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ALARMING THE CRITICAL TIMES FOR OCCURRENCE OF IMPORTANT BACTERIAL PROBLEMS IN CULTURED *OREOCHROMIS NILOTICUS* UNDER SEMI INTENSIVE SYSTEM IN SHARKIA PROVINCE

(With 8 Tables and 8 Figures)

By

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التحذير للأوقات الحرجة للمشاكل البكتيرية الهامة فى مزارع البلطى النيلى شبه المكثف بالشرقية

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أجريت هذه الدراسة علي عدد 1000 سمكة من نوع البلطي النيلي المستزرع في جمهورية مصر العربيه والتي تم تجميعها من أحواض سمكية في منطقة الشرقية (مركز بحوث الثروة السمكية بالعباسة) وبعض الأحواض السمكية الخاصة في نفس المنطقة والتى تعيش تحت نظم الأستزراع شبه المكثف. أظهرت هذه الدراسة أن العلامات الإكلينيكية الظاهرة في الأسماك المصابة كانت كالتالى: وجود أنزفة متفرقة على كامل جسم السمكة والتى تتمركز في منطقة الفم والخياشيم وحول فتحة الشرج وجذور الزعانف. بالإضافة إلي تقرحات ودكانة لون الجلد ووجود مناطق خاليه من القشور وتآكل الزعانف خاصة الذيلية منها، تمدد منطقة البطن (إستسقاء بطني)، جحوظ العين وعتمتها. أما الصفة التشريحية فبينت وجود إحمرار ونقط نزفية في معظم الأعظاء الداخلية (الكبد، الطحال، الأمعاء، والكلي) ووجود نقط تنكرز وتحلل في بعض هذه الأعظاء ، بالإضافة إلي زيادة في حجم الحويصلة المرارية ، شحوب أو إحمرار في الخياشيم مع زيادة الإفرازات المخاطية. أوضحت الدراسة إصابة عدد (420) من الأسماك بالبكتيريا وقد شملت عزل والتعرف علي كل من : إيروموناس هيدروفيلا: وجد أن معدل الإصابة بهذا الميكروب كان (56.67%) وقد كانت أعلي إصابة بكتيربة سجلت وأعلى معدل أصابة كان فى فصل الشتاء (39.9%). وبالنسبة للسيدوموناس فلوريسنس وجد أن معدل الإصابة كانت (36.91%) وكانت ثاني أعلي إصابة بالنسبة لباقي البيكتيريا وأعلى معدل أصابة كان فى فصل الخريف (48.4%) أما الستريبتوكوكس فوجد أن معدل الإصابة كان (19.77%) وأعلى معدل أصابة كان فى فصل الربيع (48.4%) واخيرآ الفلافوبكتيريم كولمنارس: وجد أن معدل الإصابة (8.34%) وأن أعلى معدل أصابة كان فى فصل الخريف (48.4%). وقد تبين من خلال نتائج العدوي التجريبية

لأسماك البلطي النيلي بالعترات البكتيرية للإيرومونس هيدروفيلا وسيدومونس فلوريسنس والتي تم حقنها عن طريق الحقن البريتوني قد أظهرت نفس العلامات الممرضة والصفات التشريحية للأسماك المصابة طبيعيا. وقد اظهرت الأصابات المرضية ومعدلات الوفاة المصاحبة للعدوى البكتيرية أهمية تلك الأمراض التي تصيب أسماك البلطي النيلي المستزرعة تحت نظام الأستزراع شبه المكثف بمنطقة العباسة محافظة الشرقية. وقد أظهرت النتائج أن أهم المسببات البكتيرية التي تم رصدها في أسماك البلطي تحت نظام الأستزراع شبه المكثف بتلك المنطقة هو : إيرومونس هيدروفيلا ثم سيدومونس فلوريسنس يليه ستريبتوكوكس وأخيرآ فلافوبكتيريم كولمنارس. كما أوضحت الدراسة من الممكن أن نهاية الشتاء وفصل الخريف وبداية فصل الربيع تمثل فترات حرجة لحدوث امراض التسمم الدموي الأرمونوسي والسيدوموناس وكذا الستربتوكوكس في أسماك البلطي النيلي المستزرعة تحت النظام سبه المكثف بينما يمثل فصل الصيف وبداية الخريف الفترة الحرجة لحدوث مرض الكولمنارس. ومن تلك النتائج يجب أن يتخذ مربو الأسماك ومسئولي الأستزراع السمكي التدابير اللازمة وبخاصة في الفترات الموسمية التي تحدث فيها تلك الأمراض وتشمل تلك التدابيرمتابعة جودة المياه والتغذية المثلى وكذا استخدام محفزات النمو ومنشطات المناعة خلال تلك الفترات.

SUMMARY

The study was carried out on 1000 *O. niloticus* collected from private and governmental semi intensive earth ponds in Sharkia province during disease outbreaks, the examined farms belongs to the Central Lab for Aquatic Research in Abbassa as well as private farms through the seasons of the year 2008. The clinical signs and postmortem lesions of diseased fish were recorded. The results revealed that (420) fish were found to be infected with bacterial agents which means that the prevalence of bacterial diseases was 42% among the examined fish. The isolated and identified bacteria were *Aeromonas hydrophila* in a percentage (56.67%) and the highest rate of infection was observed in winter season (39.9%), *Pseudomonas fluorescence* in a percentage (36%) and the highest rate of infection was observed in autumn season (48.8%). *Streptococcus* spp. in a percentage (19.77%) and the highest rate of infection was observed in spring season (54.2%). *Flavobacterium columnaris* in a percentage (8.34%) and the highest rate of infection was observed in autumn season (57.1%). Pathogencity of selected bacterial isolates belonged to *Aeromonas hydrophila* or *Pseudomonas* spp. were done through experimental infection via interperitonial and intramuscular routes. Clinical signs and P.M. lesions were recorded nearly the same clinopathological picture which noticed in naturally infected fish. From this study it could be concluded that: Diseases and resulted mortalities caused by bacterial agents represent important category among diseases affecting *O. niloticus* under semi intensive culture system in Abbasa Sharkia Province. The most important bacterial diseases affecting *O. niloticus* under semi intensive culture system in such locality

were motile aeromonas septicaemia, pseudomonas septicaemia, streptcoccosis and columnaris respectively. The aqua-culturist and fish farmers must expect the seasonal occurrence of the studied important bacterial diseases (late winter, autumn and early spring) and all prophylactic measures must be applied beginning from proper water quality, good nutrition, application of effective probiotics and immunostimulants during such periods.

Key words: *Bacterial, O. niloticus, critical time.*

INTRODUCTION

Freshwater aquaculture represents an important urgent source of animal protein for human consumption to compensate the shortage of animal proteins from other sources. Nile tilapia *"O. 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, especial in tropical and subtropical regions and the number of farms has been increasing annually all over the world (Abdelaziz and Badawy 2002; Maregoni, 2006; Newai *et al*., 2008).

Fish diseases constitute one of the most important problems and challenges confronting fish culturists. Diseases don´t occur as a single caused event but are the result of interactions of the pathogens, fish and the environment. Management of the activities of microorganisms in food webs and nutrient cycling in ponds is necessary for optimizing production, but the objectives will differ with the type of aquaculture, the species cultivated and the economics (Abdelaziz and Gihan, 2005; Woo, 2006).

Disease has become a primary constraint to sustainable aquaculture production and product trade (Subasinghe *et al*., 2000). Diseases reduce fish production by affecting the normal physiology of fish and which, if left uncontrolled, can result in mass mortalities, or in some cases, infection of man and other vertebrates that consumed them (Ezekiel *et al*., 2007).

Bacterial pathogens are the causative agents of most serious disease problems in both wild and cultured fish causing mortalities and severe economic losses (Roberts 2001; Austin and Austin 1993).

Numerous diseases have emerged as serious economic or ecological problems in aquaculture species, and increase the risk of infection establishment and spread. It has been estimated that 10% of fish loss in aquaculture is due to disease and more than 50% of these losses are due to

bacterial agents. 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). From the aforementioned data it appears that fish diseases constitute a drastic obstacle for fish production, either due to a single infection or multiple mixed infections. Therefore, the aim of the present investigation was to through the light on the following:

- Isolation and identification of bacterial agents which are commonly associated with heavy mortality among semi-intensive cultured *O. niloticus* in some private and governmental semi-intensive earth pond in Sharkia province.
- Recording seasonal prevalence of these diseases among the examined fish.
- Alarming about the critical times of occurrence of important bacterial diseases to prevent them.
- Describing the clinical picture and Postmortem lesions which associated with these infections in naturally and experimentally infected fish.
- Experimental infection of *O. niloticus* with some of the isolated bacterial agents.

MATERIALS and METHODS

Fish

Naturally infected fish:

A total number of one thousand apparently infected Nile-tilapia *(Oreochromis niloticus*) in a body weight range (25-150g) were collected during the period from January-2008 to December-2008 during the occurrence of various mortalities in private and governmental semi-intensive earth pond in Sharkia. Fish were transferred alive or freshly dead as soon as possible to the Central Laboratory for Aquatic Research laboratory (Abbassa). The live fish were placed in strong plastic bags with compressed air then packed in a large ice box surrounded with crushed ice. The recently dead fish specimens were kept on ice bags to be subjected to full clinical, PM and bacteriological examinations.

Experimental fish:

A total number of 100 apparently healthy *O. niloticus* with an average body weight of 30 gm were collected from a private fish farm. The fish were collected in prepared plastic bags and transported to the laboratory as soon as possible. The fish were kept in well equipped glass aquaria under the laboratory conditions for two weeks for acclimation. Random specimens from fish were taken for diseases examination to

ensure that fish were healthy and none infected. Fish were fed on dry pelleted ration at rate of 3% of their body weight a twice daily.

Aquaria

Glass aquaria of (80×50×40 cm) dimensions were used for the experimental work. They were supplied by aeration using RENA type 301 air pump and tap water chloride free according to (Innes, 1966).

Culture media

- Media used for isolates of bacteria:

Liquid media (Tryptic soy broth (Adwic). Semi-solid media (0.5%) nutrient agar medium (Oxoid). Solid media Tryptic soya agar (TSA) (Adwic). -Brain heart infusion agar (Bio Merieux). -MacConky agar. (Biolife). -Nutrient agar-Rimler-Shotte medium (R-S) (Shotts and Rimler, 1973). -Cytophage agar medium (Anaker and Ordal, 1959).

- Media used for the biochemical reactions:

Indol medium, Bile esculine medium (Biolife), Simon s citrate agar medium. (Difco), Starch hydrolysis medium (Difco), Triple Sugar Iron agar medium (Difco), Oxidation fermentation basal medium (O/F) (Difco), Voges Proskauer broth, Sugar fermentation media (lactose, sucrose, maltose, galactose, esculine), Argenin gelatin (Difco), Nutrient agar (Adwic), Soft agar medium (0.5%) (Carter and Colee, 1990).

Reagents and stains

- The reagent was prepared according to (Cruickshank *et al.,* 1975).
- Gram stain.
- Haematoxyllin and Eosin (H&E)**.**

I- Clinical and postmortem examination:

The fish were examined directly for clinical abnormalities and post-mortem changes according to Amlacher, (1970), Austin and Austin (1987).

II- Seasonal prevalence and critical times of occurence of bacterial infection:

Recording seasonal prevalence of bacterial diseases among the examined *O. niloticus* semi intensively cultured.

III- Bacteriological isolation and identification:

Fish were opened using the method described by (Austin and Austin 1987). The skin was disinfected using alcohol 70%. Samples were taken from lesions of the skin and gills as well as the internal organs (liver, musclature, kidneys, and spleen).

Isolation and phenotypic identification of bacteria:

The samples of skin, gills, liver, kidney and spleen were examined bacteriologically by inoculation on nutrient agar media and incubated at 22^{o}C for 24-48 hours. The suspected colonies were picked-up from the inoculated media and spread on nutrient agar and blood agar media which

incubated at 22^{o} C for 24-48 hours for purification. Then a loopful of each pure culture was inoculated on two tubes of nutrient agar media. One of them was used as stock culture on slant agar for further identification by biochemical tests, and the other tube was inoculated on semi-solid nutrient agar for motility and preservation.

Design of experimental infection:

Pathogencity of *Aeromonas hydrophila* and *Pseudomonas* spp. Were studied separately. A hundred clinically normal of *O. niloticus* were randomly divided into five equal groups, each contained 20 fish. The bacterial isolates were inoculated into BHI broth and incubated at 25^{o}C for 24hrs. 0.1 ml of each broth 24hrs culture was used for fish inoculation. *A. hydrophyla* was injected via intrperitonial and intramuscular routes in the first and second groups respectively. The *Pseudomonas* spp. was injected by the same routes in the third and fourth groups, respectively. Group 5 was designated as control and subdivided into two groups, inoculated with 0.1ml sterile broth by interperitonial and intramuscular routes, respectively. All fish were observed daily up to 3 weeks post infection. Clinical signs and P.M. lesions were recorded using the methods described by Amlacker (1970). Bacterial re-isolation from the injected fish was performed as previously described.

RESULTS

I- Clinical signs and lesions of naturally infected *O. niloticus*.

Generally, the natural infected fish showed decrease in feed intake, loss of appetite, emaciation, excessive mucus secretion forming grayish coat, sluggish movement, lethargic, scratching itself against hard object, respiratory distress, and the gill filaments appeared hyperemic, eroded (Fig.1), swollen in some cases and pale in others. Signs of asphyxia including surfacing of fish, gasping of air bubbles on the surface of water with increased opercular movements and protrusion of operculum were also observed in some examined fish. Large irregular hemorrhage on all external body surface with detached scales (Fig.2), darkening of the skin, associated with sunken eyes. Erosion and ulcer on different parts of the body (Fig.3), blood spots at the base of fins as well as laceration of membranous parts and sloughing of the free portion of them were also observed in some infected fish, detached scales with pale skin patches and more slimy skin, abdominal distention and reddish ascetic fluid in the abdominal cavity, congestion of vent opening, inflammation of digestive tract and the Intestine was voided from food. Also congestion of the internal organs were detected in some fish, Finally some diseased fish lost

their normal swimming behavior and died. In some cases intestinal prolapsed was noticed, uni or bilateral exophthalmia (Fig.4) corneal opacity (Fig.5), eye cloudiness, destruction of eye, pop-eye, and hemorrhage of the eye was observed. Some diseased fish exhibit fin rot in all fins (Fig.6). At the same time red mouth was observed in some cases (Fig.7). The internal changes correspond to the general characteristic signs of septicaemic infections, the postmortem findings were hemorrhages and congestion of gills and all internal organs and the liver was yellowish white or congested to brown color with necrotic foci, in some cases the liver was metallic in appearance. The gall bladder distended with bile and whitish to greenish colored, congested kidneys (Fig. 8). While in some cases darkness of spleen and yellowish ascetic fluid were found.

Fig. 1: Naturally infected *O.niloticus* showing Rosy red erroded gill filaments.

Fig. 2: Naturally infected *O.niloticus* showing irregular haemorrhage on the body surface with detached scales.

Fig. 3: Naturally infected *O.niloticus* showing detached scales,skin erosions and ulcers.

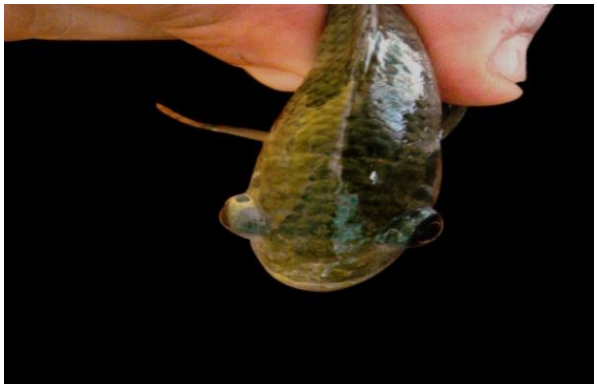


Fig. 4: Naturally infected *O.niloticus* showing bilateral exophthalmia.

Fig. 5: Naturally infected *O.niloticus* showing eye opacity.

Fig. 6: Naturally infected *O.niloticus* showing fin and tail rot.

Fig. 7: Naturally infected *O.niloticus* showing inflammed red mouth.

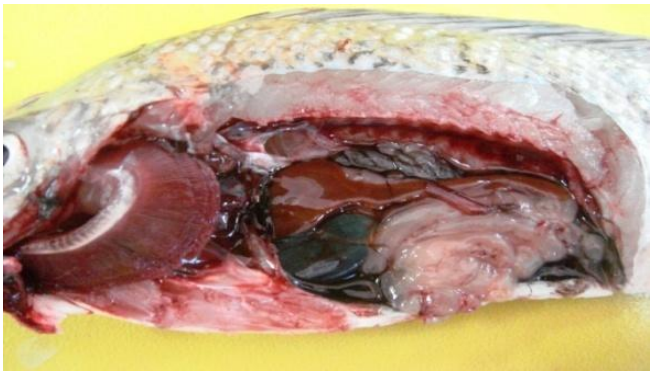


Fig. 8: Naturally infected *O.niloticus* showing congested liver and distended gall bladder.

II- Prevalence of bacterial infections in examined *O.niloticus*:

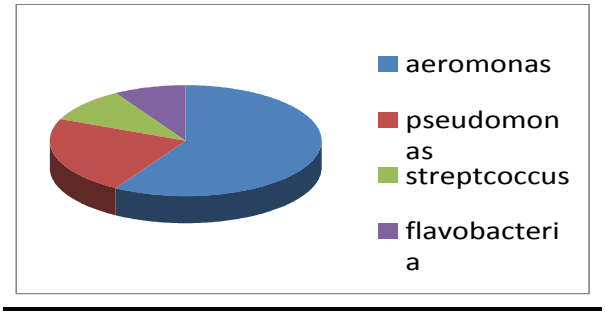
The clinical examination of naturally infected *O.niloticus* collected from the earthen ponds at Abassa revealed that the prevalence of diseases caused by bacterial agents represent 42% of the examined cases Table (1).

Table 1: The prevalence of bacterial infections in naturally infected *O. niloticus*.

Prevalence of bacterial infections	Total No. of examined Fish	No. of naturally infected fish	Percentage
	1000	420	42.00%

Table 2: Seasonal prevalence of different bacteria isolated from naturally infected *O.niloticus.*

Season	No of exam Fish	Aeromonas hydrophila		Pseudomonas flurescence		Streptococcus sp.		Flavobacteria Columnaris	
		No	%	No	%	No	%	No	%
Spring	250	46	19.3	21	13.5	45	54.2	5	14.3
Summer	250	23	9.7	14	9.0	25	30.1	10	28.6
Autumn	250	74	31.1	75	48.4	9	10.9	20	57.1
Winter	250	95	39.9	45	29.0	4	4.8	0	0
Total	1000	238	65.2	155	36	83	19.7	35	8.34



III- Identification of bacterial isolates:

The result of bacterial isolation and identification of diseased fish revealed the presence of bacterial causative agents (42%) of the examined cases. The detected bacterial infections with its prevalence were:

1- *Aeromonas hydrophila*:

A.hydrophila was isolated from naturally infected fish appeared circular yellow-colored colonies on R-S media**.** *A. hydrophila* proved to be motile, short bacilli, positive in respect to catalase, aesculin hydrolysis,

indole, and nitrate reduction (Table 3). Regarding to the experimental infection, the results showed that the interperitonial route was highly infective (80%) than the intramuscular one (60%). Re-isolation of the injected bacterial pathogen was performed from all dead and experimentally diseased fish. No mortality was recorded in the control group (Table 4).

Table 3: Morphological, cultural and biochemical characters of the isolated *A.hydrophila.*

Test	Reaction
Gram-stain	-ve
Shape	Short rod
Motility	+
Cytochrom oxidase	+
O/F	F
Growth on 0.0%NaCl	+
Growth on 5%NaCl	-
Indol	+
Nitrate reduction	-
Vogus-Proskauer	+
Methyle red	+
H2S production	-
Fermentation of:	
Sucrose	+
Maltose	+
Lactose	-
Glycerol	+
Manitol	+
Arabinose	+
Galactose	+
Fructose	+
Gelatin liqufecation	+
Starch hydrolysis	+
Citrate utilization	+
Gas from glucose	+
Arginin hydrolysis	+

Table 4: Pathogencity of *A. hydrophila*, among artificially infected tilapia.

Fish group	Bacterial pathogens	Route of inoculation	Number of infected fish	Number of dead fish	Mortality rate
I	*A. hydrophila*	I/P	20	16	80
II		I/M	20	12	60
V	Control	I/P	10	0	0
		I/M	10	0	0

2- Pseudomonas fluorescence

Pseudomonas fluorescence was isolated as a causative agent of Pseudomonadiasis. It was identified according to its morphological, culture and biochemical characters (Table 5). It showed that the suspected on BHI agar at 25C ±1C for 24hrs were circulated, convex, entire edge, glistening, creamy color and 1-2 mm in diameter, while in aged culture (72 hrs), the colonies had yellow center. Some isolates gave fluorescence pigment. On R-S medium gave dark green colonies and on Macconky agar gave pale colonies. Regarding to the experimental infection, the results showed that the interperitonial route was highly infective (60%) than the intramuscular one (50%). Re-isolation of the injected bacterial pathogen was performed from all dead and experimentally diseased fish. No mortality was recorded in the control group (Table 6).

Table 5: Morphological and Biochemical characters of isolated *Pseudomonas fluorescence* of naturally infected Tilapia

Test	Reaction
Gram-stain	-ve
Shape	Rod
Motility	+
Cytochrom oxidase	+
Growth at 37C	+
Growth on 0.0%NaCl	+
Growth on 5%NaCl	+
Indol	-
Catalase	+
Vogaus-Proskauer	-
Methyl red	-
H2S production	-
Fermentation of	
Sucrose	-
Lactose	-
Maltose	-
Trehalose	-
Manitol	-
Nitrate reduction	+

Table 6: Pathogencity of *Ps. fluorescence* among artificially infected tilapia.

Fish group	Bacterial pathogens	Route of inoculation	Number of infected fish	Number of dead fish	Mortality rate
III	*Pseudomonas flurescens*	I/P	20	12	60
IV		I/M	20	10	50
V	Control	I/P	10	0	0
		I/M	10	0	0

3- Streptococcus sp.

Streptococcus sp. was recovered from clinically naturally diseased fish as a causative agent of Streptococosis. It was gram positive cocci, arranged in pairs & short chain, non motile, grow at tryptone soya agar giving very small pen headed colony to 0.1 mm, creamy white or yellowish white color circular, entire, raised edges and glistening. Biocemically it was catalase negative, grow at media contain 6.5% NaCl, at 45 ^{o}C, Pyrrolidone arylamidase positive, hydrolyse bile esculin, gave α – or non haemolysis on 5% sheep blood agar, ferment manitol, hydrolyse arginine, ferment arabinose and not give yellow colonies (Table 7).

Table 7: Morphological and biochemical tests for *Streptococcus* species.

Test	Reaction
Gram stain	(+) cocci pairs & short chain
Motility	+
Growth on tryptic soy broth	-
Growth on MacConky agar	-
Catalase	-
Oxidase	+
Growth at 10 ˚C	+
Growth at 45 ˚C	+
Growth at 6.5% NaCl	+
Haemolysis on blood agar	+
Bile esculin	-
CAMP test	S
Sensitivity to nalidixic acid	R
Sensitivity to SXT	F
O/F	F
Argenin dihydrolase	+
Esculin hydrolysis	+
Hippurate hydrolysis	-
Pyrrolidone arylamidase	+
(Voges-Perskauer)	+
Indole production test	-
Arabinose fermentation	+
Manitol fermentation	+
Sorbitol fermentation	+
Sucrose fermentation	+
Lactose fermentation	+
Trehelose fermentation	+
Inulin fermentation	-
Raffinose fermentation	+
Gelatin liquiaction	-
Glycogen	-
Citrate utilization	-

4- Flavobacterium columnaris:

Flavobacterium columnaris was identified as causative agent of columnaris infection. It was a Gram negative, long bacilli bacterium forming typical "hay stacks" or "columns" in wet-mount preparations and motile .These bacteria have a characteristic rhizoid pattern of growth on a low nutrient agar medium. The suspected colonies on cytophaga agar at 25^{o}C for 24hrs were yellow in color, rhizoid edge, flat and 5mm in diameter. The results of Cultural and biochemical reactions of *F. columnaris* isolated from naturally infected tilapia was recorded in Table (8).

Table 8: Cultural and biochemical reactions of *F. columnaris* isolated from naturally infected tilapia.

Test	Reaction
Gram-stain	-ve
Shape	Long Rod
Motility	+
Cytochrom oxidase	+
Growth on neomycin sulfate and polymyxin media	+
Growth on 0.0%NaCl	+
Growth on 5%NaCl	+
Indol	-
Catalase	+
Vogaus-Proskauer	-
Methyl red	-
H2S production	-
Fermentation of	
Sucrose	-
Lactose	-
Maltose	-
Trehalose	-
Manitol	-
Nitrate reduction	-

DISCUSSION

The present investigation was planned to isolate and identify the most common bacterial causes of mortalities among Tilapias semi-intensively cultured in earthen ponds in Abassa fish farms in Sharkia Governorate. At the same time detect the seasonal occurrence of such common bacterial diseases among the target farms to alarming about the

critical times of occurrence of important bacterial diseases in order to prevent their negative drawback on fish. The clinical signs and postmortem lesions associated with these infections in naturally and experimentally infected fish, are also objectives of this study.

The results of bacteriological examination revealed that the prevalence of bacterial infection at percentage (42%) of total diseased fish examined. This may be due to that, various stress factors, namely abnormal water temperature, improper pond management and high density usually affect tilapia, suppress their immune response and allow most opportunistic bacteria to infect such fishes Ruth (1990); Paperna (1996); Egna and Boyed (1997); Plumb (1999); Austin and Austin (2007).

The results also revealed the isolation of *Aeromonas hydrophila* (56.67%), *Pseudomonas fluorescens* (36.91%), *Streptococcus* spp (19.77%) and *Flavobacterium columnaris* (8.3%) which mean that the most important category of bacterial diseases infecting *O. niloticus* was the septicaemic diseases(motile aeromonas septicaemia, pseudomonas and streptcoccosis) respectively and only one bacteramic disease was recorded which is columnaris disease. These isolated fish pathogens were proviously reported by Amin (1993); El-Bouhy (1995); Salah (1995); El-Gamal (2000). The predominance of motile aeromonas septicaemia among recorded bacterial diseases (56.67%) may be due to that *A. hydrophila* is considered a truly opportunistic pathogen, commonly found in the aquaculture environment and also as a part of intestinal flora of healthy fish. These results went hand in hand with those recorded by Eissa *et al*. (1990); Badran and Eissa (1991); Amin (1993); Woo and Bruno (1999); El-Ashram (2002); Austin and Austin (2007); Abou El-Atta and Tantawy (2008)

The results revealed also that the main clinical signs observed in infected *O. niloticus* included hemorrhages all over the body (fins, base of fins, mouth, eyes, gill cover and urogenital opening) and enlargement of abdomen. Also, loss of balance, excessive mucus secretion, loss of appetite, erratic scales, sluggish swimming, dullness, skin erosion and ulcer. Exophthalmia, eye cloudiness, fin and tail rot and Prolapse and congestion of the vent were also noticed. Gills were congested or pale anemic. The postmortem findings were watery asitic fluid, severe hemorrhages of all internal organs. Petechial hemorrhages in liver with necrotic foci. The gall bladder distended with bile and the spleen was dark in color. These findings was in agreement with those recorded by Plumb (1999); El-Ashram (2002); Mahmoud (2007); Austin and Austin (2007); Abou El-Atta and Tantawy (2008). The observed clinical signs could be attributed to the virulence criteria of the isolated *A. hydrophila* on affected

fish due to the fact that this organism secretes extra-cellular products that play an outstanding role in the virulence and pathogenesis of the disease and the nature of the observed signs. (Marzouk and Nawal 1991). At the same time Abd El-Ghany *et al.* (2009) mentioned that sluggish movement associated with *A. hydrophila* infection was probably the result of frayed and sloughed tail, beside hemorrhagic, edematous and ulceration of fins, in addition to anorexia which affected the vital activities of the diseased fish. Also, the over distended gall bladder could be attributed to enteritis and constriction of common bile duct.

Regarding to the artificial infection with *A. hydrophila*, the results showed that the interperitonial route was highly infective than the intramuscular one. The mortality rate was 80 and 60%, respectively. The clinical signs and post mortem changes of the artificially infected fish were similar to those of the natural infection. Similar results were recorded by Abd El-Rahman (1996); El-Ashram (2002); Attia (2004); Mahmoud (2007). To the same extent El-Ashram (2002) reported that *O. niloticus* was the most sensitive Tilapia species to *A. hydrophila* infection.

Regarding the prevalence of pseudomonas septicaemia, it came in the second position following aeromonas septicaemia. Pseudomonas is one of the most serious septicemic diseases for fish farming industry leading to sever economic losses all over the world (Plumb, 1999; Austin and Austin 2007). The presence of pseudomonas septicaemia usually lead to heavy fish mortality especially in low water temperature and when the fish were stressed. This was proved from the seasonal prevalence of pseudomonas which was high in autumn (48.39%), followed by winter (29.04%), then spring (13.55%) and finally in summer (9.04%). These findings were in agreement with Abd El-Rahman (1996); Abou El-Atta (2003).

The results revealed also that the clinical signs of pseudomonas in naturally affected *O. niloticus* were petechial hemorrhage on the external surface, slow swimming at the water surface, loss of scales, ulcer and anorexia. In some cases, showed intestinal prolapse, exophthalmia, corneal opacity and pale gills of diseased fish. The post mortem findings were hemorrhages in all internal organs and in some cases the liver was yellowish white color with necrotic areas and whitish to greenish colored gall bladder. The body fluid contained bloody ascetic fluid. The dysfunction of kidney was probably responsible for the altered fluid balance and the osmo-regulatory function. Similar pictures were previously described by Ehab (1991); El-Ashram and Abd El-Rahman (2006). Stress factors as temperature, overcrowding, poor water quality plays an important role in the incidence of pseudomoniasis (Woo and Bruno, 1999).

Regarding to the experimental infection of the isolated *Ps. fluorescens*, the results showed that the interperitonial route was shown to cause significant mortality of experimentally infected fish (60%) than the intramuscular one (50%). Some of infected fish showed peracute form and died without any marked clinical signs or gross lesions. Re-isolation of the injected bacterial pathogen was performed from all freshly dead and experimentally diseased fish. The same clinical signs and postmortem changes were similar to those of natural infection as reported by Abd El-Rahman (1996) and El-Ashram and Abd El-Rahman, (2006).

The results of bacteriological examination revealed also the occurrence of *Streptococcus* spp. at percentage (19.77%) of the bacterial infected *O. niloticus.* In general Streptococcal infections have increased in number during the last decade as a consequence of intensification of aquaculture and responsible for economic losses in the fish farms industry (*Shoemaker et al*., 2001; Hernandez *et al*., 2009). *Streptococcus* spp. is facultative pathogen that invades gills and skin tissue damaged by parasites and skin or alimentary canal damaged by environmental stress or nutritional disorders (Austin and Austin (2007). The results revealed also that the seasonal prevalence of *Streptococcus* spp. was highest in spring season (54.22%), followed by summer (30.12%), then autumn (10.85%) and lastly winter (10.53%). This result may be due to the raised water temperature which is optimum for multiplication of streptococci and result in increase the densities of *streptococcus* spp. which then increase the exposure of fish population to the pathogen. Regarding to the clinical signs, it was revealed that fish from which *Streptococcus* spp. was isolated showed loss of appetite, sluggish movement, swimming close to the surface of the water, lethargic, darkening of the skin, easily detached and lose of the scales, ulcer formation, uni- or bilateral exophthalmia, hemorrhage of the eye, in some cases change cloudy and destruct of eye (pop-eye), haemorrhages on the skin especially in the base of fins and tail. Also abdominal distention was observed. Exophthalmia was a common sign of the disease among the naturally infected fish with numerous haemorrhagic areas on the body surface particularly at the base of fins and operculum. This signs may be attributed to bacterial toxins. The common post mortem lesions observed in the diseased fish were acute septicaemia as they revealed congestion of the internal organs with pale liver and bloody ascitic fluid. These findings were in agreement with those recorded by Weinstein *et al*. (1997); Shoemaker *et al.* (2000); El- Xu *et al*. (2006); Abou El-Atta and Tantawy (2008) and Hernandez *et al.* (2009) as some signs that may be distinct for streptococcal disease, including erratic swimming, darkening of the fish, haemorrhage in the base of the fines and

operculum, pop-eyed appearance and sometimes cloudy eyes. Some infected fish showed swollen abdomen. Exophthalmia was a common feature of the disease among the infected fish, exhibited also numerous haemorragic areas on the body surface particularly at the base of the fines and operculum, this may be due to the haemolytic effect of the exotoxin produced by bacteria (Plumb, 1999).

The results of bacteriological examination revealed also the occurrence of Columnaris disease at a percentage (8.3%) of the bacterial infected *O. niloticus*. The results revealed also that the seasonal prevalence of *Flavobacterium columnari*s was in autumn season. Aly (1994) mentioned that the incidence of columnaris disease in cultured tilapia was (19%) and occurs in spring and summer when the temperature was elevated. Badran *et al*. (1994) mentioned that the columnaris disease among Nile tilapia usually occurred with high prevalence throughout the months of comparatively high water temperature, and occurred through injured tissue. The results revealed that the clinical signs of naturally infected fish with columnaris disease were loss of appetite, excessive mucus secretion, fin rot was observed in caudal, dorsal, anal, pectoral, and pelvic fins. Scale loss, skin erosions and ulcers were also observed. Lesions on the back often extend down the sides, giving the appearance of a 'saddle', typical of columnaris disease. Damage to the gills caused the fish to showing respiratory distress as breathing rapidly and 'gasping' at the surface due to lack of oxygen. These findings was in agreement with the results recorded by Bakeer (1991); Eissa (1994); El-Sayed (1996); (2006) and Austin and Austin (2007). According to Plumb (1999) proper management is essential to success of aquaculture operations, while the inadequate management is the principle factor in triggering bacterial disease outbreaks.

From this study it could be concluded that:

- Diseases and resulted mortalities caused by bacterial agents represent important category among diseases affecting *O. niloticus* in semi intensive earthen ponds in Abbasa Sharkia Province.
- The most important bacterial diseases affecting *O. niloticus* under semi intensive earthen culture system in such locality were motile aeromonas septicaemia (56.67%), pseudomonas septicaemia (36%), streptcoccosis (19.77%) and columnaris (8.34%) respectively.
- The late winter, autumn and early spring represents the critical times of occurrence of motile aeromonas septicaemia, pseudomonas septicaemia and streptcoccosis while early autumn and summer are the critical time for occurrence of columnaris disease.

- The aqua-culturist and fish farmers must expect the seasonal occurrence of the studied important bacterial diseases and all prophylactic measures must be applied beginning from proper water quality, good nutrition, application of effective probiotics and immunostimulants during such periods.

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