

## VIRULENCE OF *STREPTOCOCCUS FAECIUM* ISOLATED FROM FRESHWATER CRAYFISH, *PROCAMBARUS CLARKII*, TO NILE TILAPIA" *OREOCHROMIS NILOTICUS*

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### ABSTRACT

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The study was carried on 100 *Procambarus Clarkii* collected from the small tributaries of El-Ibrahemia canal, Assiut city. The clinical signs and postmortem lesions of red swamp crayfish were recorded. *P. clarkii* were subjected to bacteriological examinations. The isolated bacteria were identified as *Streptococcus faecium*. The percentage of infection was 36 % among the examined crayfish. Isolates were isolated from haemolymph, hepatopancrease and brain. Experimental infection was successful through intraperitoneal injection of Nile tilapia. The clinical signs were darkening in colour, haemorrhagic skin and fin. The fish showed gasping air, detachment of scales and skin erosion and ulcer. The common postmortem lesions were congestion of internal organs. The aforementioned bacterial isolate was successfully re-isolated from experimentally infected fish.

**Key words:** *Streptococcus faecium*, Nile tilapia, freshwater crayfish, hetero-species susceptibility.

### INTRODUCTION

Nile tilapia, *Oreochromis niloticus* is the most cultivated freshwater fish species due to its peculiar characteristics such as rusticity, resistance, productivity, adaptability to a wide range of environmental conditions and ability to grow and reproduce in captivity. As a result, these fishes have become excellent candidates for aquaculture, specially in tropical and subtropical regions and the number of farms has been increasing annually all over the world (Newaj- fyzul *et al.*, 2008).

Bacterial diseases induce colossal economic losses in both wild and cultured fish (Abowei and Briyai 2011). Streptococcal infection of fish is considered a re-emerging pathology affecting a variety of wild and cultured fish throughout the world (Romalde and Toranzo, 2002). Streptococcosis affects *Oreochromis spp.* as well as grey mullets (*Mugil cephalus* and *Liza ramada*) and silver carp, but not common carp (Hubbert, 1989). The major identifying feature of Streptococcus is that they are Gram-positive, aerobic and the bacterial cells appear more spherical or oval (coccus) in shape, grouped in pairs or short chains (Domenech *et al.*, 1996). Bacteriological data showed that streptococcosis is caused by various species; including *Streptococcus faecalis*, *Streptococcus faecium* & *Streptococcus agalaciae* and unclassified Streptococcus species (Kusuda and Salati, 1999).

*Procambarus clarkii* is considered the most important crayfish of the 400 species known in the world, and has been successfully introduced to all countries.

(Masser and Rouse 1997). In Egypt however, the present stock of crayfish was found to be promising although average annual yield under exploitation was estimated to be about 4.6 tons only (Emam and Khalil, 1995).

This study was done to investigate the existence of *streptococcus spp.* in red swamp crayfish in Assiut. As well as the pathogenicity of the strain recovered from red swamp crayfish to Nile tilapia.

### MATERIALS and METHODS

#### Red swamp crayfish:

A total number of 100 alive red swamp crayfish, *Procambarus clarkii*, were collected from the small tributaries of El-Ibrahemia canal, Assiut city. The body weight ranged from 10 to 20 g with total length of 9-14 cm. They were transported to the Aquatic Animals Diagnostic Laboratory, Faculty of Veterinary Medicine, Assiut University, where clinical and bacteriological examinations were conducted (Melba *et al.*, 2001).

#### Nile tilapia:

A total number of 50 apparently healthy *O. niloticus* with an average body weight of 50±10 gm were collected from a private fish farm. The collected fish were transported to the laboratory as soon as possible. The fish were kept in well aerator glass aquaria under the laboratory conditions for two weeks for acclimated. Random specimens from fish were taken for disease examination to ensure that fish were healthy.

#### **Bacteriological isolation and identification:**

Bacteria were isolated from hepatopancrease, brain and hemolymph of the collected crayfish. Samples of hepatopancrease, brain and hemolymph were bacteriologically examined by inoculation on brain heart infusion agar (BHI) (Lab-M) supplemented with 0.2 g /L sodium azide, blood agar (biolife) supplemented with 5% sterile sheep's blood and incubated at 37°C for 24-48 hours. Suspected colonies were purified and kept in refrigerator at 4°C for further biochemical identification. Pure cultures of the isolates were identified based on phenotypic and biochemical characters and enzyme activities as described (Hardie, 2001).

#### **Pathogenicity of *streptococcus faecium* to *O. niloticus*:**

##### **Bacterial strains:**

Bacterial isolates from hepatopancrease of red swamp crayfish were identified as *Streptococcus faecium* was passed three times through red swamp crayfish via haemolymph injection and used for determination of pathogenicity. *Streptococcus* isolate was grown on BHI agar and suspended in sterile distilled water to use for experimental infection.

##### **Bacterial counts and dilutions:**

A preliminary growth curve study was conducted to determine counts of colony forming units (CFU) of *Streptococcus faecium* in BHI broth at various growth phases using standard plate count method (ElKamel and Thune, 2003).

##### **Experimental challenge to *O. niloticus*:**

Recovered *streptococcus faecium* was tested for their pathogenicity in *O. niloticus* as means of evaluating their virulence profiles. A total number of 45 apparently healthy Nile tilapia were divided in to 3 groups, each of 15 fish, each group in three replicates. The fish in the first group were I/P injected with 0.33 ml of a bacterial suspension  $0.3 \times 10^8$  cfu/ml (saad El-deen and EL-Nisr 2011) (the experimental strain used for experimental inoculation was NaCl-tolerant), while fish in the second group injected with 0.33 ml of sterile saline and the third group remained un-injected. The whole experiment was repeated 3 times.

Experiments were monitored by continuous examination for clinical signs, lesions, mortalities and bacterial re-isolation throughout 15 days.

## **RESULT**

#### **1-Clinical and bacteriological examination of red swamp crayfish:**

Naturally infected *P. clarkii* with *Streptococcus faecium* revealed no gross or postmortem typical lesions of streptococcal infection, but 7 crayfish showed black spot on the back of the abdomen. Also, eleven crayfish showed necrosis at telsons and uropods. The remaining crayfish appeared to be clinically healthy.

Pure cultures of isolated bacteria were successfully done from haemolymph, brain and hepatopancrease of naturally infected *P. clarkii*. The isolates were identical biochemical characteristics suggesting that they were all the same or closely related species. Bacteriological examination of naturally infected red swamp crayfish resulted in the identification of 56 *streptococcus faecium* from 36 *P. clarkii* out of 100 examined crayfish with a percent of 36%. *Streptococcus faecium* was isolated from Haemolymph, haepatopancrease and brain of red swamp crayfish with percentage of 25, 57.14, and 17.86 % respectively.

*Streptococcus faecium* was recovered from *P. clarkii* was gram positive cocci, arranged in pairs and short chain, non motile. It grows at brain heart infusion agar with sodium azide giving pen head, dull creamy, circular, translucent, slightly convex colonies. On blood agar colonies were pale grey rounded colonies with zone of  $\alpha$ -haemolysis (greenish coloration). The biochemical patterns of the recovered isolates are summarized in Table 1.

#### **2-Experimental infection:**

The experimental inoculation of *St. faecium* in *O. niloticus* induced 33.33% mortalities within 15 days post injection. Four infected fish were darkening in colour. petechial haemorrhage in the mouth region and on the fins were noticed in 6 of fish (Fig. 1). Detachment of scales and skin erosions and ulcers were observed in 2 cases. The ulcers were usually shallow (Fig. 2). Three fish showed exophthalmia accompanied by haemorrhage of the eyes. Fin and tail rot were observed in 4 fish. Necropsy findings showed, liver was pale in colour in 6 experimental infected fish. Spleen, kidney and gills were congested in 7 fish. Gall bladder was distended with bile (fig. 3). Injected streptococcus species was successfully re-isolated from liver, kidney and spleen of the injected fishes. No mortality occurred in control fish and the bacterium was not isolated from any organ.

**Table 1:** Cultural and biochemical characters of the isolated bacteria (n=56):

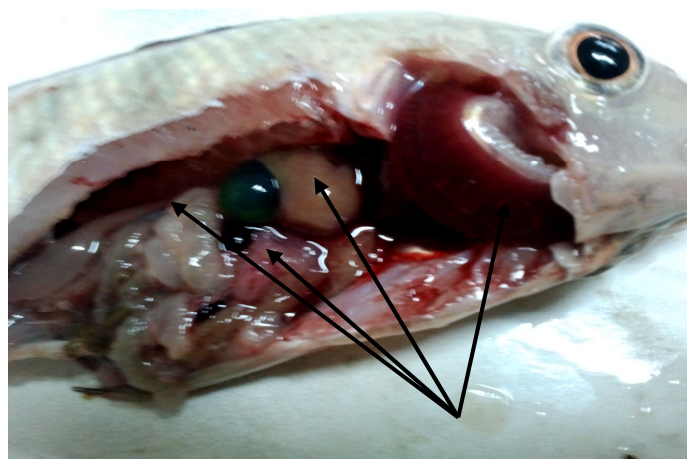
Biochemical test		Response
Gram-stain		G+ve Cocci in pair or short chain
Motility		-
Oxidase		-
Catalase		-
Growth on MacConkey agar		-
10°C		+
37°C		+
45°C		+
6.5% NaCl		48/ 56
Voges proskauer		+
Indole		-
Citrate utilization		-
TSI	H <sub>2</sub> S	-
	gas	A/A
	reaction	
Urease		-
Ornithine decarboxylase		+
Ornithine decarboxylase		+
Acid produce from:		
Glucose		+
Raffinose		+
Sucrose		+
Lactose		+
Arabinose		+
Mannitol		+
Sorbitol		-



**Fig. 1:** Nile tilapia, experimentally injected with *Streptococcus faecium* isolated from *P. clarkii* showing petechial haemorrhage in mouth region and fin



**Fig. 2:** Nile tilapia, experimentally injected with *Streptococcus faecium* isolated from *P. clarkii* showing detachment scales and skin ulceration



**Fig.3:** Nile tilapia, experimentally injected with *Streptococcus faecium* isolated from *P. clarkii* showing congested spleen, kidney, gills and pale liver

## DISCUSSION

*Streptococcus* species Causes a major disease problem in cultured tilapia and reported to cause high economic losses in both wild and cultured fish (Domench *et al.*, 1996).

Naturally infected *P. clarkii* with *Streptococcus faecium* did not show typical gross and postmortem lesions of *Streptococcus* infection. Edgerton *et al.* (2002) reported that Crayfish with asymptomatic bacteremia typically exhibit no gross or histopathological signs of disease. The condition is diagnosed by standard bacterial culture and identification techniques for aquatic bacteria.

The isolated bacteria from naturally infected red swamp crayfish were gram positive cocci, non-motile, catalase, oxidase and indole negative. From

the results of culture, morphological and biochemical tests, the isolated bacteria could be identified as *Streptococcus faecium* as guided by Hardie, (2001). Also the results agree with those reported by Pourgholam *et al.* (2010). Our result showed that *Streptococcus faecium* did not grew on MacConky agar. This result agrees with Ebtsam (2002) and disagrees with El-Bouhy (2002) who reported that *St. faecium* had variable growth on MacConky agar. The isolates had variable grew at 6.5% NaCl, these results agree with Torkey *et al.* (2006) and disagree with Ebtsam (2002). These differences may be due to strain difference.

The results revealed that the isolation rate of *S. faecium* from brain was 17.86%. This result may be explain why Clinical symptoms of streptococcosis are characteristically of the neuro-motor type; erratic swimming, swimming in circles and lack of control of

the pectoral fins, leaving fish in an erect position. (Abowei and Briyai, 2011).

Regarding to the clinical signs, it was revealed that fish infected with *Streptococcus faecium* showed darkening of body, detached scales and exophthalmia. These results are agreed with those recorded by Zeid (2004) who mentioned that some signs that may distinct for streptococcal disease, include erratic swimming, darkening of the fish, haemorrhage in the base of the fin and the operculum and pop- eye appearance. The common postmortem lesions observed in the diseased fish were acute septicemia and they revealed congestion of the internal organs. These lesions were in accordance with the finding of El-Bouhy (2002) and Safinaz (2006). This may be due to the haemolytic effect of the exotoxin produced by bacteria. During acute phase, streptococcus infection reach the blood stream of the fish and travel to all the internal organs. This will lead to septicemia and you can therefore expect to see the normal internal signs associated with septicemia, such as haemorrhages and inflammation in the eye, spleen and kidney (Rasheed and Plumb, 1984).

The successful induction of the streptococcosis experimentally indicate the potential pathogenicity of streptococcus faecium isolated from red swamp crayfish to Nile tilapia This induction was confirm by (Austin and McIntosh, 1991). This work raised some questions about the danger of *P. clarkii* to fish cultured and fish farm.

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### **ضراوة الميكروب السبحى من نوع استربتوكوكاس فيكم المعزول من استاكوزا المياه العذبة على اسماك البلطى النيلي**

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أجريت هذه الدراسة على عدد ١٠٠ من استاكوزا المياه العذبة الراكدة والتي تم تجميعها من فروع التربة الابراهيمية بمحافظة أسيوط. وقد تم تسجيل الاعراض الاكلينيكية والصفات التشريحية الموجودة فى الاستاكوزا المجمعة. وتم عزل وتصنيف البكتريا السبحية من نوع فيكم وكانت نسبة الاصابة ٣٦%. تم عمل العدوى التجريبية بنجاح باستخدام البكتيريا المعزولة من الاستاكوزا فى أسماك البلطى النيلي عن طريق الحقن بالتجويف البريتونى. وكانت الاعراض الاكلينيكية عبارة عن تحول لون الجلد للون الغامق ووجود قرح عليه مع تساقط القشور ووجود انزفة على الجلد والزعانف. وتمثلت الأعراض المرضية الداخلية فى احتقان بالأعضاء الداخلية. وقد تم بنجاح اعادة عزل الميكروب من الاسماك المصابة تجريبيا