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Research Article

INCIDENCE OF LOOSE SHELL SYNDROME IN *PENAEUS (LITOPENEAUS) VANNAMEI* FARMS IN NELLORE DISTRICT OF COASTAL ANDHRA PRADESH, INDIA

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A B S T R A C T

Shrimp farming is an important activity along the coastal regions of India. Andhra Pradesh with 974 Kms of coastline, stands first in the shrimp production. Since 2009, the area, production and sea food exports has been constantly increasing after the introduction of *L.vannamei* in the state. Inspite of steady progress in the *L.vananmei* farming, the diseases remain a major threat for production and productivity. In this context, a study was conducted on incidence of Loose Shell Syndrome (LSS) during 2014 and 2015 in *L. vannamei* farms in the three locations (Kavali-Allur (L1), Nellore (L2) and Gudur-Kota (L3)) of Nellore district of Andhra Pradesh for a total of four crops. The prevalence of LSS was shown significant variation between the three locations and also between four crops during the study period. The overall LSS infection ranged from 8% to 30% in the Nellore district. As the LSS is associated with poor water quality, mineral deficiency and other poor management practices, it can be controlled with water quality management and better management practices.

INTRODUCTION

Shrimp aquaculture is the most successful among all aquatic species. Shrimp aquaculture is an industry that has experienced a vigorous and worldwide economic growth. 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 tremendous impact on both socio-economic development and rural livelihood of people. Since 1981, succession of new viral pathogens has emerged in Asia and Latin America, causing mass mortalities and threatening the economic sustainability of the industry (Walker *et al.,* 2009). Hence, addressing the aquatic animal health issues has become an urgent requirement for sustainable growth of aquaculture.

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 rapid growth of shrimp farming led to an economic boom but, unfortunately, the outbreak of viral diseases has increased the economic risks and slowed the industry development (Flegel, 2006). 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 Strain 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. Unfortunately, the SPF shrimp was introduced in those areas where infections already existing.

Further, the pacific white shrimp *Litopenaeus vannamei* is also known to be vulnerable to a wide range of diseases and reports of mass mortalities and crop failures of culture systems have also been recorded. Numerous strategies have been used to try to control pathogens through preventive measures. The practice of bio-security measures like bird fencing, crab fencing, filtration and disinfection of water before pumping into the main pond helped to contain the spread of the disease particularly the carriers of the pathogens.

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

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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.

Loose shell syndrome is a major problem observed in *Litopenaeus vannamei* culture. Loose Shell Syndrome (LSS) has been reported in the cultured Penaeus monodon since 1998 in India (Gopalakrishnan *et al*., 2005). LSS is a chronic progressive disease of farmed shrimp. The exoskeleton of affected shrimp forms are the sort of loose covering over the abdominal musculature, with a space in between muscles and carapace (Alavandi *et al*., 2007 and Jayasree *et al*., 2008).LSS was reported in culture ponds of Litopenaeus vannamei from West Godavari, East Godavari and Prakasam, Andhra Pradesh (Masthan *et al*., 2005). The affected shrimp shows the reduction of average daily growth rate, average body weight, and poor survival, and leading to a reduction in biomass production at the affected farms (Gopalakrishnan *et al*., 2005). The growth of the shrimps is severely affected by the LSS infection in the culture ponds (Raja *et al*., 2015). LSS causes low-level mortality in India"s black tiger shrimp (Alavandi *et al*., 2008). Together with the white spot disease, Loose Shell Syndrome has caused severe economic losses for penaeid shrimp farms in north coastal AP and east Godavari district in India (Loka *et al*., 2012). The pathogen suspected is vibrio species. Hence, the present study is aimed in assessing the incidence of Loose Shell Syndrone in cultured *L.vannamei* ponds in three different areas of Nellore District, Andhra Pradesh (AP), India.

MATERIALS AND METHODS

Description of sampling sites

Among the coastal states, AP is the largest producer of shrimp *(L.vannamei)* in the country. Nellore district in the state of AP is the main district where aquaculture activities are More and their contribution to state GSDP is also significant. Nellore district is known as Aqua capital of India, where the shrimp production and culture area is significant. The district lies between 13-30' and 15-6' of the Northern latitude and 70-5' and 80-15' of the Eastern Longitude and extending over an area of 13076 Sq.Kms. Majority of farmers of the district culture *L. vannamei* because of its high market value. The study was made in three locations namely L1 (Kavali - Allur), L2 (Nellore) and L3 (Gudur- Kota) covering a total of 9 mandals of Nellore district. During the study period (January 2014 to December 2015) samples are collected from 289 culture ponds located in L1 (Kavali- Allur), L2(Nellore) and L3 (Gudur- Kota) during four crops (two summers and two winter) C1 (Crop-1), C2 (Crop-2), C3 (Crop-3) and C4 (Crop-4) during 30,60,90 and 120 days of culture (DOC).

Sample collection

Clinical observation based on the symptoms as well as clinical signs displayed by the affected shrimps (*L.vannamei*) were collected from the different culture shrimp ponds under sterilized conditions and brought to be laboratory for further study. The shrimp showing signs of LSS were collected (Figures from 2.1 to 2.4) for histological analysis and are preserved in Davidson's fixative and further processed for conventional histological techniques (Bell and Lightener, 1988).

Statistical analysis

Data were statistically analysed and comparison among different locations was done by Two-way analysis of variance (ANOVA) to find out any significant ($P<0.05$) difference among the results was done using Statistical Package for Social Sciences (SPSS; 16.0 version).

RESULTS AND DISCUSSION

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) are observed and recorded. Based on the records, it is noticed that the mean highest percentage (29.33 %) incidence of LSS was observed in L2 and the mean low percentages (8.5%) of incidences was observed in L1.

During C1, the maximum prevalence of 33.7% is found in L3 and minimum is 11.5% observed in L1 during different days of culture period. The average prevalence ranged from 9.11% to 23.15%. Like wise, during C2, the maximum prevalence of 41.8% is found in L2 and minimum is 16.7% observed in L1. The average prevalence ranged from 13.5% to 30.1%. During C3, the maximum prevalence of 33.7 % is found in L3 and minimum is 9.4% observed in L1 during different days of culture period. The average prevalence ranged from 8.07% to 20.4%. During C4, the maximum prevalence of 41.8 % is found in L2 and minimum is 21.9% observed in L1 during different days of culture period. The average prevalence ranged from 17.4% to 28.5%. (Figure-1)

The present study revealed that the overall LSS infection is 20.1% and the incidence of LSS (25.3%) is more in L2 (Nellore) than L1 (Kavali -Allur) 12% and L3 (Gudur -Kota) 23.1%. Statistical data reveal that., there was significant variation observed in the prevalence of LSS between the three locations and also between four crops (Table-1). The overall LSS infection ranged from 8% to 30.1% in the Nellore district.

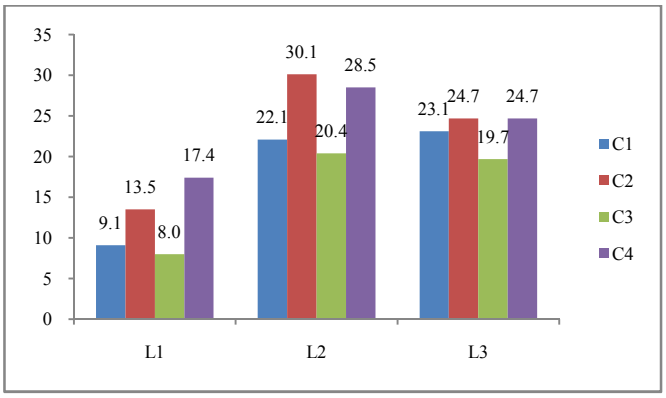

Figure 1 Crop -wise location wise average prevalence of LSS (%)

Table 1 Two factor ANOVA on the values of the Prevalence of LSS

Source of Variation	SS	df	MS	F	P-value	F crit
Rows	404.3717	2	202.1858	55.88246	0.000132	5.143253
Columns	118.3092	3	39.43639	10.89988	0.007663	4.757063
Error	21.70833	6	3.618056			
Total	544.3892	11				

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

Since, 1988, loose shell syndrome (LSS) has been reported in farmed shrimp in India. Thereafter, incidences of LSS in shrimp have been increasing every year. LSS is a chronic progressive disease of farmed shrimp. The affected shrimp has a soft carapace with a gap between the muscle tissue and exoskeleton, shrunken hepatopancreas (Alvandi *et al*., 2008).

The pathogen that affects the hepatopancrease may cause the shrimp mortality due to starvation. This might be true, as the main function of the hepatopancreas is the absorption of nutrients, storage of lipids and production of digestive enzymes (Johnson, 1980). Hence, in case of loose shell, the main target organ could be affected is hepatopancreas and the suspected pathogen is vibrio species. (Figures 2.1 to 2.4)

Loose Shell Syndrome (LSS) in Litopenaeus vannamei culture ponds at Nellore district shrimp farming areas (Fig 2.1 to 2.4)

Fig 2.1 Observation of LSS

Fig 2.2 Observation in check tray

Fig 2.3 Harvested shrimps with LSS

Fig 2.4 Affected shrimps in check tray

DISCUSSION

The MPEDA/NACA study suggested that the causes of LSS would be probably because of chronic bacterial infections and toxic pond bottom conditions. The signs and symptoms observed includes Anorexia, Soft exoskeleton, Flaccid body, Reduction in weight due to degeneration of muscle, Atrophy of Hepatopancreas with necrotic cells and very few fat bodies, Delayed blood clotting. Microscopic observation of wet mounts of Hepatopancreas, gut and hemolymph shows motile bacteria. (Ravi Kumar, 2011). The loose shell syndrome of *P.monodon*, characterized by a flaccid spongy abdomen due to muscular dystrophy, lethargic and flaccid was also found in the present study of *L. vannamei*, where the LSS infected shrimps became sluggish, soft exoskeleton, gap between muscle and shell, pigmented hapatopancreas and microbes fouling on the exoskeleton.

The studies by Ravikumar, (2011) conclude that, though the symptoms resemble like soft shell syndrome, the symptoms cannot be reversed by administration of diets containing clam meat and certain vitamins in LSS of vannamei. Studies by Gopalakrishnan *et al*., (2005) shows that there is reduction of average daily growth rate, average body weight, and poor survival, and leading to a reduction in biomass production at the affected farms

The studies by Mastan *et al*., (2015) reveal that the incidence of LSS is 41% in Prakasam district and 20% in East Godavri districts. The studies of Society of Aquaculture Professionals (SAP) in 2002 recorded the incidence of LSS in more than 50% of the farms (>1100 ha surveyed) in coastal of Andhra Pradesh. LSS can be controlled with good water management and better management practices which helps to improve quality of water. The mineral deficiency, poor water quality and high loads of vireo pathogens may be the causes of loose shell syndrome (Mastan *et al*., 2015). The loose shell

syndrome is the result of chronic toxic pond-bottom conditions due to the release of ammonia and nitrates (Baticados, 1990). In the loose-shelled shrimp, compressed hepatopancreas tubules are found. Jayasree *et al*., 2008 have isolated four species of vibrio bacteria from loose shell affected shrimps of coastal districts of AP. Prakash *et al*., reported co occurrence of LSS infection with other viral infections in cultured *P. monodon*. But such correlations were not observed in the present study. In the present study, species of vibrio bacteria were found to be associated with LSS affected L.vannamei in all the three locations. Similar findings were reported by the studies of Jayasree *et al.,* 2006 which revealed that, the disease was observed in all ages of shrimp and is present in all three regions of study i.e., Kakinada, Amalapuram and Visakhapatnam.

The LSS infected shrimps have relatively high Vibrio count in Hepatopancreas. The present study has also observed that mineral deficiency, poor water quality and high loads of Vibrio pathogens may be the causes of the Loose Shell Syndrome in *L.vannamei*. Further studies are needed to conform the causative agent(s) of the disease.

CONCLUSION

The present study revealed that the overall LSS infection is 20.1% and the incidence of LSS (25.3%) is more in L2 (Nellore) than L1 (Kavali -Allur) 12% and L3 (Gudur -Kota) 23.1%. There was significant variation observed in the prevalence of LSS between the three locations and also between four crops. The overall LSS infection ranged from 8% to 30% in the Nellore district. It is observed that though Loose Shell Syndrome does not cause mass mortalities, it posed a severe economic threat to shrimp farmers during the study period. The economic impact is in the form of forced early harvest, reduced stocking, mortality, size variation, size variation etc remains a regulating factor for the farmers. Control of the LSS can be achieved through stocking of good quality seed, water quality management, biosecurity measures besides better management practices at all stages during the culture period.

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Conflict of Interest

The authors declare that there is no conflicts of interest.

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