Assiut Vet. Med. J. Vol. 42 No. 84, January 2000

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POSSIBLE CAUSE (S) OF BLACK COLOURATION IN THE BLACK TIGER SHRIMP, *PENAEUS MONODON* (FABRICIUS) COLLECTED FROM THE RED SEA AT SAUDI ARABIA

(With 1 Table and 4 Figures)

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(Received at 7/12/1999)

الأسباب المحتملة للون الأسود في الروبيان العملاق (الجنبري) من منطقة البحر الأحمر للمملكة العربية السعودية

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تم جمع عدد ١٥ روبيانة (جنبري) عملاق Penaeus monodon من البحر الأحمر على سواحل المملكة العربية السعودية وإرسالها للمختبر وذلك إجراء الفحوص التشخيصية للحكم على سلامتها من الأمراض قبل تخزينها كأمهات للتربية . كان متوسط وزن هذه الأمهات ٧٧,٩ ± ١٧,٤ جم أما أطوالها فكانت ٢٠,٥ ± ١,٥١ سم. وجد على منطقة الذيل لهذه الأمهات بقع سوداء ذات شكل محدد يتراوح قطرها من ١-٣ سم. أجريت مسحات لهذه المناطق السوداء وصبغت بصبغة الجرام التي أظهرت أجسام قضيبية منحنية أو متعددة الأشكال وسالبة الصبغة. بعد إجراء عملية العزل على بيئة Tryptone Soya Agar تم التعرف على بكتيريا Vibrio alginolyticus وذلك بإستخدام تقنية API 20E. أظهر الفحص الهستوباثولوجي عدم وجود أي مؤشرات لحدوث المسببات الفطرية أو الطفيلية وكذلك عدم وجود الأجسام المميزة للفيروسات. إستنتج من هذه الدراسة أن سبب اللون الأسود هو حدوث إصابات ميكانيكية تبعها نفاذ لبكتيريا الفيبريو . تم تقديم النصائح للمزارعين بتلافي عملية الإصابة أثناء عمليات النقل والحصاد وبتطبيق ٣ جزء في المليون من المضاد الحيوي Furance على هيئة حمام لمدة ٣ أيام.

SUMMARY

Fifteen adult black tiger shrimp, *Penaeus monodon* collected from the Red Sea at Saudi Arabia were submitted for a diagnostic purpose prior to

stocking as broodstock. The average body weight was 77.9±17.14 g and lengths of 20.5±1.51 cm. The tail region had a small black confined areas of 1-3 cm wide. Squash smears of these areas stained with Gram stain showed pleomorphic or curved Gram-negative rods. Isolation and identification on Tryptone-Soya Agar (TSA) and API 20E, respectively were made. The isolates from all shrimps had the same characterstics of *Vibrio alginolyticus*. Histopathologically, there was no evidence for the presence of inclusion bodies specific for viral infection. Also, there was no indication for fungal or parasitic infestation. The study concluded that a mechanical damage followed by the invasion of *V.alginolyticus* are responsible for the black colouration observed. Farmers were advised to avoid mechanical damage during collection and transportation and to use 3 p.p.m. of furance as prolonged bath for three days.

Key words: *Cause(s) of black colouration – Black Tiger shrimp.*

INTRODUCTION

The aquaculture of panaeid shrimp has grown from its experimental beginnings roughly three decades ago into a major industry with a high value crop. In the mean time, increasing importance of disease especially those caused by infectious agents has been recognized. The most important diseases of cultured penaeid shrimp have had viral or bacterial etiologies, but a few important diseases have fungal and protozoan agents as their cause (Lightner and Redman, 1998).

There are extensive reports on the bacterial diseases in penaeid shrimps caused by *Vibrio species* (Lightner, 1988 and Brock and Lightner, 1990). *Vibrio species* are Gram negative bacteria especially in the marine environment and usually constitute the majority in the normal microflora of farmed and wild penaeid shrimp (Dempsey and Kitting, 1987; Lightner, 1993 and Vanderzant et al., 1971). Bacterial infections in shrimp may take three forms, localized spots in the cuticle (bacterial shell disease), localized infections of the gut or hepatopancreas, and generalized septicaemia (Lightner, 1993). Classic diagnostic techniques are still currently used for detection of various etiologies in penaeid shrimp. *Penaeus monodon* collected from the Red Sea at the shores of Saudi Arabia were submitted for routine diagnosis before stocking as broodstock. In these shrimps, black colouration in the tail area was

observed. Consequently, the purpose of this study is to investigate the possible cause (s) of these black areas.

MATERIAL and METHODS

Penaeus monodon:

Fifteen adult black tiger shrimp, penaeus monodon were caught from the Red Sea at the Saudi Arabia shores and submitted to the Fish Farming Center Laboratory, Jeddah, Kingdom of Saudi Arabia for routine diagnostic service. These shrimp were shipped in a tank filled with seawater and provided with areation. The average body weight was 77.9±17.14 g and lengths of 20.5±1.51 cm.

The gills were dissected and few filments were squashed and examined by light microscopy for identification of parasites and fungi.

Gross and Histopathology:

The exoskeleton was examined for the presence of any abnormal colouration or lesions. The cephalothorasic region was dissected and the hepatopancreas and musculature were examined for any abnormal appearance. The hepatopancreas, gut epithelium and gills were dissected and taken immediately, fixed in 10% formalin, dehydrated, embedded in paraffin, sectionned at 4-6 µ, stained with H. & E. and examined by light microscopy.

Bacteriology:

Smears from the black areas at the tail region and hepathopancreas were made and stained by Gram stain. Also, streaks of the hepatopancreas and tail region were spread on Tryptone – Soya Agar (TSA) media supplemented with 0, 2, 5% Na Cl. The agar plates were incubated for 24-48 h at 30°C. Subculture was made and selected colonies were homogenized and suspended in a sterile 0.85% Na Cl. The suspended bacteria was identified using the AP120 E commercial system (BioMeriux Laboratory, France).

RESULTS

Wet-Mount Technique:

The gills of all shrimp examined did not show any evidence for protozoa or fusarium species.

Gross and Histopathology:

The tail area had a small black confined areas of 1-3 cm wide. These areas were different from the normal black bands distributed in the body (Fig. 1). The musculature showed the normal texture and appearance. No obvious lesions were detected in the hepatopancreas.

The gills of examined shrimp did not show any evidence for protzoa, fusarium spp. or any black colouration (Fig. 2). Neither the hepatopancreas nor the gut epithelium had intracytoplasmic or intranuclear inclusion bodies specific for viral infections (Fig. 3 & 4).

Bacteriology:

The smears of the black areas and hepatopancreas stained with Gram stain showed Grame-ve curved or pleomorphic rods. The colony shape and other biochemical results are identical to *Vibrio alignolyticus* (Table 1).

DISCUSSION

Despite the development of modern techniques such as DNA pobes, polymerase chain reaction and tissue culture, simple classical method are still reliable. It is the normal, rather than the exception, to find more than a single disease causing agents (infectious or noninfectious) in individual shrimp or in populations of shrimp (Lightner and Redman, 1998). For example, viral infections are typically accompanied by secondary bacterial and epicommensal infections (Anderson et al., 1987; Brock, 1992 and Lightner, 1996).

The present study utilized simple methods such as, wet mount, histopathology and classical microbiology. It was necessary to exculde viral agents in these samples, because these shrimp will be kept as broodstock. The hepatopancreas and gut epithelium were negative for the presence of either intracytoplasmic or intranuclear inclusion bodies specific for viral infections. However, absence of inclusion bodes could not absolutely exclude viral infections as the inclusions appear morphologically during certain periods in the course of infection then disappear. Flegel et al. (1999) stated that penaeus monodon is susceptiable to different types of viruses such as hepatopancreatic parvovirus (HPV), monodon baculovirus (MBV), infectious hypodermal and haematopoietic necrosis virus (IHHNV), white-spot syndrome virus (WSSV) and yellow-head virus (YHV).

In this study, there was no evidence for fusarium spp. either by wet mount or histopathology in the gills, exoskelton, hepatopancreas and

muscles of the examined shrimp. Fusariosis is a common disease caused by Fusarium species and responsible for black gill disease or black spot disease (Sindermann, 1990). Moreover, there was no indication for protozoal infestation in the muscles or gut. Microsporidae protozoa are responsible for a condition known as cotton-shrimp (Sindermann, 1990).

Vibrio species are the predominant bacteria in the marine envionment and usually constitute the majority in the normal microflora of farmed wild penaeid shrimp. Despite the opportunisitic nature of most vibrio pathogens, recently, some occurring diseases in penaeid shrimp have been caused by *vibrio species* which behave more like true pathogens than opportunistic invaders (Lightner, 1993). Most bacterial isolates from diseased penaeid shrimp have been *vibrio species*, usually *V. alginolyticus, V. parahaemolyticus, V. spendidus, V. vulnificus* and *V. damsela* (Lightner, 1988 and Lightner, 1996).

The typical morphologicl and biochemical characteristics of *Vibrio alginolyticus* were observed in this study. Moreover, the gross lesions represented by the black areas in the absence of other etiologies suggesting the localized form of bacterial shell disease. Roald et al. (1981) stated that mechanical damage is considered to be essential to invasion by chitinolytic organisms and in these lesions *Vibrio species* have been isolated (Fisher et al., 1970).

The differential diagnosis made in this study suggests that the possible causes of the black areas in the tail are mechanical damage followed by the invasion of *V. alginolyticus*. It is necessary to avoid damage during collection and trnsportation and application of Furacin at a concentration of 3 p.p.m. as prolonged bath for three days is preferable.

CONCLUSION

Penaeus monodon shipped to the Fish Farming Center Laboratory did not show any evidence for viral, parasitic or fungal infections by means of wet mount and histopathology. However, *V. alginolyticus* was isolated and identifed in the black tail area by means of classic microbiology. The study suggested that a mechnical damage folllowed by the invasion of the bacterium had occurred. It was recommended to avoid damage as possible and the use of Furacin as a prophylactic treatment.

Table 1: Cultural and biochemical characterstics of *Vibrio alginolyticus* isolated from the black tail area of *Penaeus monodon*.

Tests	Reaction
Gram stain	-
Morphology	Rods
Motility	+
Growth with Nacl (0%)	-
Growth with NaCi (2%)	+
Growth with NaCl (5%)	+
Oxidase	+
Arginine dihydrolase	-
Lysine decarboxylase	+
Ornithine decarboxylase	-
Citrate utilization	-
H_2S production	-
Urease	-
Tryptophan deaminase	-
Indole	+
Voges-Proskauer	+
Gelatin hydrolysis	+
Acid from:	
Glucose	-
Manitol	-
Inositol	-
Sorbitol	-
Rhamnose	-
Sucrose	-
Melibiose	-
Arabinose	-
Amylodose	-

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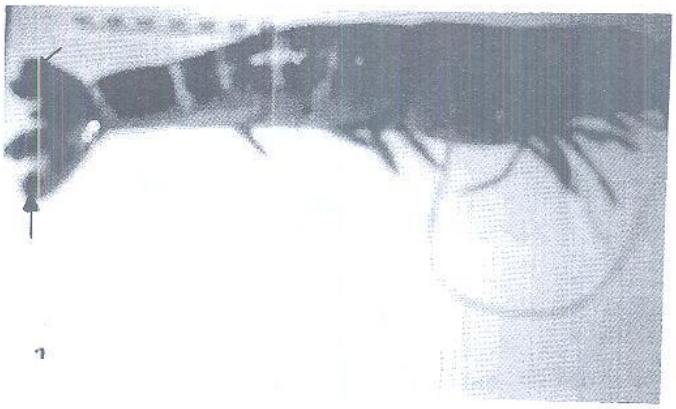
FIGURES LEGENDS

Fig. 1: Adult *Penaeus monodon* with normal black bands in the exoskeleton and black confined area in the tail (Arrow).

Fig. 2: *Penaeus monodon* gills showing the normal architecture. Haematoxylin and eosin. X 32.

Fig. 3: The gut epithelium of *Penaeus monodon* showing no evidence for the presence of intranuclear or intracytoplasmic inclusion bodies. Haematoxylin and eosin. X 32.

Fig. 4: Higher magnification of Fig. 3. Haematoxylin and eosin. X 320.



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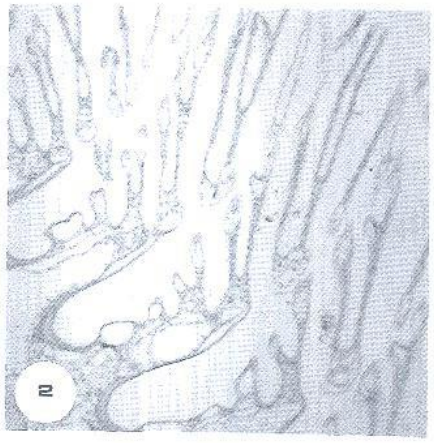

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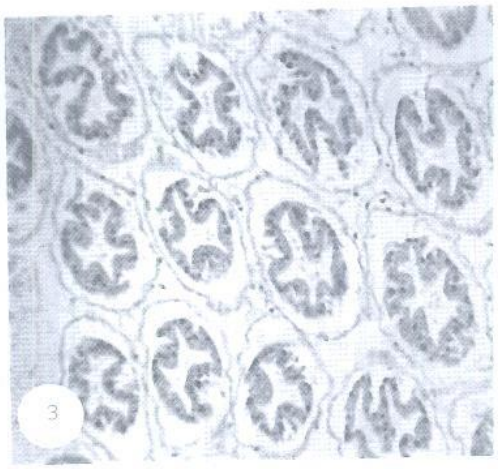

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