

## BACTERIAL INFESTATION OF *CHICOREUS RAMOSUS*

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### ABSTRACT

Infested *Chicoreus ramosus* possessed diffused cell infiltration, increase in bacterial numbers, and tissue necrosis. Bacterial infestations (*Vibrio* sp., *Pseudomonas* sp. and *Aeromonas* sp.) were more prevalent in adults than in juveniles. Animals with higher bacterial counts had heavily bored or damaged shells, but severe infestations, or mortality caused by potential pathogenic strains, were not observed.

### INTRODUCTION

The role of molluscs as intermediate hosts of parasites, and the interest in pathogenic microbes infecting economically important molluscs, stems from their potential role as biological control agents and killers of shellfish (Malek & Cheng 1974). Moreover, the study of bacterial pathogens of marine molluscs appears necessary to determine their role as primary or secondary invaders. However, reports on bacterial flora are surprisingly few. Only oysters have attracted attention from the view point of patho-biological studies. Most of the available information pertains to several species of oysters (Sprague 1971; Rosenfield 1969; Sindermann 1970). Diseases of marine snails are inadequately understood, and it remains unclear whether bacteria are specific pathogens of molluscs. Recent investigations carried out on parasites infesting the gastropod *Chicoreus ramosus*, revealed many trematodes and nematodes in various body parts, and extensive damage to the digestive gland, gonad and the foot (Emerson & Ayyakkannu 1994). This paper mainly concerns the alterations in tissue morphology and structure caused by bacterial pathogens infesting the soft body of *Chicoreus ramosus*. Despite the substantial economic importance of this gastropod, nothing is known about such infestations.

### MATERIALS AND METHODS

*Chicoreus ramosus* were collected from Cuddalore and Nagapattinam coastal waters, the south eastern coast of India, and stored in aerated sea water. The animals

were dissected, selected tissues homogenized in a sterile mortar in 2 ml sterile distilled water, transferred to a sterile test tube, 2 ml 3 % HCl and a drop of indicator (bromocresol purple) added, thoroughly mixed, left at room temperature (25 °C) for 2 hrs, 3 % NaOH added until the mixture became slightly alkaline, centrifuged at 1500 rpm for 15 min, inoculated into tubes with Dorset's egg media, and incubated at 37 °C for 24 hrs. After 24 hrs the bacterial growth was noted and the colonies were isolated for identification following the method of Simidu & Aiso (1962). For histopathological observations, the infested parts were fixed in Bouin, embedded in paraffin wax (melting point 58 °C), sectioned (4-5 µm), and stained with haematoxylin and eosin.

### RESULTS

Generally, the infested areas of *Chicoreus ramosus* possessed a low or moderate growth of bacteria, but counts were higher in adults than in juveniles. Major infestations were found in the foot, digestive gland and mantle (Fig. 1), lowest in the latter tissue. Morphological and biochemical characteristics showed that the bacteria were *Vibrio* sp., *Pseudomonas* sp., and *Aeromonas* sp. Among these potential pathogens, *Vibrio* sp. and *Pseudomonas* sp. were most common. A few animals possessed slight lesions in the foot region, and smears revealed almost pure cultures of *Pseudomonas* sp. There was no evidence of infestations which eventually could lead to the death of the animal.



**Figure 1.** Comparisons of normal and infested tissues of *Chicoreus ramosus*. 1-2 digestive gland; 3-4 mantle; 5-6 foot. Haematoxylin and eosin staining. 1 normal digestive gland. 2 arrows indicate necrosis of hepatopancreatic tubules. 3 normal mantle tissue. 4 arrow indicates lysis of basement membrane. 5 normal foot tissue. 6 arrows indicate necrosis of lining epithelium.

**The foot.** *Vibrio* sp. and *Pseudomonas* sp. infested the foot region. A few specimens showed slight lesions caused by *Pseudomonas* sp. These bacteria are potentially pathogenic, but no harmful effects were discovered. Cross section of the infected foot revealed small foci of epidermal necrosis and necrosis of the lining epithelium which appeared to be in the initial stage.

**The digestive gland.** Most of the digestive gland remained normal without much tissue alterations or any morphological changes. Also tissues adjacent to the infested part remained normal. Tissue smears of the digestive gland revealed infestation by *Aeromonas* sp. and *Vibrio* sp. Some hepatopancreatic tubules were infiltrated, necrotic, and the tubule epithelium appeared to be progressively fragmented and sloughed off from the basement membrane. However, no marked alteration in shape and structural integrity occurred.

**The mantle.** Tissue smears revealed *Pseudomonas* sp. and *Vibrio* sp. which did not cause much alteration of the tissue. Cross section of mantle tissue revealed invasion through the epithelium followed by lysis of the basement membrane.

## DISCUSSION

Sindermann (1970) suggested that microbial and parasitic diseases were the most important, but least studied factors responsible for mass mortality in culture, as well as in nature. Numachi *et al.* (1965) found that mass mortality of bivalves was caused by unidentified gram positive bacteria. However, in this study, only the gram negative *Vibrio* sp., *Pseudomonas* sp., and *Aeromonas* sp. were found. Guillard (1959) and Tubiash *et al.* (1965) isolated some of these forms from bivalves and showed that they were pathogenic. Bacterial infestation of adult *C. ramosus* was correlated with a heavily bored shell. But it is unknown whether this correlation represents a cause and effect relationship. Even though infestation of the bacteria did not cause obvious harm to the host, increased infestation of the digestive gland may eventually lead to destruction of the gland (Kinne 1980). Therefore, it is evident that a thorough understanding of the bacterial pathogens and the molluscs should be achieved.

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