following the cultivation of the prawns and by comprehensive water management involving a large input of energy. The increasingly intensive mode of prawn cultivation could have resulted in the outbreak of vibrosis, the most important of the bacterial etiology have been reported from penaeid shrimp (*Vanderzant et al.,* 1971), the majority are of a secondary etiology occurring as a result of syndromes due to ascorbic acid deficiency, toxins, wounds, extreme stress ets (Lightner & Lewis, 1983). A number of reports in literature support the latter observations.

Vibrios pose an increasing threat in raw shrimp products, because of high probablity of contamination. Most outbreak of food born illness caused by *Vibrio* species which were indigenous to the marine environment have occurred after improper storage or cross contamination from other raw seafood sources (Vanderzant *et al.*, 1971). The ecology and pathogenicity of *V. parahaemolyticus* have been extensively studied since 1950s when the species was first reported to be associated with seafood borne human disease. The frequent occurrence of this pathogen in estuarine waters has been well established (Kaneko & Colwell, 1974; Donavan & Van nette, 1989) and also reported as a causative agent of gastroenteritis through consumption of contaminated seafoods (Matte *et al.*, 1994). *V. alginolyticus* is phenotypically similar to *V. parahaemolyticus* and has been recognized as a highly virulent pathogen (Chan *et al.*, 1989).

The pathogenecity of *V. parahaemolyticus* and *V. alginolyticus* in prawns has been revealed by Martins (1991), who showed that free solutions of crude extracts of endotoxins and exotoxins of *V. parahaemolyticus* and *V. alginolyticus* injected into *P. setiferus* produced significant mortalities with gross signs similar to those observed in bacterial infections. That the population of the pathogen increase both in prawns and in their environment with a rise in water temperature is revealed and confirmed from the present study by the prevalence *Vibrio* species has already been established in the prawn culture environment. The population of pathogen in both the prawns and their environment reaching its peak, coincide with the season when deterioration of environmental condition advances. The deterioration seems to start at the middle of the culture period (July or August) as judged from the observation of the accumulation of organic wastes and hydrogen sulphide at the pond bottom. Roberts *et al.* (1984) recommended the use of TCBS agar for the

culture of vibrios as he noted that *V. alginolyticus* ferment sucrose forming yellow colonies whereas *V. parahaemolyticus* do not ferment sucrose forming green colonies on the TCBS agar. Ramasamy (1995) revealed that *V. alginolyticus* is similar to *V. parahaemolyticus* but the latter differs from it, in its ability to swarm over the surface of the agar culture media within 12 - 24 hours after inoculation at 28^{0}C and its ability to grow in media which does not contain salt (Roberts & Seiler, 1984). These isolates may not be hazardous to man as Hoashi *et al.* (1990) revealed that isolates which caused illness in human beings were thermostable direct hemolysin positive (TDH), whereas the isolates recovered from seafood were thermostable direct hemolysin negative (non-TDH).

This work emphazises that vibriosis may cause significant loss both in terms of the number of shrimps lost and in terms of revenue (Lightner, 1996). Disease should be precisely diagnosed in the early stages of infection as correct diagnosis is the most critical step in disease control programme (Baticados *et al.*, 1990). Management of the pond environment by water exchange, adjustment of feeding, liming, aeration as well as antibiotic treatment may regulate the population of endemic vibrios reaching the damaging threshold level to infect the shrimps and bring mortality.

REFERENCES

BATICADOS, M.C.L., LAVILLA-PITOGO, C.R., CRUZ-LACIERDA, E.R., DELA-PENA, L.D. & SUNAZ, N.A. 1990a. Studies on the chemical control of luminous bacteria *V. harveyi and V. splendidus* isolated from diseased *P. monodon* larvae and rearing water. *Diseases of Aquatic Organism.* **9** : 133 - 139.

BLAKE, P.A., WEAVER, R.E. & HOLLIS, D.G. 1980. Diseases of Humans (other than *V. cholera*) caused by vibros. *Ann. Rev. Microbiol.* **34** : 334 - 367.

BECHTELLER, C. & HOLLER, D. 1996. Vaccination against vibrosis in the prawn. *Animal Res. & Development.* **43/44** : 140 - 149.

CHAN, K.Y., WOO, M.L., LAM, L.Y. & FRENCH, G.L. 1989. *Vibrio parahaemolyticus* and other halophilic vibrios associated with seafood in Hongkong. *J. Appl. Bateriol.* **66** : 57 - 64.

CURTIS, J.H., SLOAN, E.M., LANCETTE, G.A., PEERLAND, J.T. & SOFOS, J.N. 1994.

DONAVAN, T.J. & Van NETTEN, P. 1995. Enumeration of *V. parahaemolyticus and V. vulnificus* in various seafoods with two enrichment Broths. *Jl. Food Prot.* **57** : 403 - 409.

HOASHI, K., OGATA, K., TANIGUCHI, H., YAMASHITA, H., TSUJI, K., MIZUGUCHI, Y. & OHTOMO, N. 1990. Pathogenesis of *V. parahaemolyticus* : intraperitoneal and oragastric challenge experiment in mice. *Microbiol. Immunol.* **34**(4) : 355 - 366.

HONDA, S., MATSUMOTO, S., MIWATANI, T. & HONDA, T. 1992. A survey of urease positive V. parahaemolyticus strains isolated from traveller's diarrhea, sea water and imported frozen seafoods. *Eur. J. Epidemiol.* **8**(6) : 861 - 864.

JOHNSON, S.K. 1990. Handbook of Shrimp Diseases. Sea Grant Publ. No. TAMU-SG. 90-601, Texas A & M Univ. pp. 25.

JOSEPH, S.W. & COLWELL, R.R. 1974. *Vibrio parahaemolyticus* and related halophilic vibrios. *CRC Crit. Rev. Microbiol.* **10** : 77 - 124.

KANEKO, T. & COLWELL, R.R. 1974. Distribution of V. parahaemolyticus and related organisms in the Atlantic Ocean of south Carolina and Georgia. *Appl. Environ. Microbiol.* 28 : 1009 - 1017.

KAPER, J.B., BRADFORD, H.B. ROBERTS, N.C. & FALKOW, S. 1982. Molecular epidemiology of *Vibrio cholera* in the U.S. Gulf coast. *J. Clin. Microbiol.* **16** : 129 - 134.

KAYSNER, C.A., ABEYTA, C. Jr., STOTT, R.F. LILJA, J.L. & WEKELL, M.M. 1990. Incidence of urea-hydrolyzing *Vibrio parahaemolyticus* in Willapa Bay, Washington. *Appl. Environ. Microbiol.* **56**(4) : 904 - 907.

LIGHTNER, D.V. 1996. *Vibrios-Culture and Identification.* A Handbook of pathology and diganostic procedure for diseases of Penaeid shrimp. pp. 180 - 206.

LIGHTNER, D.V. & LEWIS, D.H. 1983. Diseases of cultured Penaeid shrimps. pp. 283 - 320. In : *Handbook of Mariculture* (McVey Ed.). Vol. I. Crustacean Aquaculture, Ist edn., CRC Press, Boca Raton, Florida.

MARTINS, M.T. 1991. Toxins of the family Vibrionaceae. pp. 188 - 201. In : *Proceedings of the first Brazilian congress on Proteins* (Oliveira, B. de Jr. & Sgarbieri, V.C. Eds.). Unicamp., campinas S. Paulo, Brazil.

MATTE, G.R., MATTE, M.H., RIVERA, I.G. & MARTIUS, M.T. 1994. Distribution of potentially pathogenic vibrios in oysters from tropical region. *Jl. Food Prot.* **57** : 870 - 873.

RAMASAMY, P. 1995. Diseases of shrimps in aquaculture systems : Diagnosis and Therapeutic measures. pp. 40 - 61.

ROBERTS, N.C. & SEILER, R.J. 1984. Methods of monitopring vibros in the environment. pp. 269 - 275. In : *Vibros in the environment* (Colwell, R.R. Ed.). John Wiley & Sons Inc., New York.

TISON, D.L. & SEIDLER, R.J. 1983. *Vibrio aestuarianus* : a new species from estuarine water and shell fish. (complete referencedesired/delete)

VANDERZANT, C.R., NICKELSON. & JUDKINS, P.W. 1971. Microbial flora of pond reared shrimp (*Penaeus aztecus*). *Appl. Microbiol.* **21** : 916 - 921.

TAKAHASHI, Y., YAN, Y. & HAMI, T. 1990. Pathogenecity and charascteristics of a Brown pigment producing *Vibrio* sp. isolated from Kuruma prawn, *P. japonicus* Bate. *The Jl. Shimonoseki Univ. of fisheries.* **39** : 109 - 118.