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Prevalence of Vibriosis in *Penaeus (Litopenaeus) vannamei* in three different locations of Nellore district of Coastal Andhra Pradesh

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Abstract

Shrimp aquaculture is an industry that has experienced a vigorous and worldwide economic growth. The shrimp aquaculture at present is almost synonymous *Penaeus* (*Litopenaeus) vannamei* culture. Viral and bacterial diseases have emerged as a serious economic problem for shrimp farming in many countries including India. Vibriosis which was a serious problem in marine aquaculture, and is now a continuous problem in *Penaeus vannamei* culture throughout the world. In the present study, incidences of vibriosis was observed during 2014 and 2015 in *L. vannamei* farms of Kavali-Allur (L1), Nellore (L2) and Gudur-Kota (L3) Region of Nellore district of Andhra Pradesh for a total of four crops. Among the three locations, the mean highest percentage (18.1 %) incidence of Vibriosis was observed in L2 and the mean low percentages (7.5%) of incidences was observed in L1. The study revealed that, overall Vibrio infection during the period ranged from 5.2% to 27.6 % in the Nellore district.

Keywords: P *vannamei*; Disease, Vibriosis; Nellore,

Introduction

Shrimp aquaculture is the most successful among all aquatic species. The gradual increase of such activity is most prominent in tropical and subtropical countries (Joventino *et al*., 2008). Shrimp farming has grown from a traditional, small scale business in South East Asia into a global industry than other culture systems (Joseph Selvin *et al*., 2009). With the progress and expansion of shrimp culture, disease incidence has become one of the most serious threats to the aquaculture industry and has a tremendous impact on both socio-economic development and rural livelihood of people. Indian shrimp farming has been evolved as a leading shrimp culture industry in the international scenario, using locally available crustacean species such as *Fenneropenaeus indicus* and *Penaeus monodon* growing in both in salt and brackish waters. The culture of tiger shrimp *P. monodon* was in a steady progress globally until early 90's, when it was struck by white spot syndrome disease (Flegel, *et al*., 1998). Difficulties in captive breeding of tiger shrimp could not make it possible for the development of Specific Pathogen Free (SPF) and genetically improved stains with disease resistance (Otta *et al*.,2014). In this context SPF status of pacific white leg shrimp (*Litopenaeus vannamei*) was introduced in India in 2009 which revived the shrimp culture in India. The SPF status does not ensure that it is free from all the diseases and therefore, the threat of shrimp diseases continued to remain. The pacific

white shrimp *Litopenaeus vannamei* is also known to be vulnerable to a wide range of viral and bacterial diseases and reports of mass mortalities and crop failures of culture systems have also been recorded. Unfortunately, the SPF shrimp was also introduced in those areas where infections already existing. Numerous strategies have been used to try to control pathogens through preventive measures. The implementation of Better Management Practices (BMP) at every stage helped to contain the spread of the disease.

However, of late some unregistered hatcheries supplied the post larvae developed from domesticated SPF brood stock led to deterioration of quality in the seed creating the stress leading to viral and bacterial infections. The present scenario of shrimp culture in Andhra Pradesh is beset with all types of diseases. The major ones are from Viruses, then the Bacteria. Disease out breaks do occur in intensive system because of the stress on environment due to high stocking densities, spurious seed and use of large quantities of feed materials and chemicals etc. even though many disease-carrying pathogens are present, epidemic diseases occur only when the environment is favourable and the host is weak due to malnutrition or under stress. Injury, fluctuation in dissolved oxygen, temperature, pH, salinity and inadequate nutrition and variety of toxins in the water cause stress on the prawns making them vulnerable to disease. When the population of stressed prawns in the pond increases it gives the needed impetus to the pathogen to enter exponential phase of growth increasing its numbers enormously, leading to diseases. Hence, addressing the aquatic animal health issues has become an urgent requirement for sustainable growth of aquaculture.
Vibriosis is one of the most common disease problems in marine aquaculture and on several occasions it has caused massive mortalities (Karunasagar *et al*., 1994; Vandenberghe *et al*., 1998). 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).

Disease caused by Vibrio species was considered as a secondary infection, that usually proliferates when shrimp has become immunologically weak (Moriarty,1998). *V. harveyi* continues to cause chronic mortalities up to 30% among shrimp larvae, post larvae and adults under stressful conditions. 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). In aquatic environment, the microorganisms population normally consists of non-pathogenic bacteria, as well as obligatory and opportunist organisms (Schulze *et al*., 2006) and maintains an equilibrium This equilibrium may break sometimes due to an inadequate handling and sometimes may favour pathogenic bacteria reproduction (Karunasagar *et al*., 1994) and can cause rare mortality. Two types of Vibrio infections namely Septic Hepatopancreatic Necrosis and Systemic vibriosis are more commonly observed in shrimp farms. 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). The main clinical symptoms include lethargy, muscle opacity, atrophy of Hepatopancreas with discolouration and melanised foci in/on cuticle/gills/appendages.

The present study was aimed at identifying the prevalence of Vibrio infection in cultured crustacean i.e, *L. vannamei* in three different locations of Nellore District, Andhra Pradesh, India. The study revealed diseases like vibriosis is still present in exotic species, *L. vannamei* besides new emerging diseases.

Materials and Methods

Sample Collection: The present study was carried out for four crops during 2014 and 2015. For bacteriological analysis water samples were collected following the method of Dalmin *et al.* (2001). Water was collected in 100 ml sterilized screw–capped PVC bottles just below the water surface. Necessary precautionary measures were taken to minimize the contamination through handling. In order to avoid clustering of bacterial cells, the container with water sample was shaken vigorously for 2 min to ensure complete mixing and homogenous distribution of bacteria. Water and soil (sediment) samples were collected separately from culture ponds at 30, 60, 90 and 120 days of culture for microbial analysis using standard procedures.

Estimation of Total Vibrio Count (TVC): Total vibrio count (TVC) was determined following the procedure of Dalmin *et al.* (2001). TCBS (Thiosulphate citrate bile salts sucrose) agar medium was used for isolating *Vibrios* from the water and tissue samples. Spread plate method was used to

inoculate bacteria from the samples into agar Petri plates. This method could provide bacterial colonies for identification and further characterization. 1 ml of sample was spread over cool, solidified surface of agar medium using a sterilized bent glass rod. After inoculation the Petri plates should be heated with a spirit lamp for prevention of contamination from the environment. The petriplates were incubated in an inverted position at 36°C for 20-24 hr. Bacterial colonies could be seen after 18 h. TVC was expressed as colony forming units / ml (cfu/ml). The more number of yellow colonies observed as disease prevalence.

Results

The results obtained on the disease occurrence on 30,60,90 and 120 days of culture (DOC) in *L. vannamei* culture ponds of three different locations (L1: Kavali- Allur; L2: Nellore; L3: Gudur-Kota) of Nellore district shrimp farming areas during four crops (two summers and two winter) C1 (Crop-1), C2 (Crop-2), C3 (Crop-3) and C4 (Crop-4).

In the present study, incidences of vibriosis was observed during 2014 and 2015 in *L. vannamei* farms of Kavali-Allur, Nellore and Gudur-Kota Region of Nellore district of Andhra Pradesh. Among the three locations, the mean highest percentage (18.1 %) incidence of Vibriosis was observed in L2 and the mean low percentages (7.5%) of incidences was observed in L1 (Figure 1). During C1, the maximum prevalence of 23.5% is found in L2 and minimum is 8.3% observed in L1. The average prevalence ranged from 9.21% to 21.68%. During C2, the maximum prevalence of 27.6% is found in L2 and minimum is 7.3% observed in L1. The average prevalence ranged from 8.5% to 23.4%. During C3, the maximum prevalence of 16.3 % is found in L2 and minimum is 6.3% observed in L1. The average prevalence ranged from 6.75% to 14.25%. During C4, the maximum prevalence of 27.6 % is found in L2 and minimum is 5.2% observed in L1. The average prevalence ranged from 6.25% to 23%. The results are shown in Table-1. It is evident that, the two factor ANOVA shows there is significant (P <0.05) differences in Vibrio occurrence in locations and also in crops (Table 2).

Table 1 : Percentage of vibriosis disease affected *L. vannamei* culture ponds of three different locations (L1, L2 & L3) of Nellore districts in for four crops

Locations	Crop-1	Crop-2	Crop-3	Crop-4
L1	9.9	8.5	7.0	6.5
L2	21.6	23.4	14.5	23.4
L3	9.2	8.9	10.0	6.5

Table 2: Two factor ANOVA on the values of Prevalence of Vibriosis

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Rows	411.3854	2	205.6927	25.59408	0.001155	5.143253
Columns	19.50269	3	6.500897	1808898	0.00533508	4.757063
Error	48.22038	6	8.036731			
Total	479.1085	11				

The rows (locations) have a significant (p <0.05); The columns (crops) have significant (p <0.05)

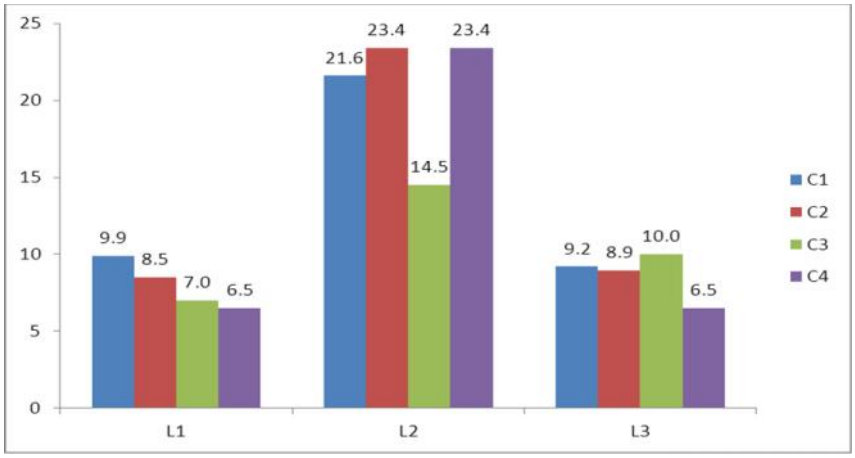

Figure-1: Crop -wise location wise prevalence of Vibriosis (%)

Discussion

Viral and bacterial diseases, together with poor soil and water quality, are the main causes of shrimp mortality (Liao, 1989; Chamberlain,1997), although deficient environmental management of shrimp farms is another important determinant (Flegel,1996).

Vibriosis is one of the most common disease problems in marine aquaculture and on several occasions it has caused massive mortalities (Karunasagar *et al.*, 1994; Vandenberghe *et al.*, 1998). Disease caused by Vibrio species was considered as a secondary infection, that usually proliferates when shrimp has become immunologically weak (Moriarty, 1998). *V.harveyi* continues to cause chronic mortalities up to 30% among shrimp larvae, post larvae and adults under stressful conditions. In aquatic environment, the microorganisms population normally consists of non-pathogenic bacteria, as well as obligatory and opportunist organisms (Schulze *et al.*,2006) and maintains an equilibrium This equilibrium may break sometimes due to an inadequate handling and sometimes may favour pathogenic bacteria reproduction (Karunasagar *et al.*, 1994) and can cause rare mortality. Two types of Vibrio infections namely Septic Hepatopancreatic Necrosis and Systemic vibriosis are more commonly observed in shrimp farms. The main clinical symptoms include lethargy, muscle opacity, atrophy of Hepatopancreas with discolouration and melanised foci in/on cuticle/gills/appendages.

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, water logging, drainage problem, sludge loads which increase organic load, 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* young juveniles infected by *Vibrio harveyi*, *V. parahaemolyticus* and *V. penaeicida* (Aguirre-Guzman *et al.*,2001). Adult shrimps suffering vibriosis may show the symptoms of 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). Melanised foci in /on cuticle/gills/appendages, atrophy of Hepatopancreas with discolouration are also observed.

The present study revealed that the overall Vibrio infection is 12.5% and the incidence of vibriosis (20.7%) is more in L2 (Nellore) than L1 (Kavali – Allur) 8% and L3 (Gudur –Kota) 8.7%. There was significant variation observed in the prevalence of vibriosis between the three locations and also between four crops. The overall Vibrio infection ranged from 5.2% to 27.6 % in the Nellore district. However, a similar study (Biju and Gunalan, 2016), reported a higher rate of incidence of vibriosis i.e., 29.01 %. The studies of 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. 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.

Conclusion

Vibriosis continuous to be common problem in shrimp faring in Andhra Pradesh particularly in Nellore district. Majorly the problem arises as secondary infection. Compared to Viral disease, the epidemics and losses are less in vibriosis. The main reasons attributed for the outbreak of vibriosis include stocking of poor quality seed, poor water quality management, poor pond design and high stocking densities. Vibriosis can be controlled with good water management and sanitation which helps to reduce the stress on shrimps. Use of Probiotics and Immunostimulants is also suggested to control the vibriosis.

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Conflict of Interest

The authors declare that there is no conflicts of interest regarding publication of this manuscript.

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