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## VIBRIOSIS IN TILAPIA SPECIES AT ASSIUT GOVERNORATE (EGYPT)

(With 3 Tables & 3 Fig)

By

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### مرض الفيبريوزس / الضمة في أسماك البلطي بمحافظة أسيوط - مصر

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شملت هذه الدراسة على عدد مائه من أسماك البلطي النيلى المريضه وقد أوضحت الأعراض الاكلينيكيه عن ظهور بقع نزفيه على سطح الجلد وخاصة فى المنطقه السفلى للبطن ، عتامه مع جحوظ فى العينين ، ظهور انتفاخ أوديمى فى البطن والتهابات حول فتحة الشرج بالاضافه الى احتقان فى جميع الأعضاء الداخليه .  
 بالفحص الميكروبيولوجى تم عزل خمسة عشر عترة من الميكروب المسبب للمرض ومع دراسة الصفات المورفولوجيه والبيوكيميائيه للميكروب المعزول اتضح أن الميكروب يتبع ميكروب الفيبروكوليرا .

وكذلك تم دراسة ضراوة الميكروب باستخدام الحقن البريتونى والعضلى وجد أن هناك تطابق بين الأعراض الاكلينيكيه والصفه التشريحيه المعروفه عن المرض فى السمك المحقون بالعترات المعزوله وكذلك امكن عزل الميكروب من السمك النافق بعد الحقن .  
 وأوضحت الدراسة مدى حساسية الميكروب الى الاستربتوميسين والنيوميسين والخلتاميسين والاوكتترسيكلين وكذلك الكلورمفينكول . توصى الدراسه باستخدام احدى هذه المركبات مع تجنب العوامل المحفزه لانتشار المرض وذلك للتحكم فى انتشار هذا المرض فى المزارع السمكيه .

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## SUMMARY

Vibriosis in *Tilapia* spp. is not sufficiently documented especially under Egyptian conditions. One hundred diseased fishes were clinically and microbiologically investigated. The clinical signs of the disease included, peticheal haemorrhages on body surface especially on the abdomen, cloudiness of the eyes, exophthalmos, ascities, inflammation of the anal orifice and congestion of the internal organs. Fifteen isolates of *Vibrio* spp. were isolated from internal organs and identified by studying cultural, morphological and biochemical features. The isolates were closely related to *Vibrio cholerae*. Transmission experiments with these isolates using *Oreochromis niloticus* showed similar clinical and post-mortem pictures after intraperitoneal and intramuscular inoculation. Pathogenic *Vibrio* spp. were highly sensitive to streptomycin, neomycin, netilmicin, oxytetracycline and chloramphenicol. According to these findings one can safely advise that these drugs can be safely used to control *Vibrio* infection among fishes.

**Keywords:** Vibriosis, tilapia.

## INTRODUCTION

Vibriosis is the most important disease of marine, estuarine and freshwater fishes (POST, 1983). It causes serious losses among fish in both fresh and seawater aquaculture (EQUISA, 1969; CISAR and FRYER, 1969; KITAO et al., 1983 and AUSTIN and AUSTIN, 1987).

*Vibrio anguillarum* is the most commonly identified aetiologic agent in different species of fresh-saltwater fishes (ANDERSON and CONROY, 1970; BULLOCK and CONROY, 1971; COLWELL and GRIMES, 1984 and FRYER and ROHOVEC, 1984). Other strains have been reported which include *Vibrio cholerae*, *V. alginolyticus*, *V. Carcharie*, *V. damsela*, *V. ordalii* and *V. vulnificus* as pathogens of fishes (AUSTIN & AUSTIN, 1987).

A scanty literature could be traced concerning vibriosis in Egypt. Clinical signs of the disease is not sufficiently documented or described. The aim of this investigation is to give a rather detailed picture on isolation, identification, clinical signs and postmortem findings of *Vibrio* infection in *Tilapia* spp. Experimental induction of the disease with trials

to determine chemotherapy drugs using antibiotics sensitivity test will be also included.

## MATERIAL and METHODS

### Case history:

One hundred fishes (*Tilapia spp.*) were brought to the department of animal medicine which were previously collected from different localities at Assiut Governorate. Fishes were delivered to the laboratory in plastic bags with enough water.

Fishes were kept in clean aquaria containing dechlorinated tap water and provided with electric aerators. After 48 hours, fishes became dark in colour, haemorrhagic patches appeared over the skin and some fishes died without appearance of clinical signs. Twenty out of the total fish were put in a separate aquaria to study the progress of the disease. The rest of fishes were subjected to the following examinations.

#### 1- Clinically and post-mortem examination:

Diseased fishes were examined for clinical and post-mortem findings according to the methods described by AUSTIN and AUSTIN, (1987).

#### 2- Bacteriological examination:

Aseptic samples of liver, kidney and spleen from diseased fishes were streaked on brain heart infusion agar and sheep blood agar (5%). The plates were incubated at  $23 \pm 1^\circ \text{C}$  for 48 hours. Isolated colonies were examined for cell morphology and motility by Gram stain and hanging drop method respectively. Biochemical identification was carried out according to the methods of COWAN and STEEL, 1974 and BAUMANN, FURNISS and LEE, 1984. The determination of biochemical tests of *Vibrio* were made by methods of FARID & LARSEN, (1980) & WEST, LEE & BRYANT, (1983).

#### 3- Parasitological examination:

Parasitological studies included naked eye examination, direct smear from gills lamellae and skin scrapings following the methods of KABATA (1985).

#### Pathogenicity test:

A total of 30 apparently healthy tilapia (*Oreochromis niloticus*) with an average body weight  $50 \pm 5$  gr. were collected from River Nile. Fishes were kept one week in a clean aquaria containing dechlorinated tap water before starting the experiment. The aquaria were provided with electric aerators and thermostatically controlled to adjust water temperature at  $22 \pm 1^\circ \text{C}$ . Fishes were divided equally into three groups. First and second groups were inoculated respectively (I.P and I.M.

routes) which 1.0ml of 24 hours broth culture containing  $10^7$  ml viable *Vibrio* (McCARTHY et al., 1974). The third group was treated with sterile broth as control. Before inoculation. anaesthesia of the fishes with MS 222 (Tricaine methane sulphonate) was done according to Roberts, (1989). All experimentally infected fishes were observed daily for any clinical signs and mortality. Post-mortem examination was carried out on dead fishes to detect the gross lesions. Reisolation of *Vibrio* was attempted from died fishes to complete Koch's postulates.

**Drug sensitivity test:**

Sensitivity test for isolated *Vibrio* were applied using different types of antibiotics and sulpha discs according to AMTSBERG et al., 1973. The sensitivity to the vibriostatic agent O/129 at concentration of 150,50 and 10 ug/ml was determined by the method described by LEE et al., (1981). The interpretation of inhibition zone was estimated according to the limits given by FINEGOLD and MARTIN, 1982.

**RESULTS**

**Clinical signs and post-mortem findings:**

The clinical signs and post-mortem findings of Vibriosis in *Tilapia spp.* were characterized by dark colouration of the skin, petechiae on body surface especially on the belly. Whitish spots surrounded with erythema at the side of the body, bloody congestion at the base of fins with finrot especially tail fin, cloudiness of the eyes and complete opacity with exophthalmos. Gills were congested, swollen and in some fishes gills were anaemic. Anal orifice was inflamed and congested, ascities was observed in some fishes. Internal organs were congested and the intestinal tract filled with yellowish exudate especially the lower part.

**Parasitological examination:**

The fishes were lightly infested with *Ichthyophthirius multifiliis* and *Dactylogyrus spp.*

**Bacteriological examination:**

Fifteen bacterial isolates were recovered from liver, kidney and spleen of diseased fishes. According to morphological cultural and biochemical features (Tab. 1), The isolates were identified as *Vibrio spp.* and were closely related to *Vibrio cholerae*.

**Pathogenicity test:**

The results of experimental inoculation test indicated that, the isolated *Vibrio* was pathogenic to tilapia. The mortality rates, time of death/ day postinoculation and clinical signs were described in table (2).

The inoculated bacteria were reisolated from internal organs of dead fishes. No any alternations were observed in control fishes.

**Drugs sensitivity test:**

The isolated strains of *Vibrio* were highly sensitive to streptomycin, neomycin, netilmicin, oxytetracycline and chloramphenicol as shown in table (3).

**DISCUSSION**

Vibrionaceae is a heterotrophic group of organisms with the main habitat in water. *Vibrio cholerae* non-01 was first reported as the causative agent of vibriosis in the fresh water fish ayu in Japan by MUROGA *et al.* (1979). Recently, the *Vibrio cholerae* non-01 was isolated from diseased ayu fish collected from rivers by KIIYUKIA, *et al.* (1992). In general the prevalence of vibrionaceae in environment are influenced by temperature (thermal pollution), presence of organic compounds (organic pollution) and crowding of water living animals (aquaculture) LARSEN and FARID (1980).

*Tilapia* species are one of the most important species for Egyptian fisheries and constitutes about 0% of the catch of the Nile associated lakes in Egypt. BALARIN (199) and ISHAK (1980).

The clinical signs and post-mortem findings of vibriosis in *Tilapia* species were similar as described during *Vibrio* infection in different species of fishes by McCARTHY (1976). RICHAROS (1980). FRYER and ROHOVEC (1984). MUROGA, *et al.* (1984). RASHEED (1989) and BADRAN, and EISSA (1991).

The clinical signs, gross lesions and reisolatio of *Vibrio* from dead fishes during pathogenicity test cleared that, the cause of the disease in the present study can be attributed to vibrio infection. Susceptibility to this type of infection is an additional support to our findings.

Transportation, improper handling of fishes, high temperature and parasitic infestation are stress factors, which may enhance the infection and lead to appearance of the clinical picture of vibriosis on *Tilapia*. Susceptibility of tilapia to bacterial infections seems to increase under above mentioned stress conditions as reported by ROBERTS and SHEPHERD (1974) and BEJERANO (1985).

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Occurrence of relatively small straight or curved gram negative bacteria from the internal organs of infected fish with described signs of the disease might be indicative for the implication of a member belonging to family vibrionaceae. This could be confirmed by culturing on blood agar plates, Oxidase positive, o/F, decarboxylase tests, esculin, urea degradation, Bgalactosidase, voges-proskauer, sucrose, mannitol fermentation, in addition to the sensitivity to vibriostatic agent O/129, confirmed that the isolates closely related to *Vibrio cholerae*. These findings coincided with the work of MUROGA, et al. 1979 and KIIYUKIA, et al., 1992.

The result of the drugs sensitivity proved that, the isolated strains of *Vibrio* were highly sensitive to streptomycin neomycin, netilmicin. oxytetracycline and chloramphenicol. These results are more or less agree with that reported by McCARTHY (1976). Analysis of these findings one can safely advice that those drugs can be safely used to control *Vibrio* infection among fishes.

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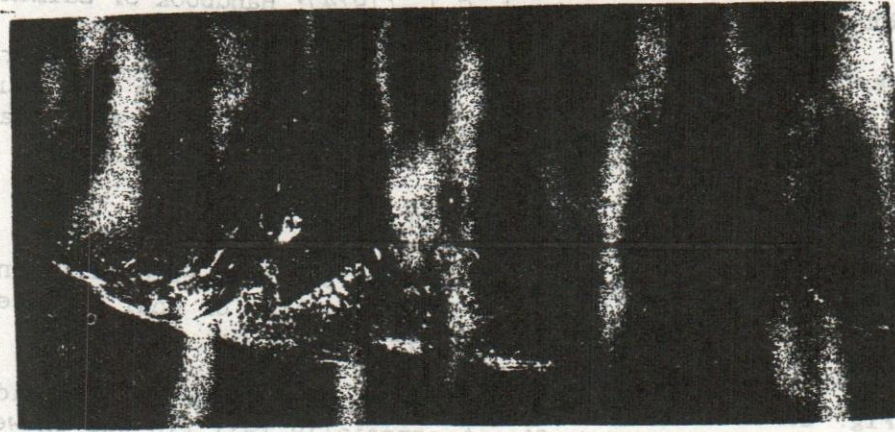
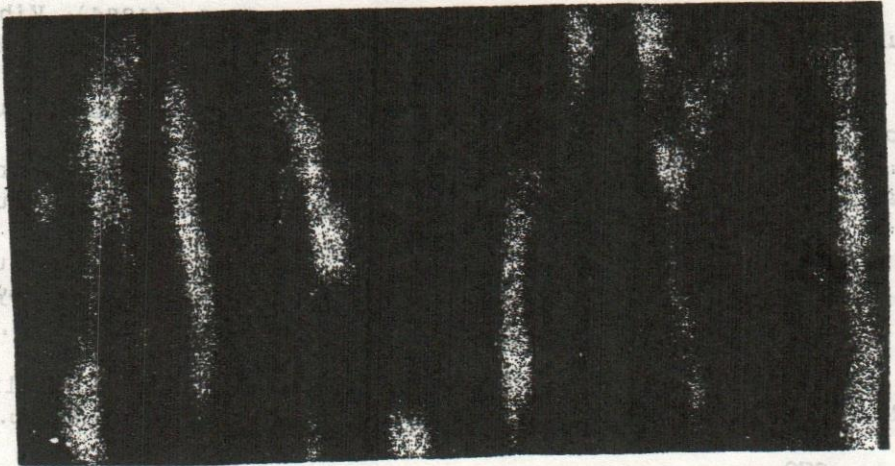
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### LEGENDS OF FIGURES

- Fig. 1: Petechiae on the surface of the body especially on the belly, inflammation and congestion of anal orifice was showed in *Oreochromis niloticus* infected with vibriosis.
- Fig. 2: Whiteish spot surrounded with erythema at the side of the body with finrot especially tail fin was showed in *Oreochromis niloticus* infected with vibriosis.
- Fig. 3: Complete opacity with exophthalmos was showed in *Oreochromis niloticus* infected with vibriosis.





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Table (1): Morphological and biochemical characteristics of *Vibrio* spp. isolated from diseased fish

| Tests                   | Results | Tests                       | Results |
|-------------------------|---------|-----------------------------|---------|
| Gram-stain              | -       | H <sub>2</sub> S production | -       |
| Motility                | +       | Methyl red                  | +       |
| Haemolysis              | +       | Voges-proskauer reaction    | +       |
| Cytochrome oxidase      | +       | Gas from glucose            | -       |
| Catalase                | +       | Production of acid from     |         |
| O/F-test                | -/+     | Glucose                     | +       |
| Hydrolysis of Aesculin  | -       | Salicin                     | -       |
| Starch hydrolysis       | +       | Trehalose                   | +       |
| Gelatin Liquefaction    | +       | Arabinose                   | -       |
| Arginine dihydrolyase   | -       | Lactose                     | -       |
| Lysine decarboxylation  | +       | Inositol                    | -       |
| Ornithine decarboxylase | +       |                             |         |
| Urease                  | -       | Raffinose                   | -       |
| Indole                  | +       | Mannitol                    | +       |
| Growth in % NaCl        |         | Sucrose                     | +       |
|                         |         | Sorbitol                    | -       |
|                         |         | Sensitivity to:             |         |
| 0                       | +       | 0/129 (10 U $\mu$ /ml)      | +       |
| 6                       | +       | 0/129 (50 U $\mu$ /ml)      | +       |
| 8                       | -       | 0/129 (150 U $\mu$ /ml)     | +       |
| 10                      | -       |                             |         |
| ONPG*                   | +       |                             |         |

- + character present for 100% of isolates
- character absent for at least 80% of isolates
- \* ONPG. O-nitrophenyl-B-D-galactopyranoside.

Table (2): Pathogenicity of isolated *Vibrio spp.* in inoculated fish

| Fish groups    | No. of inoculated fish | Routes of inoculation | Mortality rate | Time of death 1 day post inoculation | Clinical signs and post-mortem findings  |
|----------------|------------------------|-----------------------|----------------|--------------------------------------|--|
| 1              | 10                     | I/P                   | 10             | 2 - 4                                | Petichae over the body surface especially on the belly. Redening and inflammation of the anal orifice.             |
| 2              | 10                     | I/M                   | 10             | 3 - 6                                | Paleness of the liver swelling of the kidney and spleen. inflammation of the intestinal tract coldness of the eye. |
| 3<br>(Control) | 5                      | I/P                   | -              | -                                    | No any alterations   |
|                | 5                      | I/M                   | -              | -                                    |  |

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**Table (3): Sensitivity of *Vibrio spp.* to some antimicrobial drugs**

| Drugs        | Results | Drugs                         | Results |
|--------------|---------|-------------------------------|---------|
| Ampicilin    | 25 Ug   | Penicillin                    | 10 i.u  |
| Erythromycin | 15 Ug   | Oxytetracycline               | 10 Ug   |
| Streptomycin | 10 Ug   | Chloramphenicol               | 30 Ug   |
| Neomycin     | 30 Ug   | Nitrofurantoin                | R       |
| Netilmicin   | 10 Ug   | Slufamethoxazole/Trimethoprim | R       |

R = Resistant  
S = Sensitive