World Journal of Agricultural Sciences 4 (2): 137-139, 2008
ISSN 1817-3047

Outbreak of Vibriosis in Mantis Shrimp (*Squilla* sp.)

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INTRODUCTION

Mantis shrimp (*Squilla* sp.) is a marine crustacean live in sea bed. Some of aquarist assumes it as pest due to the fact that it can easily sneak into tank and hide in rock [1]. It eats fish, small crustacean and coral in the aquarium tank. However, some of aquarists kept it as pet [1]. Commercial fishermen from United States considered mantis shrimp as nuisance during fishing operation, besides, having no commercial value. On the contrary, mantis shrimp is favorite seafood among Malaysian especially for the Chinese community. In Malaysia, fresh caught mantis shrimp costs around USD 1 to USD 2 per kg [2] whereas the prices of ready eat mantis shrimp within USD 4 to USD 6 per kg in the seafood restaurant. In Taiwan, the price of fresh caught mantis shrimp can reach up to USD 4/kg. Mantis shrimp also can be found regularly in fish markets of Spain, Italy, Egypt and Morocco [3]. Therefore, it indicates mantis shrimp has a great potential in seafood industry especially in Asia. To date there has been little study on disease in mantis shrimp. Thus, this project was carried out to investigate causative agent in the outbreak of mantis shrimp as well as the antibiogram of the agents. Approximately fifty mantis shrimp (*Squilla* sp.) were bought from local fisherman and brought back and maintained in marine hatchery of Universiti Malaysia Terengganu, Malaysia. After a week in the hatchery, 100% of mantis shrimp became lethargic and loss of appetite. Grossly mantis shrimp showed black and brown circular lesions on the carapace and abdomen whereas melanization was found on the telson and uropod of the mantis shrimp. Their eyes were also become black. All the diseased mantis shrimp were brought back into the laboratory for further investigation. Sterile cotton bud was used to swab onto the lesion and hepatopancreas of the diseased mantis shrimp separately. They were then spread onto blood agar, cytophaga agar, glutamate starch phenol red (GSP) agar (Merck, Germany), xylose lysine deoxycholate (XLD) agar (Merck, Germany) and MacConkey agar without crystal violet (Difco, USA). After 24 h incubation at room temperature, the inoculated plates were examined for presence of bacterial colony. The bacteria were then kept in Trypticase Soy Agar (TSA) (Merck, Germany) deep tube for the identification purpose. The identification of the suspected bacteria was done using commercial identification kit (BBL Crystal, USA) and conventional biochemical tests such as Gram staining, oxidase, catalase and motility test. The bacterial isolates were cultured in Trypticase Soy Broth (TSB) (Merck, Germany) for 24 h at room temperature. Bacterial suspension was then adjusted to 10^6 cfu/ml using a biophotometer (Eppendorf, Germany) and spread plated on Mueller Hinton (MH) agar (Oxoid, England). After placing antibiotic disks on the MH agar plate, the plate was incubated for 24 h at room temperature. After incubation period, the diameter of inhibition zones of the each tested antibiotic was measured and interpreted as 'sensitive' or 'intermediary sensitive' or 'resistant' based on National Committee for Clinical Laboratory Standards (NCCLS) provided by the manufacturer. Each antibiotic test was run in triplicates. Antibiotic disks that applied in the present study were erythromycin 15 μg/disk, furazolidone 15 μg/disk, sulphamethoxazole 25 μg/disk, chloramphenicol 30 μg/disk, kanamycin 30 μg/disk, nalidixic acid 30 μg/disk and oxytetracycline 30 μg/disk. In the present study, majority of the isolates were identified as *Vibrio* spp. They were eight isolates of *Vibrio alginolyticus*, three isolates of *V. mimicus*, two isolates of *V. parahaemolyticus* and one isolate of *V. hollisae*. The

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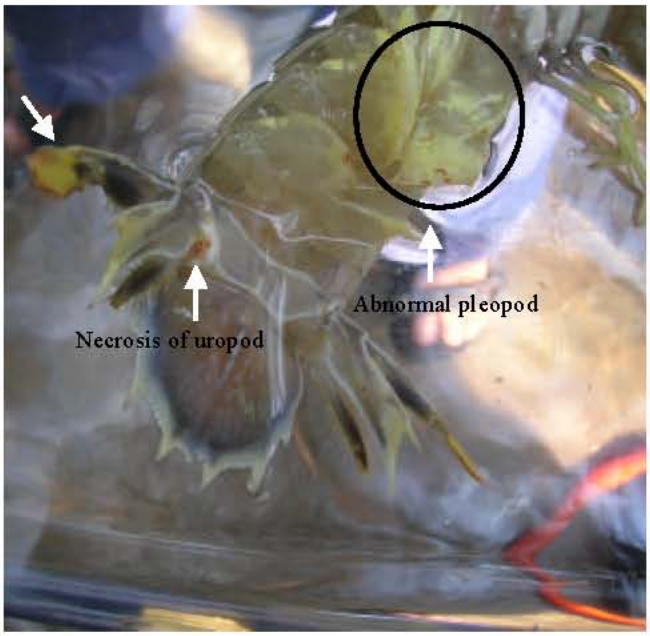

Fig. 1: Abnormal pleopods and necrosis of uropod

Table 1: Antibiotic Sensitivity Test of the bacterial isolates from diseased mantis shrimp (*Squilla* sp.)

Isolate	E15	FR15	RL25	C30	K30	NA30	OTC30
Flavobacterium sp.	I	S	I	S	I	S	S
Flavobacterium sp.	I	S	R	S	S	S	S
Shigella sp.	R	S	R	S	S	S	S
Weeksella virosa	R	S	R	S	S	S	S
Weeksella virosa	S	I	I	S	S	S	S
Weeksella virosa	S	S	S	S	S	S	S
Weeksella virosa	S	S	R	S	I	S	S
Weeksella virosa	S	S	S	S	S	S	S
Weeksella virosa	S	S	S	S	S	S	S
Weeksella virosa	S	S	R	S	S	I	S
Vibrio alginolyticus	S	S	S	S	S	S	S
Vibrio alginolyticus	S	S	R	S	I	S	S
Vibrio alginolyticus	S	S	R	S	S	S	S
Vibrio alginolyticus	I	S	I	S	S	S	S
Vibrio alginolyticus	S	S	R	S	S	S	S
Vibrio alginolyticus	S	S	R	S	S	S	S
Vibrio alginolyticus	R	S	R	S	S	S	S
Vibrio alginolyticus	S	S	I	S	S	S	S
Vibrio holissae	S	S	R	S	S	S	S
Vibrio mimicus	S	S	S	S	S	S	S
Vibrio mimicus	S	S	S	S	S	S	S
Vibrio mimicus	R	S	R	S	S	S	S
Vibrio parahaemolyticus	S	S	R	S	S	S	S
Vibrio parahaemolyticus	I	S	R	S	S	S	S

Key:
R = resistance, I = intermediately sensitive, S = sensitive
E = erythromycin 15µg/disk, FR = furazolidone 15 µg/disk, RL = sulphamethoxazole 25 µg/disk, C = chloramphenicol 30 µg/disk, K = kanamycin 30 µg/disk, NA = nalidixic acid 30 µg/disk, OTC = oxytetracycline 30 µg/disk

other bacteria were seven isolates of *Weeksella virosa*, two isolates of *Flavobacterium* sp. and one isolate of *Shigella* sp. Gram positive bacteria were not successfully isolated from the mantis shrimp. *Vibrio* spp. were reported commonly isolated from both marine environment and organisms. These bacteria are well known as vibriosis causative agent and caused mass mortality of marine culture especially cultured marine shrimp. *Weeksella virosa* isolates were also identified from diseased mantis shrimp which could be typical mantis shrimp microbiota. For instance, 19.05% of the isolated bacteria from diseased cultured bluefin tuna (*Thunnus thynnus*) were identified as *W. virosa* in the study of Kapetanovic *et al.* [4]. In present study, symptoms and gross appearances of the mantis shrimp were similar to vibriosis infected *Penaeus monodon* and other crustaceans as reported by Lavilla-Pitago *et al.* [5] and Prayitno and Latchford [6]. The infected shrimp will exhibit decreased appetite, became darker and light or dark brown focal lesion and necrosis appeared on appendage tips. So far, vibriosis outbreak among mantis shrimp has not been reported. Therefore, this study was the first reported vibriosis outbreak in mantis shrimp. Figure 1 showed abnormal pleopods and necrosis on uropod of the mantis shrimp in the present study. Table 1 showed antibiogram of 24 bacteria isolated from mantis shrimp against 7 types of antibiotics. In the present study, percentage of antibiotic 'resistance' was reported as 10.7% whereas 'intermediary

sensitive' and 'sensitive' were 6.5% and 82.8%, respectively. Four and 14 cases of antibiotic bacterial resistance were found for both erythromycin and sulphamethoxazole. 5 out of 7 tested antibiotics were found effective to control the isolated bacteria. They were kanamycin, oxytetracycline, chloramphenicol, nalidixic acid and furazolidone. From the study, most of the bacterial isolates were resistant to sulphamethoxazole and most sensitive to furazolidone, oxytetracycline and chloramphenicol. Although only a few bacterial isolates were found to be resistant to erythromycin and sulphamethoxazole, this could warrant more thorough study should be done on wild mantis shrimp. There is possibility that the catching area was overexposed to both antibiotics which could be due to sewage or drainage especially from aquaculture sites. This was supported by the finding of Tendencia and de la Pena [7] which bacteria isolated from water of ponds that have previously used oxolinic acid were more resistant to the antibiotic compared to those bacteria that were isolated from water of ponds that have not used any antimicrobial. To date, none of these bacteria have been directly related to disease outbreak in mantis shrimp, thus emphasing the need for further studies on bacterial diversity and antibiogram on mantis shrimp.

ACKNOWLEDGEMENT

This project was funded by Fundamental grants No. 59025 and 59088. The authors wish to thank Mr Eric Kon for his help in sampling diseased mantis shrimp.

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