J. mar. biol. Ass. India, **45** (2) : 178 - 185, July - Dec., 2003

Histopathological survey of cultured shrimps in Cochin, Kerala

Liya Ambipillai, K. S. Sobhana, K. C. George and N. K. Sanil

Central Marine Fisheries Research Institute, Cochin – 682 018, India

Abstract

A disease survey was conducted using histopathological tools in a cross section of shrimp farming area-in Cochin during March to June, 2001. Shrimp samples collected from 26 farms comprised of *Penaeus indicus, P. monodon,* and *Metapenaeus dobsoni.* The major pathological condition recorded was the presence of chronic inflammatory lesions characteristic of systemic bacterial infection and was observed in 42% of the farms surveyed. In 19% of the farms, hepatopancreatic pathology, typical of oral/enteric vibriosis was recorded. Mortality of *P.monodon* due to mixed infection of MBV and vibriosis was recorded in one farm. Fouling of shrimps by the ciliate protozoan, *Zoothamnium* could be detected only in 8% of the cases. Abnormal conditions such as spontaneous muscle necrosis, dark coloured gills and brown discolouration of the shell were recorded in 36% of the farms. White spot viral inclusions were not recorded in the target tissues of the samples examined during the period of survey. No apparent pathological conditions were recorded in 27% of the farms surveyed.

Introduction

The Indian shrimp farming industry witnessed a rapid expansion during the period 1988 –'94. With the expansion of the industry, aquaculture waste management became a very serious issue. The unorganised and unregulated expansion with lack of scientific consideration resulted in the outbreak of diseases leading to the collapse of the industry during 1994 and '96. Viral diseases due to Monodon baculovirus(MBV), Infectious hypodermal and haematopoietic necrosis virus(IHHNV), Yellow head virus(YHV) and White spot virus(WSV) have been reported at several times in India (Shankar *et al.*, 1994; Ramasamy *et al.*, 1995; Ruby Sheela *et al.*,1998; Shankar and Mohan,1998). Mortalities due to luminous vibriosis and filamentous bacterial infections and larval mycosis were reported in hatcheries (Karunasagar *et al.*, 1994; Abraham *et al.*, 1997 and Felix, 2000). Bacterial diseases due to vibrios and filamentous bacteria, fungal infection (*Lagenidium*) and epicommensal infestation, abnormal conditions such as black gill and tail rot have also been reported (Panchayathapani, 1997). Many a times, outbreak of the disease may be the sole factor limiting the success of the culture. Often these outbreaks can be avoided by proper management measures. In order to formulate useful control strategies, it is

essential to identify the diseases prevalent in an area. Knowledge of current disease problems is also essential if the changing pattern of disease associated with widespread intensification is to be monitored and controlled (Turnbull *et al.*, 1994).

Histopathology is an ideal tool for routine health monitoring and diagnosis, where the changes at the cellular and tissue level due to the pathogen is interpreted to arrive at diagnosis. The present histopathological survey on cultured shrimps in Cochin area was undertaken to identify the occurrence of disease conditions and pathogens involved, in a cross section of shrimp farming area at a given period and also to study the tissue level pathological changes and host responses in different disease conditions.

The authors are grateful to Dr. R. Paul Raj, Scientist-in-charge, P.G. Programme in Mariculture for the constant encouragement and guidance throughout the study period. The authors also express their gratitude to the Director, C. M. F. R. I., Cochin for providing facilities to carry out the work. The award of a Junior Research Fellowship to the first author by the Indian Council of Agricultural Research, New Delhi, during the study period is gratefully acknowledged.

Material and methods

Shrimp samples were collected from 26 farms, mainly comprising of the modified extensive systems in and around Cochin, having populations reported to be suffering from diseases (those observed to be weak or with abnormal gill or shell colouration), with a previous history of disease outbreak or reportedly having healthy populations. Samples were collected randomly by operating cast net. The samples collected comprised of *Penaeus indicus*, *P. monodon* and *Metapenaeus dobsoni*.

The samples were fixed in Davidson's fixative, subsequently processed, embedded in paraffin wax, sectioned and stained with haematoxylin and eosin (H&E) for light microscopy following routine histological techniques (Bell and Lightner, 1988). Mid-line sections of the cephalothorax, sections of the gill, cross sections of abdomen and slant sections of the last two abdominal segments were examined from all the shrimps.

Results

Chronic inflammatory lesions in the form of melanised or non- melanised haemocytic nodules were identified in the gills, connective tissue, muscle and

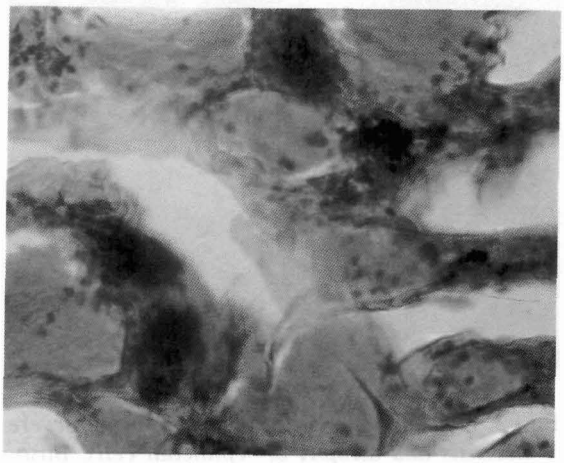

Fig. 1 - *Chronic inflammatory lesions in the form of melanised haemocytic nodules in the gills. H&E. X 400.*

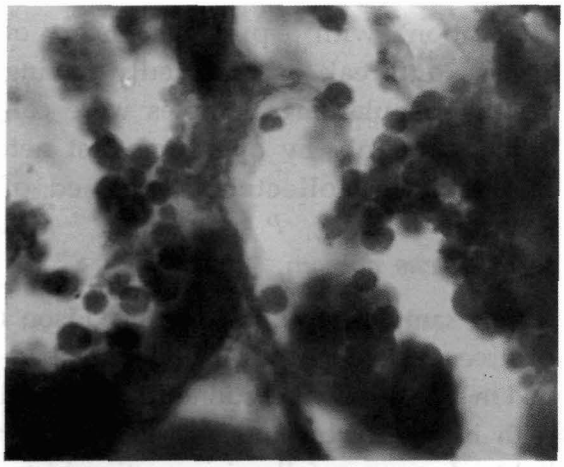

Fig. 2. *Pathology of hepatopancreas in* P.monodon *showing severe necrosis, rounding and sloughing of cells, indicating bacterial infection. H&E. X 1000.*

haemocoel spaces in samples from 42% of the farms surveyed (Fig. 1). Pathology of hepatopancreas showing severe necrosis, loss of structure, atrophy of tubule epithelial cells, vacuolation and rounding and sloughing of cells into the lumen were recorded in 19% cases (Fig.2 to 4). No marked haemocytic infiltration was noticed in any of these cases. Normal hepatopancreas (midgut gland) in shrimps contain numerous tubules with star shaped lumen lined with various types of epithelial cells (Fig. 5).

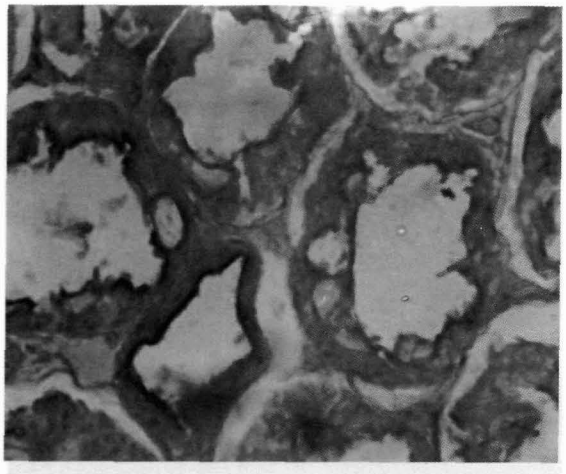

Fig. 3. *Hepatopancreatic tubules showing severe necrosis and atrophy of epithelial cells. Basophilic inner margins indicate presence of bacterial plaques suggesting extension of oral bacterial infection to enteric region. H&E. X 400.*

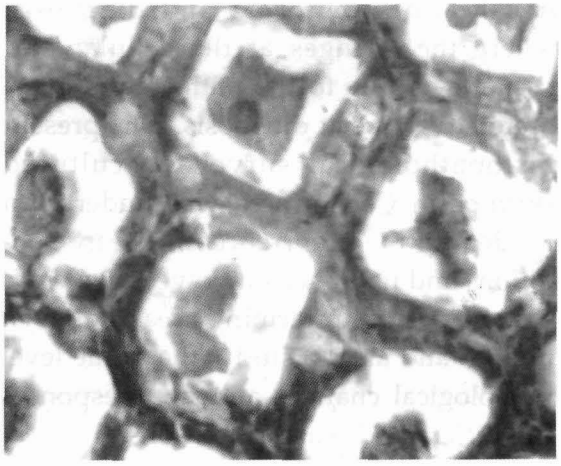

Fig. 4. *Section of hepatopancreas showing pathology, severe necrosis, atrophy of cells and sloughing into the lumen with no inflammatory cells indicating oral bacterial infection. H&E. X 400.*

Fig. 5. *Tubular epithelial cells of the hepatopancreas from normal shrimp. H&E. X 1000.*

Presence of eosinophilc, spherical, intranuclear occlusion bodies characteristic of MBV was recorded in the hepatopancreatic tubule epithelial cells (Fig. 6) of samples from one farm, where mortality was recorded during the period of study. The samples also showed hepatopancreatic pathology of severe necrosis, rounding and sloughing of cells into the lumen, vacuolation and loss of structure. Other than this, there was no histopathological evidence of viral inclusions in any of the samples examined. The target tissues of WSV, such as cuticular epidermis, connective tissue, gills, lymphoid organ, haematopoietic tissue, ventral nerve cord *etc.* were free from inclusion bodies characteristic of WSV .

Infestation by the epicommensal ciliate protozoan, *Zoothamnium* in the gills and cuticular surface was observed in histological sections of samples collected from 8% of the farms surveyed (Fig. 7).

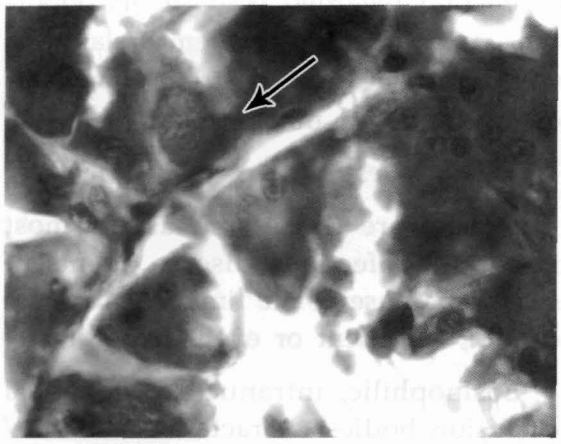

Fig. 6. *Eosinophilic, intranuclear occlusion bodies (arrow), characteristic of MBV in the tubular epithelial cells of hepatopancreas from diseased* P.monodon. *H&E. X 1000.*

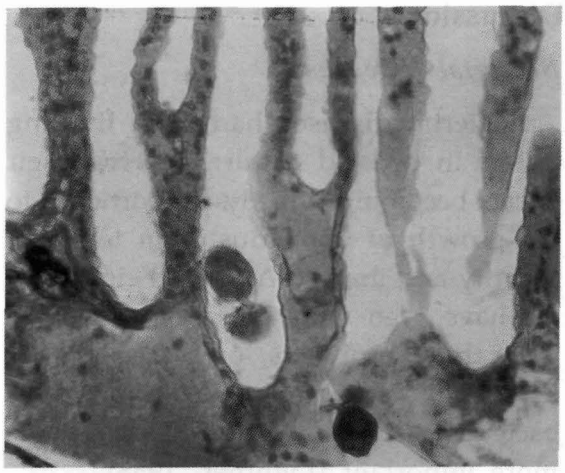

Fig. 7. *Infestation by* Zoothamnium *in the gills of* P.indicus. *H&E. X 400.*

Abnormal conditions such as spontaneous muscle necrosis, showing several whitish, opaque areas in the muscle (Fig.8), dark coloured gills and brown discolouration of the shell were recorded in 36% of the farms. There were no apparent histopathological features in samples examined from 27% of the farms surveyed.

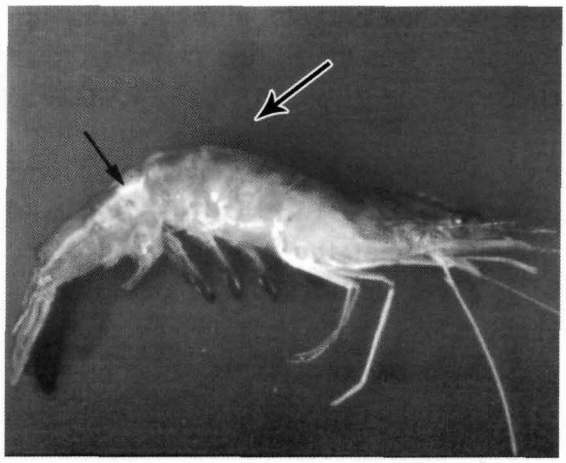

Fig. 8. *Spontaneous muscle necrosis (arrow) in* P.indicus.

Discussion

Bacterial diseases

Bacterial diseases had been limiting factors in penaeid culture systems, their effects becoming directly proportional to the growth of the industry in terms of severity and impact. Although eight genera have been reported to be associated with these problems, only two groups occur quite commonly, the filamentous bacteria and vibrios, with the latter being more important (Lightner, 1988).

Results of the histopathological examinations in the survey showed chronic inflammatory lesions in the form of haemocytic nodules typical of systemic bacterial infections in gills, haemocoel spaces and in the loose connective tissue in majority of the cases. Such multifocal nodules were melanised or non-melanised and in some cases showed presence of fibrous tissue. Severe hepatopancreatic pathology typical of oral or enteric vibriosis, such as necrosis of tubule epithelium and rounding and sloughing of cells into the lumen was the second major problem encountered. However in most of these cases with hepatopancreatic pathology, no marked haemocytic response was noticed. This indicates bacterial colonisation of the mouth and foregut region, which can lead to pathological changes in the hepatopancreas probably due to the action of bacterial toxins without causing any inflammatory response. Heavy bacterial colonisation of the mouthparts or the cuticular lining of the oesophagus and foregut can lead to rounding up and sloughing of hepatopancreatic tubule and midgut epithelial cells in to their lumens leading to typical enteritis. Following cuticular colonisation, bacterial invasion of the midgut and hepatopancreas may occur. At this stage haemocytic inflammation is very commonly seen. The conditions were typical of the systemic and enteric vibriosis described by Egusa *et al.*(1988); Nash *et al.* (1990) and Jiravanichpaisal and Miyazakir (1994).

Due to the economic losses encountered following epizootics, bacteria are considered as one of the most economically significant disease agents of shrimp (Lavilla- pitogo, 1995). Bacterial disease in cultured shrimp usually occurs in conjunction with other disease processes or reflects an outcome of a breakdown in the ecological balance within the culture system. Many of these bacteria are normal inhabitants of the marine and brackishwater environment. As majority is generally regarded as secondary opportunistic pathogens, problems related to vibriosis can be traced to stress, poor water quality and bad management.

Viral diseases

Viruses are considered as the most important infectious disease agents of shrimp because of the limited means for control, treatment or even diagnosis.

Eosinophilic, intranuclear, spherical occlusion bodies characteristic of MBV were detected in the hepatopancreas of shrimps sampled from one farm, where mortalities were recorded in one month old *P. monodon*. The hepatopancreas was

badly affected showing severe pathology. The samples were also positive for pathogenic vibrios. The affected shrimps showed signs of reduced feeding, reduced growth, movement to pond margins and heavy mortality. MBV is widely distributed in cultured penaeid shrimp population in India (Panchayuthapani, 1997). There were reports of mass mortality of *P.monodon* larvae in hatcheries (Felix and Devaraj, 1993; Ramasamy *et al.*, 1995). Lightner *et al.* (1983) recorded presence of occlusion bodies typical of MBV infection in apparently healthy post larval samples. MBV infections are common in wild brooders and elimination is not practical. However, contamination of PL could be easily overcome by good sanitary practices in hatchery. Control methods include screening of broodstock for presence of MBV occlusion bodies, individual spawning of gravid females, separation of eggs and larvae from brooders followed by rinsing in seawater and chemical disinfectants.

Disease outbreak due to the dreaded white spot virus still prevails in many regions and poses a major threat to Indian shrimp farming. Mortalities due to this disease have been reported throughout the East and West coasts since October 1994 (Mohan and Shankar, 1995; Krishna *et al.*, 1997). The disease is caused by a systemic ectodermal and mesodermal non-occluded baculovirus. The virus replicates in the nucleus of cells of ectodermal and mesodermal origin tissues producing the characteristic basophilic intranuclear inclusion bodies (Wongteerasupaya *et al.*, 1995). Jasmin and Manissery (2000) have reported prevalence of WSV from the shrimp culture systems of Cochin area. During the present study, WSV inclusions were not observed in any of the shrimp samples examined. Histological sections of the important target tissues showed normal appearing nucleus without any hypertrophy or inclusion bodies. However, due to the limited number of samples taken and examined, it cannot be concluded that the shrimp farms in this area were free of white spot virus disease during the period of survey.

Epicommensal infestation

Surface or gill fouling by protozoan ciliates like *Zoothamnium, Epistylis* and *Vorticella* are very common in shrimp farming (Lightner, 1988; Turnbull *et al.*, 1994). Such a condition can result in mortality by interfering with water flow over gills, gas exchange over the gill surfaces, moulting, feeding and locomotion, especially when the pond water quality is not good (Lightner, 1988). However no mass mortality due to epicommensal ciliate infestation has been reported in India (Panchayuthapani, 1997).

In the present study, infestation with *Zoothamnium* was identified in the histopathological sections of samples from only 8% of the farms. Mixed infection of *Zoothamnium* and bacteria (as evidenced by presence of chronic inflammatory lesions) was observed in one case. Infections of mixed aetiology are serious but less conspicuous. It usually involves viruses, bacteria (principally vibrios), cili-

ates such as *Zoothamnium*, microsporidians *etc.*, coupled with nutritional and environmental stresses. In the case of viral diseases, infectious agents such as Vibrios, *Zoothamnium* and factors leading to stress such as high stocking density, wide fluctuations in pH, temperature and salinity can become component causes for disease outbreak. Eradication of the virus or the susceptible shrimp species from the culture environment is impractical. Hence the rational approach would be to monitor and control component causes such as epibiont *Zoothamnium* infestation and proper feed and water quality management.

Abnormal conditions such as discolouration of gills and spontaneous muscle necrosis were observed in 36% of farms, without any histopathological evidence of infectious aetiology. Hence it is assumed that these are all non-specific clinical signs due to some unknown factors. The occurrence of spontaneous / idiopathic muscle necrosis is characterised by the presence of whitish opaque areas in the striated musculature, especially in the distal abdominal segments (Lightner, 1988). It has been reported that the condition typically follows periods of severe stress from overcrowding, low dissolved oxygen levels, sudden temperature or salinity changes and rough handling. It is reversible in its initial stages, if the stress factors are reduced, but it may be lethal if large areas are affected. When irreversible change occurs and opportunistic bacteria invade the necrotic areas of the abdomen, the condition is called tail rot (Lightner, 1993).

In 27% of the farms surveyed, no histopathological evidence for any disease conditions were identified. In the present study, statistical sampling protocol could not be followed and due to the limited number of samples examined from each farm, absence of any disease/pathological condition in the samples need not necessarily mean that it is from a disease free population.

Although shrimp culture has advanced in leaps and bounds, production could become unpredictable because of an array of poorly understood processes in the ponds. It is obvious, however, that the shrimp farmers have become more concerned with the environment in general, and its effects on the cultured organisms in particular. With this realization, the farmers began to demand for more effective, accurate, rapid and environment friendly solutions to their production problems. Research has to come up with novel ideas and user-friendly products for disease prevention, surveillance, diagnosis and control.

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