International Journal of
Life Sciences
International Peer Reviewed Open Access Refereed Journal

Int. J. of Life Sciences, 2020; 8 (1):191-193
ISSN:2320-7817(p) | 2320-964X(o)

Original Article Open Access

Histopathological changes in hepatopancrease of crab *Paratelphusa jacquemontii* by Vibrio spp.

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Manuscript details:

Received: 14.02.2020
Accepted: 25.03.2020
Published: 10.04.2020

Cite this article as:
Ghaware AU and Jadhao RG (2020) Histopathological changes in hepatopancrease of crab *Paratelphusa jacquemontii* by Vibrio spp*, Int. J. of Life Sciences*, Volume 8(1): 191-193.

Available online on
http://www.ijlsci.in
ISSN: 2320-964X (Online)
ISSN: 2320-7817 (Print)

ABSTRACT

Increasing pollution of rivers and other water bodies has become a matter of great concern in recent years. Further crabs, fishes are the non-target organisms are affected. The accumulated toxicants damage their body parts and may reach the consumers. In the study aimed to histopathology of freshwater crab *Paratelphusa jacquemontii* (Rathbun), was investigated in the under laboratory experimental conditions. The results obtained *Vibrio spp.* affect in the disease imparted damage to hepatopancreas.

Key words: *Paratelphusa jacquemontii*. Histopathology, hepatopancreas

INTRODUCTION

Microorganisms are widely distributed in nature and diversity of microorganisms may be used as an indicator for organic pollution. The impact of changes in the water quality on flora and fauna may be differential, favouring some forms but detrimental to others, affecting natural system (Cooke *et al.,* 2002). Faecal contamination of water leads to higher bacterial load and subsequent water-borne diseases. Infection in crabs is more likely to occur at polluted water as reason of stress factor. Understanding the transfer of contaminants through the food web is critical to predict the exposure of humans to contaminants either through existence or commercial consumption of aquatic food and the possible health consequences of such exposure. In addition, such information is crucial in making accurate risk assessment for aquatic food safety. Botkin and Keller (2003) state that sewage pollution that has been observed in all aquatic ecosystems, affecting water resources if not properly treated before discharge. Due to sewage pollution has an important effect on benthos; crustaceans are mostly known as bio-indicators in various aquatic ecosystems, especially for polluted waters (Rinderhagen *et al.,* 2000). Such microbial activities result in a decrease in the economic value of crustaceans, such as lobsters and crabs, because of the unsightly effects on the cuticle of the animals.

MATERIAL AND METHODS

Microbiological parameters

Isolation of bacteria:

One ml of the water sample from water body was serially diluted with distilled water. Appropriately diluted water samples were plated on Nutrient Agar (NA) medium using spread plate technique. The inoculated plates were incubated at 37^0C for 24-48 hours. After incubation plates with countable range (30-300 colonies) were selected for counting and the bacterial load in the sample was expressed as total colony forming units (cfu) per ml of water sample.

Selection of Bacteria for experiment:

Vibrio sp., isolated from the water samples of Nal-Damyanti Sagar dam were selected as test micro-organisms for the experimental study. *Vibrio sp.* was selected considering their ubiquitous presence in the aquatic environment and their role as an important crab pathogen in fresh water crabs. These micro-organisms were selected hypothesizing that in a stressed environment the different bacterial species could negatively affect the crab fauna.

Preparation of inoculum:

Pure cultures of *Vibrio sp.* was inoculated separately in to 10 ml sterile nutrient broth and incubated at 37^0C for 20 to 24 hours. After incubation the cells were harvested by centrifugation at 3000 rpm for 15 minutes. The cell pellets were washed in isotonic saline. The initial inoculum density was determined by spread plate method on nutrient agar after desired dilution of inoculum in sterile distilled water or isotonic saline. Selected doses (0.5ml/crab) of 10^3, 10^5 and 10^7 dilutions of *Vibrio sp.* was injected through walking legs of crabs.

Experimental set up:

The fresh water crabs, *Paratelphusa jacquemontii* collected from Nal-Damyanti Sagar dam were brought to the laboratory. The crabs were kept in properly aerated large tanks contains well water. Water from the campus well was selected as control medium as the water sample was found to be clean with least bacterial concentration. Crabs of about the same weight and size irrespective of sex were selected for the experiment. Crabs were fed with dead prawn and the water was changed daily and was acclimatized for 15 days in the prevailing room temperature.

The crabs were divided into two tanks of 10 crabs each with well water. Tank-A stand as a control. Crabs in Tank-B were injected with *Vibrio sp*., The experiments were conducted for a period of 15 days. For the analysis, the crab was caught individually using a small hand net from aquarium tanks. The analysis was carried out on the days 2,4,6,8,10,12,15.

Histological analysis

Tissue processing:

To determine whether bacterial infection results in structural changes in internal tissues such as hepatopancreas of both control and infected crabs were examined histologically.

The excised tissues were fixed in aqueous Bouins fluid. After fixation for 24 hrs, the tissues were further processed to study histological details as per procedure of Bancroft and Stevens (1982). In brief, the tissues were dehydrated through 30-100% different alcohol grades and cleared in xylene. Cold and hot impregnations were followed by embedding the tissue in paraffin wax (Melting Point 58-60^{o}C). Serial sections were cut at 7 µm serial using rotary microtome. The sections were stained using Harris Haematoxylin and Eosin-Y as counter stain (Bancroft and Stevens**,** 1982). Damage to the tissues of treated crabs is recorded by comparing the data obtained from control.

RESULTS AND DISCUSSIOINS

Hepatopancreas pathology:

Histologically, each tubule of the hepatopancrease was surrounded by haemal spaces that contained haemocytes and apparent blood vessels. The hepatopancreatic tubules were enclosed by a basal lamina and contained a central lumen as shown in (Fig. 4.5-A). *Vibrio spp.* was more pathogenic in leaving severe inflammation of the submucosa, haemorrhagic ulceration and large scale degeneration of both mucosa and submucosa (Fig.4.5-B) Haemocyte infiltration, wandering of phagocytes and extensive degranulation were observed to be the potential immunopathologic response towards *Vibrio sp.* challenge. In severely infected crabs, epithelial cell boundaries were often not as clear as those observed in control animal. Pathological symptoms included intertubular accumulation of haemocytes (Fig.4.5-B). Cell necrosis and reduction of intertubular spaces were noticed in hepatopancreas infected with *Vibrio sp*.

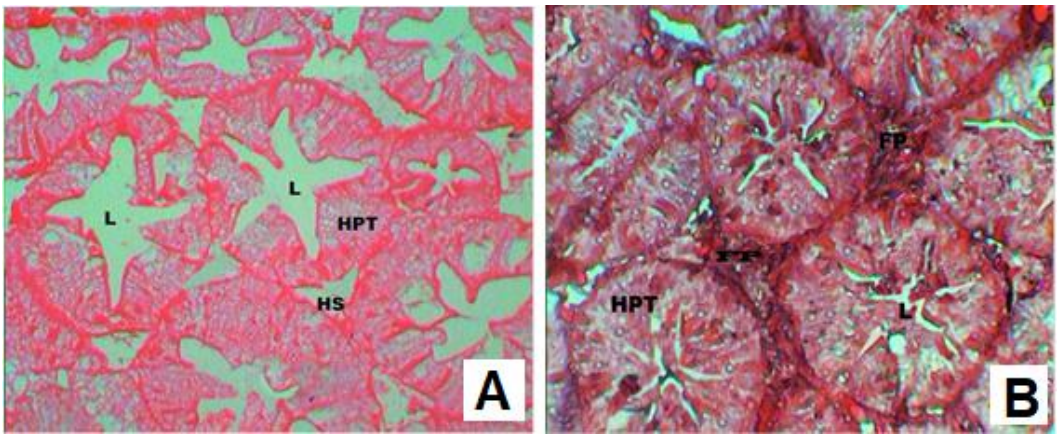

Figure 1: A: Normal hepatopancreaease of fresh water crab *P. jacquemontii*.
B: Hepatopancreatic pathology *P. jacquemontii* infected with *Vibrio sp.*

CONCLUSION:

In the present investigation, experiment infection of the freshwater crab *P. jacquemontii* by the gram negative strains of bacteria *Vibrio spp.* HikimaSonomi,(2003) believes that *Vibrio spp.* a gram negative bacteria infecting the hemolymph is an opportunistic pathogenic effect of this strain affects greatly the physiological condition of the organism. *Vibrio spp.* have been demonstrated as pathogenic over a few marine arthopods like *Limulus polyhemus* (Bang, 1956). Lightner (1977) has reported that species of *Vibrio, Pseudomonas* and *Aeromonas* are involved in the disease syndrome in penaeid shrimp. Newman and Feng (1982) have also identified that *Vibrio spp.* are pathogenic to *Cancer irroratus.*

Histological studies of hepatopancreas infected by *Vibrio spp.* shows severe damage, characterized by aggregation of hemocytes, migration of phagocytes and hemocytic encapsulation of injured tissues. In *Vibrio* infected hepatopancreas, the tissue damage is serious leading to glandular necrosis. Such observations have been reported by Johnson,(1983). Thus, it may be concluded that all detrimental associations of bacteria with crustaceans are more serious. The presence of Vibrio sp in crabs which are potential human pathogens indicates the need for public enlightenment, campaign and general education on proper handling and thorough cooking of crabs is important in reducing the outbreak of the disease.

Conflict of Interest
The author declares that there is no conflict of interest.

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