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ASSOCIATION OF *VIBRIO HARVEYI* WITH
MORTALITIES IN THE BROWN-MARbled
GROUper, *EPINEPHELUS FUSCOGUTTATUS*
(FORSSKAL) RAISED UNDER GROwOUT
CULTURE CONDITIONS
(With 4 Figures)

By

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بكتيريا الفيبريو المصاحبة لوفيات الهامور البني المبرقش
تحت ظروف تربية النمو

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تم دراسة أسباب الوفيات لأسماك الهامور البني المبرقش تحت ظروف تربية النمو في المياه العالية الملوحة على سواحل البحر الأحمر للمملكة العربية السعودية. كان معدل النفوق هو سمكة واحدة يوميا لمدة ١٥ يوما متتالية. أظهر الفحص الخارجي للأسماك النافقة خسوف العينين أو فقدان لكل العين من جهة واحدة. كان هناك أيضا فرح في الجلد مما أدى إلى تعرض العضلات للبيئة الخارجية. أظهرت الأعضاء الداخلية لحتقان وكذلك إنتفاخ ملحوظ في المثانة الهوائية. أما الفحص الميكروسكوبي أظهر إصابة شديدة للخياشيم بطفيل *Cryptocaryon irritans*. صاحب هذا الطفيل زيادة في عدد الخلايا المبطنية للقناة الثانوية للخياشيم وكذلك إلتحام هذه القائق. كان هناك نشاط ملحوظ لتصنيع الخلايا الدموية بالكلية الخلفية. أظهرت العضلات تحلل وتجمع من كرات الدم الحمراء ويعض الخلايا الليمفاوية. تم عزل بكتريا *Vibrio harveyi* من الكلية الخلفية ومناطق التفرح على بيئات TSA, BHI ثم التعرف على الخصائص البيوكيميائية باستخدام تقنية API 20E.

SUMMARY

Mortality in the brown-marbled grouper, *Epinephelus fuscoguttatus* raised under growout culture conditions in the hypersaline waters of the Red Sea Coast of Saudi Arabia were investigated. The number of dead

fish was one per day and continued for successive fifteen days. The morbid fish showed exophthalmia or unilateral destruction of the whole eye. Also, ulcers were detected and resulted in exposing the underneath muscles of the tail region. Congestion of parenchymatus organs and over inflation of the swim bladder were observed. Histopathologically, the gills had severe infestation with *Cryptocaryon irritans*. The infested gills showed hyperplasia and lamellar fusion. Marked activation of the haematopietic tissue of the posterior kidneys was also observed. The muscles showed myolysis with focal accumulation of red blood cells and few lymphocytes. *Vibrio harveyi* was isolated from the posterior kidneys and ulcerated areas on Brain-Heart Infusion agar (BHI) and Trypton-Soya Agar (TSA) and identified biochemically using the commercial API 20E.

Key words: *V. harveyi*, Brown – marbled grouper – Growth culture.

INTRODUCTION

The epinepheline serranid grouper are commercially marine food fish in several countries, especially in Southeast Asia and the Middle East (Quinito and Toledo, 1991). The Fish Farming Center at Jeddah, Kingdom of Saudi Arabia raised successfully *Epinephelus fuscoguttatus* under growout culture conditions using a flow-through tank culture system. The growth performance of these fish was averaged 578.01 g within 7 months (James *et al.*, 1998). However, mortalities occurred especially when the fish were fed on trash fish. The number of dead fish was one fish per day and continued for fifteen days. The purpose of this study was to identify possible causes of mortalities.

MATERIAL and METHODS

Fish:

Epinephelus fuscoguttatus raised in earthen ponds of 600m² and fed to satiation on trash fish 1-2 times a day. The water temperature ranged between 30-35°C and salinity of 42 ‰. The average body weight of these fish was 578±17 g.

Gross and Histopathology:

The dead fish were examined for the presence of any lesions. Samples of the gills, posterior kidneys, and muscles were taken immediately, fixed in 10% formalin, embedded in paraffin and

sected at 4-6 μ . The sections were stained by H & E and examined by light microscopy. The intensity scale of the protozoan infestation in the gills of *E.fuscoguttatus* was based on the number of parasites per microscopic field at 400X. A number higher than 50 protozoan per microscopic field was considered severe degree of infestation.

Bacteriology:

Fish were dissected aseptically and streaks of the posterior kidneys and ulcerated areas were made on BHI and TSA supplemented with 0,2,5% NaCl and incubated at 20-25°C for 24-48h. Identification of the isolates was made using the commercial API20E (BioMeriux Lab., France).

RESULTS

Gross and Histopathology:

The morbid fish showed exophthalmia. In some fish, unilateral destruction of the whole eye was noticed. The stomach was empty of food. The parenchymatous organs showed congestion. The skin of the tail region had ulceration, which resulted in exposing the underneath muscles. Light microscopy showed severe degree of infestation with *C.irritans* in the gills. The organism was found between the secondary gill lamellae (interlamellar) and appeared rounded to oval-shaped with distinct nucleus and several food vacuoles (Fig. 1). The infested gills had hyperplasia and lamellar fusion (Fig. 2). The posterior kidneys showed slight degenerative changes of the tubules with pronounced activation of the haematopoietic tissue (Fig. 3). The muscles had myolysis with focal accumulation with red blood cell and few lymphocytes (Fig. 4).

Isolates of the posterior kidneys and ulcerated areas showed the following features: Gram negative motile rods, able to grow in media containing 2 & 5% NaCl. The organism was positive for the following tests: Oxidase, B-galactosidase, Lysine decarboxylase, Ornithine decarboxylase and Indole; and negative for Arginine dihydrolase, Citrate utilization, H₂S production, Urease, Tryptophan deaminase and Voges-Proskauer. It was also able to ferment, with acid production only glucose, manitol, sorbitol and sucrose, but did not ferment inositol, rhamnose, melibiose and arabinose. According to Baumann *et al.* (1980) the features of the isolated match with *Vibrio harveii*.

DISCUSSION

Epinephelus fuscoguttatus is considered one of the most desirable food fish in the Kingdom of Saudi Arabia. The Fish Farming Center successfully cultured this species under the hypersaline water conditions (James et al., 1995). However, the growout culture in earthen ponds is facing many constraints. For example, spontaneous individual mortality was commonly observed. This study showed severe degree of infestation with *C. irritans*. Sindermann (1990) stated that *C. irritans* is an obligate pathogen affecting several marine species and homologue to *Ichthyophthirius multifiliis* of freshwater fishes. Moreover, the optimal temperature of this protozoan is considered high and ranged between 25-30°C (Sindermann, 1990). In this study the temperature is optimal for this protozoan. The hyperplasia and lamellar fusion observed in this study suggested an irritation induced by the protozoan had occurred. Hyperplasia is a quick response to a wide variety of causes i.e. toxins, chemical and infectious agents (Clark et al., 1997). Likewise, it is known that *C. irritans* followed closely by an array of bacterial agents which caused dermatitis and exophthalmia (Sindermann, 1990). Similar observation was observed in this study.

Vibrio spp. have been isolated and related to mortalities in cultured marine fishes, such as seabream, gilt-head seabream, silvery black and brown-spotted grouper (Rasheed, 1989; Saced, 1995 and Balebona et al., 1998). The kidney changes observed in this study was previously reported and suggested that the kidney is the main target organ for the bacteria (Rasheed, 1989). While, the ulceration and myolysis observed in this study suggest a direct action of the bacterial toxins.

Despite, the opportunistic nature of the bacteria, there are several reports which indicated that *V. harveyi* can be a primary pathogen (Balebona et al., 1998 and Baticados et al., 1990). In this study, the gross lesions, the abundant bacterial growth and the microscopic changes are supporting the pathogenic nature of the *V. harveyi* which was isolated and identified from these lesions.

CONCLUSIONS

The main causes of mortalities in *Epinephelus fuscoguttatus* are *Vibrio harveyi* and *Cryptocaryon irritans*. The cultural and biochemical characteristics are indicating the type of *Vibrio spp.* involved in mortality. The histopathology supports the pathogenic nature of *V. harveyi* rather than opportunistic.

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FIGURE LEGENDS

- Fig. 1:** *Epinephelus fuscoguttatus* gills infested with *C. irritans* between the secondary lamellae. The organism appeared rounded-oval shaped with distinct nucleus and several food vacuoles (Arrow). Haematoxylin and Eosin. X 64.
- Fig. 2:** The infested gills showing hyperplasia (H) and lamellar fusion (L). Haematoxylin and Eosin. X 32.
- Fig. 3:** The posterior kidneys of *Epinephelus fuscoguttatus* showing pronounced activation of the haematopoietic tissue (Arrow). Haematoxylin and Eosin. X 32.
- Fig. 4:** The muscles of the tail region had myolysis (M) with accumulation of RBCs ® and few lymphocytes (Arrow). Haematoxylin and Eosin. X 64.



