

## STUDIES ON SOME PREVAILING BACTERIAL FISH DISEASES AMONG CULTURED OREOCHROMIS NILOTICUS

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### ABSTRACT

The present study was planned to evaluate the seasonal prevalence of bacterial diseases among cultured Nile Tilapia (*Oreochromis niloticus*) and Mirror carp. Collection of 450 fish was carried in different seasons. Recording the clinical signs and post-mortem lesions followed by isolation and identification of the isolated organisms was applied in addition to cultural and biochemical reactions of the organisms. It was found that the seasonal prevalence of bacterial diseases in *Oreochromis niloticus* in spring, summer, autumn and in winter was (73 & 82 & 58 and 34%) respectively. Besides, histopathological alterations were studied.

### INTRODUCTION

Fish diseases constitute one of the most important problems and challenges confronting fish culturists. The disease occurrence is a complex interaction between the host species, disease agent and the environment (Sniaczko, 1974).

With increasing intensification, the incidence of diseases is also expected to increase. This explains why intensive tilapia production is susceptible to infectious agents including bacterial, fungal and parasitic agents (Woo, 2006).

Bacterial pathogens are the causative agents of most serious disease problems in both wild and cultured fish causing massive mortalities and severe economic losses (Austin and Austin, 1993).

It is important to point out that common diseases in freshwater aquaculture are due to

different bacterial organisms namely Aeromonads and Streptococci (Alicia et al., 2005).

Motile Aeromonads are considered to be a significant pathogen of human beings associated with gastroenteritis, septicemia and isolated from children with diarrhea (cholera like disease) (Albert and John, 2000 and Khalifa et al., 2008).

Streptococcus species is an important bacterial pathogen causing serious damage in fish culture industry. Recently, it has been associated with human infection. (Dodson et al., 1999 and Shuto et al., 2006).

The present study was carried out to through out the light on the following.

Clinical signs and postmortem lesions of naturally infected fish, isolation and identification of bacterial agents associated with high mortalities in cultured *O. niloticus*, seasonal

prevalence of the prevailing bacteria among infected fish and studies on histopathological alterations in gills, liver, kidneys and spleen of infected fish.

## **MATERIAL AND METHODS**

### **Materials:**

#### **(1) Fish (Naturally infected fish)**

A total number of 450 fish were collected alive from freshwater aquaculture from a semi-intensive earthen private fish farm at Dakahlia governorate within the period from April 2009 to March 2010 suffering from various mortalities. The collected fish specimens represent 450 *Oreochromis niloticus* with body weight 80-150 g and length 8-18cm.

The fish were transported alive or freshly dead as soon as possible to the laboratory of Fish Disease and Management, Faculty of Veterinary Medicine, Mansoura University. The live fish were placed in large tanks containing water from the same farm and supplied with battery aeration then kept in glass aquaria provided with aerated dechlorinated tap water and water temperature at 25° C ±1.

#### **(2) Aquaria**

Fully prepared glass aquaria (80 X 50 X 40 cm) dimensions and capacity of 120 liters water, were used during the experiments. These tanks were supplied with chlorine free tap water (Innes, 1966) and electric air pumping compressors for supplying water with oxygen. Also Electric filter for filtration of water.

#### **(3) Diet**

Fish were fed in the rate 3% of body weight twice daily on artificial commercial pellets ration containing 25 % protein.

### **[1] Clinical examination**

Gross and Postmortem examination

The clinical picture was performed externally and internally according to (Austin and Austin, 1987).

### **2] Bacteriological examination**

#### **a) Bacterial isolation**

Naturally infected fish were aseptically opened according to the methods described by Stockopf (1993). Samples for bacteriological examination was taken using sterilized platinum loop from liver, kidneys, spleen and intestine as well as skin lesions and ulcers also from bloody ascetic fluid. Samples were inoculated in nutrient agar, tryptone soya agar, brain heart infusion agar and incubated at 25°c for 24-48 hrs. The colonies were picked up and streaked on semi-solid agar for further purification and further identification according to Pikul et al., (2009).

#### **b) Identification of the isolated bacteria**

##### **(1) Microscopical examination**

Gram stain was used for staining suspected colonies and examined microscopically for morphology and staining affinity according to Collins et al., (1991).

##### **(2) Primary identification of isolated bacteria**

The obtained colonies were cultured into tryptic soya broth, incubated at 27°c for 18 - 48 hrs, then streaked on different laboratory media. Pseudomonas and Aeromonas Selective Agar base, R-S media, Selective Streptococcus agar. Another colonies were inoculated onto nutrient agar slant for further identification, colony and growth appearance, morpho-

logical characters and motility test using semi-solid agar (Berthe et al., 1995 and Pikul et al., 2009).

### (3) Biochemical reactions

They were carried out according to Collins et al., (1991)

## RESULTS

### 1- Clinical picture

#### a) Clinical signs

The clinical examination of naturally infected *O. niloticus* included skin, gills, fins, eyes and other external signs were recorded. Generally, the naturally infected fish showed loss of appetite, emaciation, stunted growth, sluggish movement, and excessive mucus secretion. The gross external signs showed dark discoloration, exophthalmia and abdominal distension. Skin erosion and loss of scales with hemorrhagic patches (Fig. 1). Finally they lost their normal behavior and died.

#### b) Postmortem findings

The internal changes observed in *O. niloticus* showed congested liver and kidneys with distended gall bladder (Fig. 2).

### 2- Histopathological findings

The histopathological findings of the tissue specimens collected from liver and kidneys of naturally infected *O. niloticus* revealed that the liver of naturally infected *O. niloticus* showed congestion of portal vein, besides hydropic degeneration of hepatocytes Fig (3) and the kidneys showing chronic nephritis with marked lymphohistiocytic exudates Fig.(4).

## DISCUSSION

The occurrence of septicemic bacterial dis-

eases in a fish farm causes high losses due to heavy mortalities and difficult eradication of disease. Septicemic bacterial diseases are considered to be an important problem which cause not only high economic losses but also public health hazards.

The present study was carried out on naturally infected *Oreochromis niloticus* suffering from septicemic bacterial diseases

The clinical signs appeared in the collected *Oreochromis niloticus* were in the form of loss of appetite, emaciation, stunted growth, sluggish movement, and excessive mucus secretion with dark discoloration, exophthalmia, abdominal distension. Skin erosion and loss of scales. Finally they lost their normal behavior and died. This agreed with Badran and Elasa (1991), Amin (1993) and Austin and Austin (2007).

The postmortem examination revealed congested liver and kidney and distended gall bladder. This agreed with Abou El-Atta et al. (2008) and Austin and Austin (2007).

These signs and lesions may be attributed to septicemia resulting from bacterial toxins.

The isolated bacteria were *Aeromonas hydrophila* which appeared white yellow or creamy color on R-S media, circular convex with entire edges 2 mm in diameter. The isolated bacteria were Gram negative motile short bacilli. Also *Streptococcus* sp. was isolated which characterized by creamy or yellowish white color, circular very small pen headed colony to 0.1mm. It was Gram cocci arranged in pairs and short chains. The bac-

teria appeared non motile, glistening with entire raised edges. In addition to *Pseudomonas* sp. Which was in young culture after 24 hrs at 25°C circular convex with entire edges, glistening and creamy color. The colonies were gram negative short motile bacilli. In old culture the colonies appeared dark green in color

Concerning the seasonal prevalence, it was found that the seasonal prevalence of bacterial diseases in *Oreochromis niloticus* was highest in summer was (82%) followed by in spring (73%), in autumn was (58%) and in winter (34%). In Mirror carp the prevalence of bacteria in spring 80%, in summer 95%, in

autumn 70% and 80% in winter

Concerning the histopathological findings of the tissue specimens, the results revealed that the gills of naturally infected *Oreochromis niloticus* showing hyperplasia of primary lamellae besides congestion of blood vessels. Also the liver of naturally infected *Oreochromis niloticus* showing congestion of portal vein, besides hydropic degeneration of hepatocytes.

It can be concluded that *Aeromonas hydrophila*, *Streptococcus* sp. And *Pseudomonas* were prevailing in cultured fishes especially in summer.

**Table (1): Seasonal prevalence of bacteria isolated from naturally infected *O. niloticus***

Season	Number of collected fish	Number of infected fish	%
Spring	100	73	73
Summer	150	123	82
Autumn	100	58	58
Winter	100	34	34

**Table (2): Seasonal prevalence of bacterial species isolated from naturally infected *O. niloticus***

Season	Number of examined fish	Aeromonas		Streptococcus sp.		Pseudomonas	
		No.	%	No.	%	No.	%
Spring	100	39	39	44	44	15	15
Summer	150	69	48	54	36	24	16
Autumn	100	32	32	12	12	45	45
Winter	100	19	19	6	6	26	26

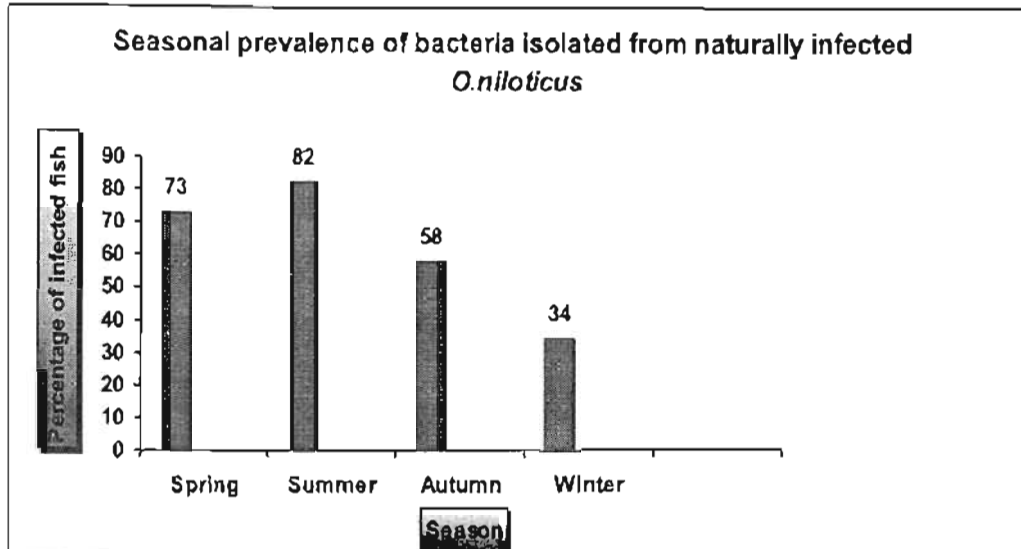


Fig. (5) Seasonal prevalence of isolated bacteria from naturally infected *O. niloticus*

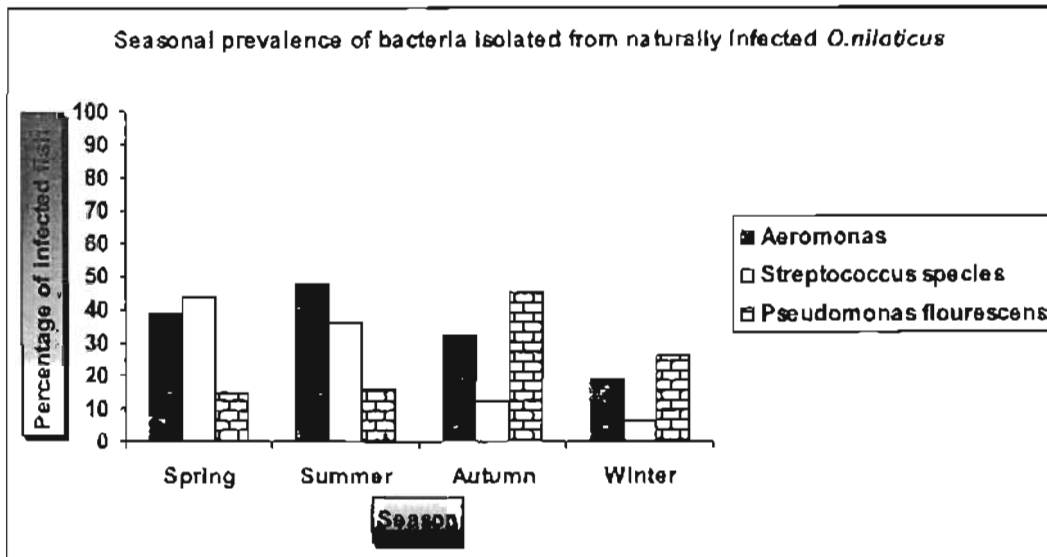


Fig. (6) Seasonal prevalence of isolated bacterial species from naturally infected *O. niloticus*

Table (6): Cultural and biochemical reactions of the isolated *Aeromonas hydrophila*, *Streptococcus* and *Pseudomonas* sp.

Test	<i>Aeromonas hydrophila</i>	<i>Streptococcus</i> species	<i>Pseudomonas</i> species
Gram stain	- ve	+ ve	- ve
Shape	Short rods,	Cocci in pairs or short , chain	rods
Motility	+	- ve	+
R-S media	Yellow, convex	- ve	Dark green colonies
Nutrient agar	Convex and smooth	White flat and round	Yellow green and convex
Pigmentation	- ve	- ve	+
Cytochrome oxidase	+	- ve	+
Indol	+	- ve	- ve
Voges -Proskauer	:	+	- ve
H <sub>2</sub> S production	- ve	- ve	- ve
Methyl red	+	- ve	+
Gelatin liquefaction	+	- ve	+
Starch hydrolysis	+		
Sucrose fermentation	Acid and gas	- ve	+
Lactose fermentation	- ve	-ve	- ve
Glucose fermentation	Acid and gas		Acid production
Maltose fermentation	Acid and gas	±	
Mannitol fermentation	Acid and gas		+
Catalase	+	-ve	+
Oxidase		+	
TSA & BHIA	+	+	+
Citrate utilization	+	-ve	- ve
Growth 5% on Na Cl	-ve	+	+

TSA= Tryptone Soya Agar

BHIA= Brain Heart Infusion Agar

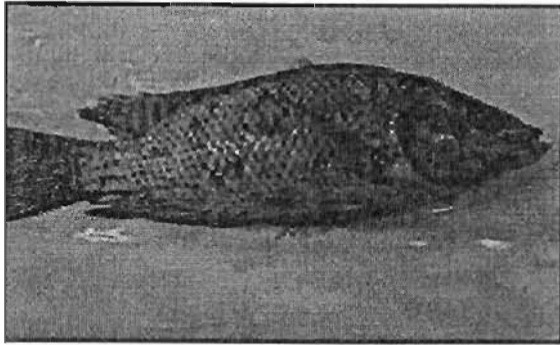


Fig. (1): Naturally infected *O. niloticus* showing skin erosion and loss of scales with hemorrhagic patches

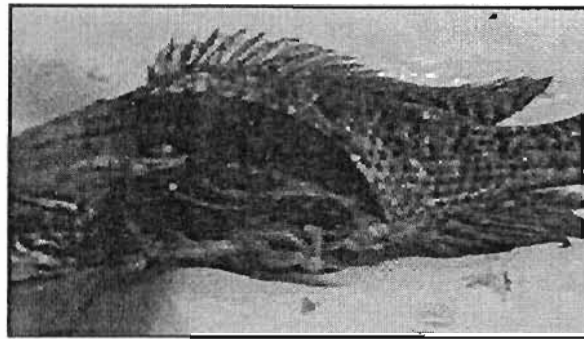


Fig. (2): Naturally infected *O. niloticus* showing congested liver and kidney and distended gall bladder

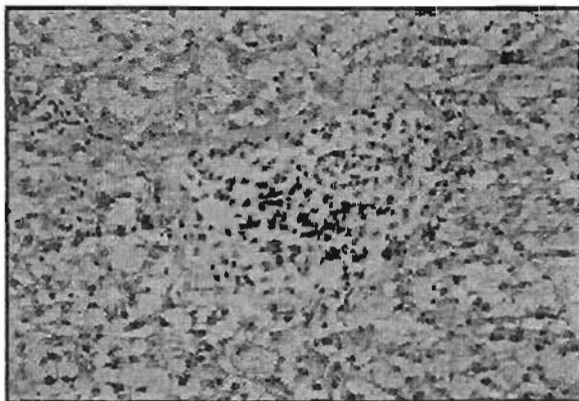


Fig. (3): Liver of naturally infected *O. niloticus* showing congestion of portal vein, besides hydropic degeneration of hepatocytes.

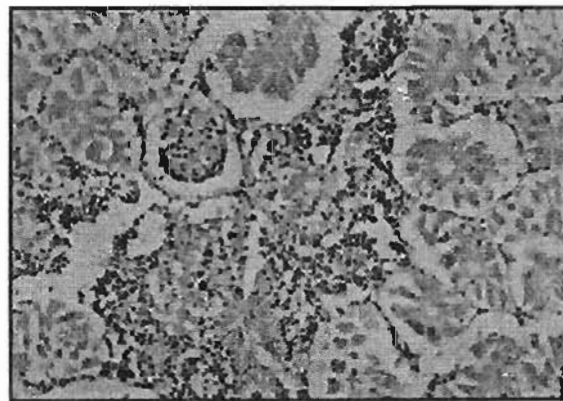


Fig. (4): kidney of naturally infected *O. niloticus* showing chronic nephritis with marked lymphohistiocytic exudates.

## REFERENCES

- Abou El-Atta, M. E. I. and El. Tantawy, M. M. (2008)** : Bacterial causes of skin affection in tilapia nilotica (*Oreochromis niloticus*) with special references to its control. 8th International symposium on Tilapia in Aquaculture 2008.
- Albert, M. and John, M. (2000)** : Prevalence of enterotoxin genes in *Aeromonas* Spp. Isolated from children with diarrhea, healthy controls, and the environment. *Journal of Clinical Microbiology*. 38(10), 3785-3790.
- Alicia, E. T.; Beatriz, M. and Jesus, L. R. (2005)** : A review of the main bacterial fish disease in mariculture systems. 246,1-4, 37-61.
- Amlacker, E. (1970)** : Text book of fish diseases. T.F.H. Publ., Neatune city, New Jersey, 117-135.
- Amin, N. E. (1993)** : Studies on *Aeromonas hydrophila* isolated from fish reared in reused waste water in Egypt. *J. Egypt. Vet. Med. Ass.*,53 (182) :129-132.
- Austin, B. and Austin, D. A. (2007)** : Bacterial Fish Pathogens: Diseases in farmed and wild fish. 4<sup>th</sup> ed. Praxis Publishing Ltd, Chichester, UK, Printed in Germany.
- Austin, B. and Austin, D. A. (1993)** : Bacterial fish pathogens: Diseases in farmed and wild fish. Chichester, UK: Ellis Horwood. Second edition.
- Austain, B. and Austin, D. A. (1987)** : Bacterial fish pathogens Disease in farmed and wild fish. Ellis Horwood Ltd., chinchter, England
- Badran, A. F. and Elssa, I. A. (1991)** : Studies on bacterial diseases among cultured fresh ware fish (*Oreochromis niloticus*) in relation to the incidence of bacterial pathogens at Ismailia Governorate J. Egypt. Vet. Med.Assoc., 51,(4) : 937-847.
- Carter, G. R. and Colee, J. R. (1990)**: Diagnostic procedures in fish, Newjersy city, NJ, TFH publications.
- Collin, C. H.; Lyne, P. M. and Grange, J. M. (1991)** : Collins and lyne microbiology method. 6<sup>th</sup> Ed. Butter worth- Helnemann, Oxford, London.93.
- Cruickshank, R.; Duguid, J. P.; Marimion, B. P. and Swain, R. H. (1975)** : Medical Microbiology, 12<sup>th</sup> ED.Vol. 11, Churchill living stone. Edinburgh, London and Newyork.
- Dodson, S. V.; Maurer, J. J. and shotts, E. B. (1999)** : Biochemical and molecular typing of streptococcus iniae from fish and human cases. *Journal of Fish Diseases*. 22 (5-6), 331-336.
- Innes, W. T. (1966)** : Exotic aquarium fishes. 19<sup>th</sup> Ed aquariumincorporated. New Jersey. 12-25.
- Isenberg, H. D. (2004)** : Clinical Microbiological procedures handbook vol 1,2, and 3. 2<sup>nd</sup> Ed. American Society for microbiology. Washington, D.C.
- Khalifa, S. G.; Salwa, F. A.; Rania, A. E. K. tef A. G. and John, K. (2008)** : *Aeromonas*-Associated infections in Developing countries. *J. Infect. Developing Countries*, 2(2). 81-98.
- Pikal, J.; Stefan, R.; Lennart, E.; Hatpeng, L. and Kenneth, S. (2009)** : A highly virulent pathogen, *Aeromonas hydrophila*, from the fresh water cray fish *pacifastacus leniusculus*. *Journal of Invertebrate pathology*. 101(1), 56-66.
- Roberts, R. J. (2003)** : Fish pathology. 3rded. W.B. Saunders, Philadelphia P.A.
- Shotts, E. B. and Rimler, R. (1973)** : Medium for the isolation of *Aeromonas hydrophi-*



la college of veterinary Medecine, university of Georgla. Appl. Microbial. October, 26 (4), 550-553.

**Shutou, K.; Kanai, K. and Yoshikoahi, K. (2006)** : Virulence attention of capsular polysaccharide-deleted mutants of streptococcus Iniae in Japanese flounder paralichthy alivaceus. Faculty of Fisheries, Japan, Nagasaki university, 8520-8521.

**Snieszko, S. F. (1974)** : The effect of environmental stress on outbreak of infectious diseases of fish. J. Fish Biol., 6 : 197-208.

**Stoskopf, M. K. (1993)** : Bacterial diseases of goldfish, Koi and carp. Fish Medecine.

**Woo, P. T. (2006)** : Fish diseases and disorders. ABI publish, London, U.K.

## الملخص العربى

### دراسات على بعض الأمراض البكتيرية الشائعة بين أسماك البلطى النيلية

مدحت سمير شقوير فيولا حسن زكى

قسم الأمراض الباطنة والمعدية والأسماك

كلية الطب البيطرى - جامعة المنصورة

تم إجراء هذه الدراسة على ٤٥٠ سمكة من أسماك البلطى والتي تم تجميعها من إحدى المزارع بمحافظة الدقهلية فى فصول السنة الأربعة، بعد إجراء الفحص الظاهرى على هذه الأسماك تبين أن بعض الحالات تعاني من تأخر النمو ووجود بقع نزيفية على الجسم والزعانف وعنامة فى العينين.

عند إجراء الصفة التشريحية تبين وجود إحتقان وزيادة فى حجم الكبد وتضخم فى الطحال والكلى فى بعض الحالات يوجد سائل أصفر مدمم فى التجويف البطنى.

تم عزل وتصنيف البكتيريا المسببة للتسمم الموى فى أسماك البلطى والمبروك اللامع المصابة وإجراء الاختبارات البيوكيميائية بعد أخذ عينات من الحياشيم الكبد والكلى والطحال وتم التعرف على ميكروبات الايرومونات هيدروفيللا والاستريبتوكوكس والسودوموناس.

وقد سجلت الدراسة أن الإصابة الكلية فى أسماك البلطى كانت ٦٤٪ بينما كانت ٧٦٪ فى أسماك المبروك، لوحظ أن الإصابة بالايرومونات كانت فى أعلى معدلاته فى فصل الصيف يليه الربيع ثم الخريف وأخيراً الشتاء.