Journal of Drug Metabolism and Toxicology

Dinakaran et al., J Drug Metab Toxicol 2013, 4:4
DOI: 10.4172/2157-7609.1000155

Research Article Open Access

Tail Rot Disease in *Macrobrachium idella idella* (Hilgendorf, 1898)

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Abstract

Shell disease may also be referred to as black spot, brown spot or spot disease and was recognized early this century as a problem in impounded population of aquatic animals. The chitinoclastic bacteria which cause shell disease are ubiquitous and infect a wide range of crustaceans, including prawns. All wild and cultured prawns are susceptible to infections; however disease rarely in wild crustaceans due to a lack of overcrowding and less mechanical damage. In the present study the Tail rot disease noticed in the M. idella idella (prawn), which is not common for prawns. The tail rot diseased prawn was tested to bacteriological for enumeration, isolation and biochemical identification of the microorganism.

Keywords: *M. idella idella*; Tail rot disease; Bacterial prawn disease; Spot disease; Brown spot disease

Introduction

Aquaculture is one of the fastest growing food production sectors in the world [1]. According to FAO statistics, over 80% of fish produced by aquaculture comes from Asia, with the production valued at $38.855 billion. However, disease outbreaks have caused serious economic losses in several countries. According to a World Bank Report, global losses due to prawn disease are around $3,000 million [2]. Thus, health management is of major importance in aquaculture. Bacterial disease may cause a range of problems from mortalities to growth retardation and sporadic mortalities. *Vibrio* spp. are the most important bacterial pathogens of prawn. *Vibrio* spp. are aquatic bacteria that are widely distributed in fresh water, estuarine and marine environments. Over 20 species are recognized, some of these are human pathogens (*V. cholerae*, *V. parahaemolyticus* and *V. vulnificus*) while some species are pathogens of aquatic animals including shrimp (*V. harveyi, V. splendidus*, *V. penaecida*, *V. anguillarium*, *V. parahaemolyticus*, *V. vulnificus*). *Vibrio* spp. are commonly observed in prawn hatcheries, grow-out ponds and sediments [3,4]. Mortalities due to pathogens in prawn hatcheries were already reported from Asia [5-8]. Filamentous bacteria such as *Leucothrix mucor*, *Thiothrix* sp., *Flexibacter* sp., *Flavobacterium*, *Cytophaga* sp. may cause infection to penaeid prawn larvae. In the present study a work was designed to know the reasons for the tail rot disease in edible prawn, *M. idella idella*.

Materials and Methods

Samples collection

The live samples of *M. idella idella* were randomly collected from Vellar estuary. Then it was cultured in the laboratory in fiber glass tank. During the course of culture tail rot disease was noticed which appears like green and black in colour in the prawn which is not common for prawns. Immediately the sample was tested for bacteriological enumeration. Various type of processing was done to find out the correct etiological agent of the disease. Processing varies in accordance with the aim of the study and the pathogens looked for identification. Isolated microorganism was subjected to pure culture by using various media's like Nutrient agar and selective media's like Macconkey agar, Blood agar and TCBS. Selected colonies from selective and differential media were subjected to macroscopy, microscopy and biochemical tests like IMViC, Urease, Nitrate, Catalase, Oxidase, and carbohydrate fermentation tests for identification.

Bacteriological examination

Preparation of the samples for bacteriological examination: A diseased prawn was taken aseptically and transferred to the microbiological laboratory. By means of sterile swab, exoskeleton of the prawn was touched and culturing it into the sterile nutrient agar plates. All plates were inverted and incubated at 28°C for 18-24 h. After incubation, the isolates were subjected to Gram's staining method for bacterial identification. For identification of isolates selective medium were used like TCBS, Blood agar etc. All the isolates were identified for further identification by means of using biochemical tests, such as IMVIC tests, Urease, TSI and Carbohydrate tests.

Results and Discussion

Tail rot disease are said to be a type of shell diseases. This disease affected 100% of the spent females after being held in the tanks. Shell disease appeared initially as coloured patches on the tail that later spread over the body. Many parts of the exoskeleton were soft and black lacking calcified tissue underneath. These areas easily became perforated, exposing underlying tissues. Several species of Gram negative organisms were identified such as *Vibrio parahaemolyticus, V. anglinotenus, Pseudomonas aeroguinosa, Aeromonas sps* and gram positive *Staphylococcus aureus* (Table 1).

Bacterial disease agents are common among fishes and shellfishes. However, it should be noted that the presence of these organisms does not necessarily always produce an outbreak of disease. For examples, species of *Aeromonas* and *Pseudomonas* which are amongst the normal flora of healthy fish and shellfish often cause diseases when they are under stress (eg: high population densities, enhances the changes of a potential pathogen to invade the host, thus provoking disease) [9].

The shell disease in the first batch of crabs was considered severe

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Received July 18, 2013; **Accepted** September 06, 2013; **Published** September 09, 2013

Citation: Dinakaran GK, Soundarapandian P, Varadharajan D (2013) Tail Rot Disease in *Macrobrachium idella idella* (Hilgendorf, 1898). J Drug Metab Toxicol 4: 155. doi:10.4172/2157-7609.1000155

S.No.	Test	*S. aureus*	*Vibrio parahemolyticus*	*Vibrio alginolyticus*	*Pseudomonas aeroguinosa*	*Aeromonas hydrophila*
1	Gram staining	+	-	-	-	-
2	Shape	Cocci	Rod	Rod	Rod	Rod
3	Motility	-	+	+	+	+
4	Indole test	-	+	+	-	+
5	Methyl red test	+	ND	ND	-	+
6	Vogesproskaur test	-	-	+	-	-
7	Citrate utilization test	NP	+	+	+	D
8	Urease test	NP	v	ND	V	-
9	TSI test	NP	NP	NP	K/K	K/A
10	H2S	NP	NP	NP	-	-
11	Gas	NP	NP	NP	-	-
12	Catalase tests	-	NP	NP	-	+
13	Oxidase test	+	+	+	+	+
14	Carbohydrate test					
15	Glucose	NP	+	ND	-	+
16	Lactose	NP	-	ND		
17	Sucrose	NP	-	+	-	+

NP: Not performed; ND: Not determined

Table 1: Identification of Bacteria from prawn.

because more than 75% of the dorsal region of the shells was affected. Many parts of the exoskeleton were soft and black lacking calcified tissue underneath. Several species of sucrose-fermenting and non-sucrose fermenting *Vibrios* were identified such as *V. vulnificus,V. splendidus,* and *V. orientalis.* It is probable that the microbiological aggregate formed on the shell provided a good environment for chitinoclastic *Vibrios* to settle, caused gradual damage and resulted to perforation. Shell disease in crabs and other crustaceans with a chitinous exoskeleton is rather common in older animals. This is due to longer intermoult periods in older animals. Injury inflicted during handling and crowding, and exposures to pollutants are some of the predisposing factors implicated [10,11].

An early sign of shell disease is the presence of small, darkened, sometimes frible, or cratered area on the cuticle. As the disease progresses, the lesions deepen, become larger, and eventually coalesce. The edges of the lesions, where bacteria are most active, may be whitish in colour. The affected areas are softened and easily broken. The darkening of affected areas is presumably due to the deposition of melanin. Blackening is particularly prominent in shell disease of shrimp, and infected shrimp are also likely to lose parts of their appendages [12]. In lobsters and blue crabs, lesions usually are confined to the cuticle. Nonetheless, shell disease is involved in mortalities in captive lobsters, and reported a mortality rate of 71% in diseased lobsters, but only 6% in lobsters unaffected by shell disease [13].

Through the examination of over 3000 mud crabs the disease (shell lesions) called 'rust spots' appeared as orange coloured areas on the dorsal carapace, which in severe cases would ulcerate to expose underlying soft tissues which was prevalence in Port Curtis compared to a number of other locations in Queensland, was determined. The normal background prevalence of 5% reported for shell disease in other crustacean populations. The lesion pathology of rust spot lesions was determined to be unique compared to the described pathology of other shell diseases, where there is an external erosion of the shell primarily due to pathogenic organisms. Of these, 82.9% had rust spot lesions on the carapace. The majority of rust spot-affected crabs (78.8%) were females [14,15] reported that aside from chitinase, *Vibrios* also possess the enzymes gelatinase and lipase considered as compounding factors that enhance shell degradation.

Shell disease may also be referred to as black spot, brown spot or spot disease and was recognized early this century as a problem in impounded populations of aquatic animals. The chitinoclastic bacteria which cause shell disease are ubiquitous and infect a wide range of crustaceans, including prawns. All wild and cultured prawns are susceptible to infection; however disease rarely occurs in wild crustaceans due to a lack of overcrowding and less mechanical damage [16].

The expression of shell disease as a chronic condition or as an acute condition associated with mortality depends on host susceptibility, the pathogen involved and environmental conditions. Numerous other bacterial and fungal organisms invade shell disease lesions as secondary pathogens [17]. *Aeromonas* sp. has received increasing attention due to the epidemiological evidence which suggested that these organisms cause sporadic human gastroenteritis outbreaks followed the consumption of shellfish [18,19]. These organisms were including in the list of enteric pathogenic bacteria [20].

In conclusion, tail rot disease is not a disease caused by a single pathogen and should not be considered a disease solely restricted to the exoskeleton. Numerous bacteria within the marine environment are capable of degradation of the chitin component of the crustacean cuticle and it is likely that the collective effects of the lesion community lead to further exoskeleton degradation. If breach of the cuticle occurs,

infection of the body cavity of the crustacean may result, with the internal symptoms differing depending on the nature of the penetrating bacteria and this may ultimately leads to the death of the animal. It is important to study if such a condition leads to problems like delayed clotting, impaired defense mechanisms, and increased susceptibility to other infectious microorganisms.

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