International Journal of
Science Inventions Today
www.ijsit.com
ISSN 2319-5436
Research Article

PREVALENCE OF VIBRIO INFECTION IN *PENAEUS (LITOPENAEUS) VANNAMEI* FARMS

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ABSTRACT

Penaeus (*Litopenaeus) vannamei* is one of the most farmed shrimp species globally. Disease caused by viral and bacterial agents are one of the major impeding factors for profitable shrimp aquaculture. Vibrio infection is a continuous problem in *Penaeus vannamei* culture throughout the world. The current study estimates the prevalence of Vibrio infection and associated mortality observed in three different shrimp farming areas of Nagapattinam district. The maximum prevalence of Vibrio infections in *Penaeus vannamei* were observed from Sirkazhi (29.5%), followed by Nagapattinam (29 %) and Vedaranyam (28.7%). Pond level mortalities associated with Vibrio infection were ranged from 17 % to 43%. The study revealed that Vibrio infections are a major cause of shrimp production loss in Nagapattinam district.

Keywords: *Penaeus vannamei*; Vibrio infection; shrimp disease

INTRODUCTION

Introduction of specific pathogen free (SPF) *Penaeus vannamei* into India has led to a surge in aquaculture production of shrimp from around 70,000MT in 2008-9 to 400,000 MT in 2015-16. On one side total shrimp aquaculture production keep raising due to rapid intensification of culture, on the other side, economic losses due to White spot disease and various other causes too witnessed. Disease caused by viral and bacterial agents are a major hurdle to the shrimp production. The shrimp culture industry has faced serious losses due to infectious diseases in last few decades. Since the mid-nineties of the previous century, shrimp aquaculture in Asia are subjected to many problems such as disease out-breaks, environmental degradation, poor pond soil and water quality and is highly correlated with poor management practices in the pond (Lightner 1993; Subasinghe 1977). Shrimp reared in controlled conditions with artificial feed and high stocking densities often leads to disease outbreak causing crop losses to shrimp farmers who do not adopt better management practices. Diseases are caused by virus, bacteria, protozoan, fungus, toxins etc.

Bacterial infections of shrimp have been observed for many years, bacterial infection usually occurs in the shrimp when they are weakened. Bacterial disease is most serious threat and often caused mass mortality in shrimp larvae which greatly influenced the sustainable supply of healthy fry (Ganesh *et al*. 2010). Diseases with bacterial etiology, particularly Vibrio species, have inflicted loss to the shrimp farming industry worldwide especially in Asian countries (Chiu *et al* 2007; Jory 2014; Loy 2011; Magbanua *et al* 2000; de la Pena *et al* 2003).

Vibriosis, a disease caused by gram-negative bacteria is one of the major disease problems in the aquaculture of shellfish and finfish (Adams 1991; Chen *et al* 2000; Lavilla-Pitogo *et al* 1996; Lavilla-Pitogo *et al* 1998; Lightner *et al* 1992; Lightner & Lewis 1975). Some Vibrio species identified to cause vibriosis include *V. harveyi, V. vulnificus, V. parahaemolyticus, V. alginolyticus, V. penaeicida* (Brock and Lightner 1990; Ishimaru *et al* 1995). These bacteria are part of the natural microflora of wild and cultured shrimps. They become opportunistic pathogens once the environment becomes favorable for their growth such as poor water quality, crowding, low dissolved oxygen (DO), high water temperature, low water exchange and suppressed natural defense mechanisms of the animal (Brock and Lightner 1990; Lewis 1973; Lightner and Lewis 1975; Sizemore and Davis 1985). The organs exposed frequently to the bacteria get infected more often like the digestive system including hepatopancreas and gut from where the infection is suspected to spread to other organs. Among the bacterial diseases Vibriosis, filamentous bacterial fouling, black spot disease, septic hepatopancreas necrosis are the most common in culture *Penaeus vannamei*. Shrimp body fluids are most often infected by the bacterial group named Vibrio. Infected shrimp show discoloration of the body tissues in some instances, but not in others. Vibrios are the well-known bacteria responsible for devastating economic losses both in shrimp hatchery and culture ponds and it causes mass mortality both in larval cultures and shrimp production (Lavilla-Pitogo, 1995; Saulnier *et al.,* 2000).

Vibrio species cause infection at all life stages (from eggs to brood stock); generating in most cases 100 % mortality (Prayitno and Latchford, 1995; Harris and Owens, 1999). The major types of bacteria which

affect the shrimp are *Vibrio harveyi, V. parahaemolyticus*, *V. alginolyticus* and *V. splendidus*. Among the Vibrio strains, *Vibrio harveyi* is recognized as an important pathogen of cultured penaeid larvae throughout the Southeast Asian region (Karunasagar *et al*. 1994). The disease phenomenon caused by these strains is commonly referred to as luminous bacterial disease or luminous vibriosis (Lavilla-Pitogo *et al*. 1990). The present study was undertaken to estimate the prevalence of Vibrio infection and associated shrimp mortality in *Penaeus vannamei* from Nagapattinam district.

MATERIALS AND METHODS

Sample Collection:

The present study was carried out for one crop from March 2015 to June 2015. Cultured shrimp *P. vannamei* (4 to13 g) were obtained from shrimp farms in three different areas (Sirkazhi, Vedaranyam, Nagapattinam) in Nagapattinam district of Tamil Nadu, India and were analyzed for pathogens associated with the mortality. Shrimp ponds reporting mortality of shrimp were selected for the sampling (n= 85). Shrimp samples were preserved for bacteriology, molecular diagnosis and for histology. The samples for PCR were preserved in 95% ethyl alcohol. The samples for histological analysis were preserved in Davidson's fixative and processed for histology (Bell & Lightner, 1988). Besides shrimp sample, water and sediment samples from pond were collected every month throughout the culture operation.

Bacteriological analysis:

The samples (shrimp, pond water and sediments) were analyzed by plating on thiosulfate citrate bile salt sucrose agar (TCBS, HiMedia, Mumbai) and tryptic soy agar (TSA, HiMedia, Mumbai) with 2% NaCl. The plates were incubated at 30 °C for 24 h and typical colonies obtained were subjected to biochemical tests for identification of Vibrio spp.

The morphology and the number of colonies on TCBS were recorded for all samples (Lightner, 1996). Dominant bacterial colonies were purified by streak plate technique and identified followed by standard procedures (Baumann and Schubert, 1984; Lightner 1996).

RESULTS

Moribund shrimp samples collected during the study exhibited clinical signs such as lethargic movement, surface swimming and pale red shells and appendages. White spots on the carapace surface were observed in some moribund animals and muscles were appeared as opaque.

Vibrio species were isolated from moribund shrimp samples from three major shrimp farm villages of Nagapattinam district, with an overall occurrence of 29.01 %. The maximum 29.5 % prevalence of Vibrio infection was observed during 2015 in Sirkazhi whereas minimum 28.7 % was recorded in Vedaranyam. All three cultured areas of *P. vannamei* were observed with Vibriosis and associated mortality (Table. 1 and Fig. 1). Shrimp mortality caused by Vibrio infection varied from pond to pond. The mortality ranged from 17% to

42 %.

Prevalence (%)-2015			
Month	**Sirkazhi**	**Vedaranyam**	**Nagapattinam**
March	28	29	28
April	30	31	29
May	31	27	28
June	29	28	31
	29.5	28.7	29

Table. 1. Prevalence of Vibrio infection in *L. vannamei*

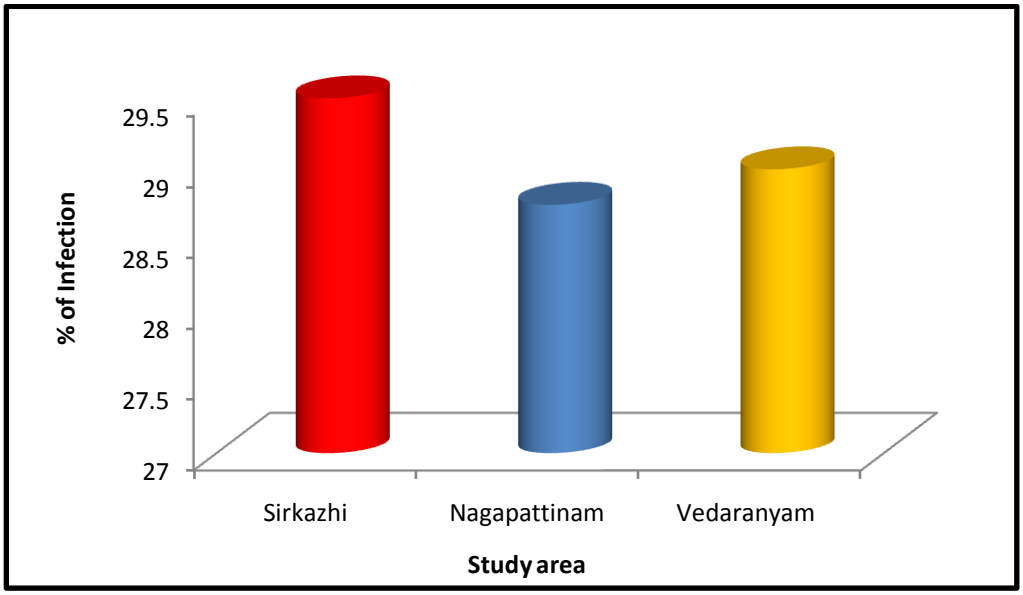

Figure 1: Prevalence of Vibrio infection in *L. vannamei*

Shrimp samples obtained from shrimp ponds with mortality were observed with more number of Vibrio colonies on the TCBS agar plate (Fig. 2). Total Vibrio count ranged from 10^5 to 10^7 colony forming units in most cases. The samples were tested negative by PCR for viral pathogens.

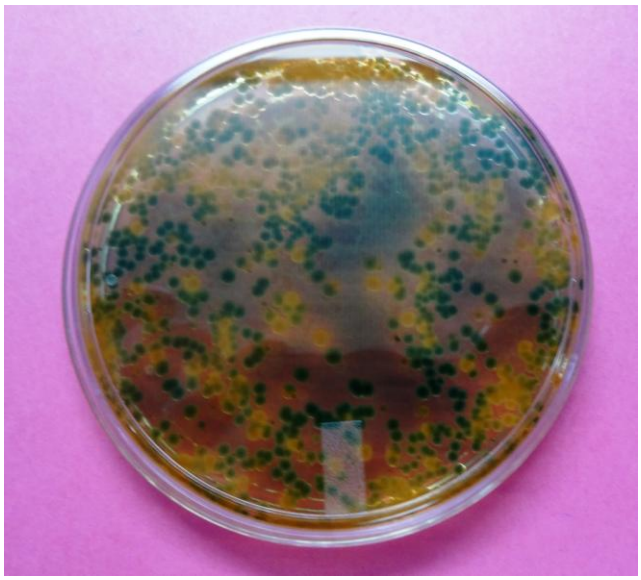

Figure 2: Growth of Vibrio colonies on TCBS agar medium

Figure 3: *P. vannamei* with Vibrio infection

DISCUSSION

Disease incidences are currently an important constraint to growth of aquaculture, which has impacted both socio-economic development and rural livelihoods. The results of the present study showed that, Vibriosis is one of the leading causes of shrimp mortality. No significant variation was observed in the prevalence of Vibrio between the three farming areas and between four months of study. Higher organic load in the shrimp culture system provides opportunity for Vibrio spp. to multiply fast. Whereas shrimp immune system is often compromised in shrimp ponds with higher unionized ammonia level and lower dissolved oxygen level. This provides Vibrio spp. opportunity to invade and cause mortality (Brock and Lightner 1990;

Lewis 1973; Lightner and Lewis 1975; Sizemore and Davis 1985; Moriarty,1997). Bacterial diseases, mainly Vibrio species are often associated with low survival rates in hatchery or grow-out conditions. In the present study the major Vibrio spp. isolated were *Vibrio parahaemolyticus*, *Vibrio harveyi*, *Vibrio vulnificus* and *Vibrio campbelli* as has been documented by various researchers. *V. harveyi* have been documented in *Penaeus monodon* and *P. vannamei* in Indonesia Sunaryanto and Mariam, 1986, Thailand Jiravanichpaisal *et al*., 1994 and India Karunasagar *et al*., 1994.

The present study, revealed that the overall Vibrio infection is 29.01%. However, a similar study (Abraham and Palaniappan, 2004) reported a higher rate of isolation of luminescent bacteria (59.68%) from samples including source water, eggs, broodstock, larvae, larval rearing tank water, algal culture tanks, Artemia nauplii and swab samples from water distribution systems in hatcheries of Tamil Nadu, India. Generally the gram-negative bacteria were found to be the dominant forms in the shrimp culture ponds (Sung *et al*. 2003). Otta *et al*. (1999) also reported that 5.2 to 36% of the Vibrio spp present in the pond water of shrimp farms of the east and west-coast of India. Similarly, in our study the Vibrio infection occur ranging from 28.7 to 29.5 %. Sindermann (1979) has pointed out that *Vibrio* spp. is the major disease causing bacteria normally found in the environment (Yasuda and Kitao 1980; Sharmila *et al*. 1996). Approximately, 30 types of pathogenic *Vibrio* spp were identified from shrimp culture farm (Jayasinghe *et al*. 2008). This may be due to variation in the season and condition of sea water and soil in the area.

The water quality parameters and culture pond management play a vital role in the Vibrio infected ponds. Low water exchange in farms to prevent water contamination through intake water and utilization of high amount of organic manure, inorganic fertilizer, high stocking density, feed waste, fecal matter, algal bloom and human interference are the main reasons for this situation (Moriarty 1997; Lloberra *et al*. 1991). Mortalities due to vibriosis occur when shrimps are stressed by factors such as: poor water quality, crowding, high water temperature, low DO and low water exchange (Lewis, 1973; Lightner and Lewis, 1975; Brock and Lightner, 1990). High mortalities usually occur in postlarvae and young juvenile shrimps.

Body opaqueness, necrosis and lethargy have been observed in *Litopenaeus vannamei* larvae and postlarvae infected by *Vibrio harveyi*, *V. parahaemolyticus* and *V. penaeicida* (Aguirre-Guzman *et al*. 2001). Similar gross signs and histopathology were seen in our specimens collected from the suspected ponds. Adult shrimps suffering vibriosis may appear hypoxic, show reddening of the body with red to brown gills, reduce feeding and may be observed swimming lethargically at the edges and surface of ponds (Anderson *et al*., 1988; Nash *et al*., 1992). The eyeballs of infected shrimps turn brown and fall away and mortality occurs within a few days (Chen, 1992).

CONCLUSION

Vibriosis is a common problem world-wide particularly in India. Highly pathogenic strains of Vibrio spp are also emerging and continuous to cause mortalities among culture shrimp. Problems caused by secondary Vibriosis are common, but are considered minor compared to viral epidemics. Vibriosis can be controlled by rigorous water management, use of probiotics and sanitation to minimize the Vibrio load in the

cultured water and to reduce stress on the shrimps. Good site selection, pond design and pond preparation are also important. Draining, drying and administering lime to ponds following harvest is also recommended to control the Vibrio sp in shrimp farming systems.

Acknowledgement:

Authors are thankful to Director, CAS in Marine Biology, Faculty of Marine Sciences, Annamalai University for the facility provided and engorgements during the study period.

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