Egyptian Journal of Aquatic Biology & Fisheries
Zoology Department, Faculty of Science,
Ain Shams University, Cairo, Egypt.
ISSN 1110 – 6131
Vol. 24(5): 467 – 482 (2020)
www.ejabf.journals.ekb.eg

Pathological, bacteriological and seasonal prevalence of *Aeromonas hydrophila, vibrio vulnificus, Proteus vulgaris* and *Pseudomonas aeruginosa*; infecting *Oreochromis niloticus* in some Egyptian fish farms

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ARTICLE INFO

Article History:
Received: July 20, 2020
Accepted: Aug. 17, 2020
Online: Aug. 20, 2020

Keywords:
Pathology;
A. hydrophila;
V. vulnificus;
P. vulgaris;
P.aeruginosa;
O. niloticus

ABSTRACT

In this study, ninety fish samples showing clinical signs of septicemia were collected from two private fish farms of *Oreochromis niloticus*; forty fish were sampled from fayoum and fifty were from sharkia governorte, Egypt, during four seasons (autumn 2018 to summer 2019). The sampled diseased fish represented 10% of the fish, showing clinical signs and the mortality rate along the year was 15 %. In postmortem examination, the signs of septicemia on the affected fishes were clear in the most of the internal organs. The histopathological results were fully described in different organs of the affected fish. Bacteriological examination revealed isolation of *Aeromonas hydrophila* with prevalence rate along the year 77.33 % with the highest prevalence in summer (100%) in El Fayoum and it was 29.72 % along the year with the highest prevalence in autumn 33.33% in El Sharkia governorate. *Vibrio vulnificans* in El Fayoum governorate was not isolated but it was isolated from El Sharkia with prevalence rate of 16.22%; with the highest prevalence in autumn (33.33%,). *Proteus vulgaris* was identified in both governorates with overall prevalence rate of 6.67% in El Fayoum and the highest prevalence was recorded in both autumn and spring seasons (11.11%). In El Sharkia it was 27.03% but with the highest prevalence in summer (100%). *Pseudomonas aeruginosa* was also recorded with the prevalence rate of 20% along the year in El Fayoum governorate with the highest prevalence in winter 55.56% and it was 27.03% in El Sharkia governorate with the highest prevalence in winter 43.75%. In antibiotic sensitivity testing, each microorganism showed resistance against erythromycin except *Vibrio vulnificus* that showed sensitivity. *Aeromonas hydrophila* and *Proteus vulgaris* showed resistance to colistin sulphate and susceptibility to sulpha-trimethoprime. *Vibrio vulnificus* and *Pseudomonas aeruginosa* showed resistance against sulpha-trimethoprime and sensitive against colistin sulphate.

INTRODUCTION

In Egypt, fish production is representing 20 % of white animal protein production. The most common cultured fish is *Oreochromis niloticus*. (**Younes *et al.*, 2015**). Bacterial diseases are most important causative agent causing high mortality and great economic losses in aquaculture (**Scarpellini *et al.*, 2004).** *Aeromonas hydrophila*, *Vibrio vulnificus*, *Proteus vulgaris* and *Pseudomonas aeruginosa* are Gram negative microorganisms causing general signs of septicemia with severe economic losses in aquacultures. *Aeromonas hydrophila* is the main cause of hemorrhagic septicemia in aquaculture system in Egypt and causing great economic losses (**Alyahya *et al.*, 2018)**. Motile aeromonas septicemia caused by *Aeromonas hydrophila* is a disease persisting to several weeks causing gradually mortality **(Zhang *et al.*, 2016).** Histopathologically, the lesions of the aeromonas infection are characterized by degenerative changes in kidneys, liver, spleen, gills and stomach **(Dong *et al.*, 2017; Hassan *et al.*, 2017)**; Vibriosis is causing common postmortem lesions including skin depigmentation, hemorrhagic spots, congested liver, spleen and stomach; the disease usually cause economic losses and mortality **(Younes *et al.*, 2016).** *Proteus vulgaris* was isolated from ulcers in the fresh water fish **(Mandal *et al.*, 2002)** and the naturally infected fishes with *Proteus vulgaris* showed hemorrhages in buccal cavity, body surfaces and base of the fins, fin rot, protruded hemorrhagic anus, congested gills and internal organs together with focal hemorrhage on the surface of the liver **(Aya, 2013).** The naturally infected fishes with *pseudomonas aeruginosa* showing hemorrhage in body surfaces especially at base of the fins, fin rot, loose of scales, skin ulceration and distended abdomen; postmortem lesions are hemorrhagic ascetic fluid, enlarged pale liver, enlarged congested spleen and hemorrhagic enteritis **(Magdy *et al.*, 2014).** The prevalence rates of infection with *Aeromonas hydrophila*, *Vibrio vulnificus*, *Proteus vulgaris* and *Pseudomonas aeruginosa* in fishes are greatly different and are multifactorial dependent; it may change from time to time even within the same fish species. The prevalence rates of infection with *Aeromonas hydrophila* was recorded by **Ahmed (2002)** as 47.3 % in *O.niloticus*, and it was 14% as recorded by **Ebeed *et al.*, (2017)**. The prevalence rate of *Vibrio vulnificus* was 8 % in fresh water fish **(Saad *et al.*, 2015)** and 12.5% in Cultured *Oreochromis niloticus* around Qarun Lake **(Younes *et al.*, 2016)**. In case of *Proteus vulgaris*, the prevalence rate was 12% **(Aya, 2013)**.and it was recorded as 2% by **Rabab *et al.*, (2019)**; while in *P.aeriginosa*, the Prevalence rate of infection in *O.niloticus* during the period between 2015-2016 was 60 % in winter and 24 % in summer **(Elham *et al.*, 2017)** while the prevalence rate of *Pseudomonas* spp. In *O.niloticus* Was 13.8 % **(Manal and Ahmed, 2016)**. Thus the need of updating information of the microbial prevalence in fish aquaculture is very important and could help in the diseases control at different locations.

The aim of this work was isolation and identification of the bacterial infection causing mortalities in some *O. niloticus* aquaculture at some locations in Egypt, recording the seasonal variation of the infection and studying the antibiotic sensitivity for these isolates.

MATERIALS AND METHODS

This study has been approved by the Institutional Animal Care and Use Committee, Faculty of Veterinary Medicine, Cairo University, Egypt (Vet. CU. IACUC, approval No. Vet CU20022020164).

1. Fish sampling

Two private fish farms of *Oreochromis niloticus* at El Fayoum and El Sharkia governorates showed signs of septicemia with a history of recorded mortality ranged from 10-20 % in each farm during autumn 2018 to summer 2019. Ninety fish that weighted 80 ±5 g representing about 10% of fishes showing clinical signs were collected from the infected ponds that showing mortalities and/ or clinical signs. Alive fish were transferred in boxes supplied with aerators, while Moribund and freshly dead fish transported in icebox to wet laboratory unit of Fish Diseases Department; Animal Health Research Institute (AHRI), Dokki, Giza, Egypt. Lesions and postmortem findings of naturally infected fish were recorded according to (**Conroy and Herman 1981**; **Austin and Austin, 2012)**. The specimens from each region were analyzed for both bacteriological and histopathological examination.

2. Bacteriological examination

Loopfuls aseptically taken from liver, kidney, spleen and brain according to (**Austin and Austin, 2012)** directly streaked on the different selective media as Thiosulfate-citrate-bile salt- Sucrose (TCBS) agar, Salmonella Shigella (SS) Agar, Medium Base (Oxoid, Ltd.), Pseudomonas Agar Base (LabM, UK) an XLD (Oxoid, Ltd), followed by incubation at 25°C/ 24 hrs. Identification of pure bacterial isolates were identified by biochemical characterization following the criteria described in Bergey's Manual of Determinative Bacteriology (**Holt *et al.,* 1993)** and performed by commercial API *20NE, API*20E kits (Bio-Merieux,France) following the criteria described by **Elmer *et al.,* (1998)**. Pure colonies were transferred to glycerol broth 20% at -80 °C (**Pujalte, *et al.,* 2003)**.

3. Antibiotic sensitivity test

Sensitivity was determined by the agar diffusion method **Quinn, *et al.,* (2002)** using 6 mm diameter commercial discs (Oxoid) included the following antibiotics' discs. The antibiotic discs were Amoxicillin, Gentamicin, Nalidixic acid, Colistin suphate, erythromycin, nitrofurantoin, sulpha-trimethoprime, ciprofloxacin and Vibriostate O/129. Antibiotic sensitivity was tested on Mueller-Hinton agar with or without 3%NaCl . Inhibition zones diameters were interpreted as sensitive, intermediate and resistant according to **CLSI (2010)**.

4. Pathological examination

4.1. histopathological examination

Tissue specimens from spleen, kidney, liver and brain of the same fishes used in bacteriological examination were immediately sampled and immersed in neutral buffer formalin10 %. After proper fixation, the specimens were trimmed, washed, dehydrated in ascending grades of ethyl alcohol, then cleared in xylol and embedded in paraffin. Thin

sections about 4-6 microns in thickness were prepared and stained with Haris haematoxylin and Eosin, gram's stain and prussion blue for microscopic examination **(Bancroft and Marilyn, 2002).** The tissue changes were described and photographed using research light microscope Olympus BX-53.

4.2. Immunohistochemical evaluation:

The immunohistochemistry for paraffin section was performed according to **Zhang *et al.*, (2016)**. Briefly, rehydration of paraffin section by passing the sections in descending grades of alcohols (alcohol 100%, 95% for 5min. to each, 80%, 70% for 3 min. to each), antigen retrieval was occurred by immersion of tissue section in antigen retrieval buffer then placed in autoclave at 121 ^{o}C then leave the slides to cool down at room temperature, washing 3 times by PBS, removing of excess blocking buffer and incubate the section with primary antibodies in humid box at 4 ^{o}C for 12- 16 hrs, KJand incubate at 25 ^{o}C for 1 hour, remove excess of secondary antibody and washing 3 times by PBS and finally adding adequate amount of counterstaining dye.

RESULTS

1. Clinical and postmortem examination

This research planned to investigate the main causes of mortalities in some *O. niloticus* farms. The lesions of naturally infected fishes of each strain were nearly the same, with evidence of septicemia. The infected fished showed abnormal swimming, gasping, off food, loss of scales, petechial hemorrhage on all body surfaces, loss of scales, congestion of the blood vessels on the surface of all body fins and gills (Figure 1A). In visceral organs, the postmortem examination of internal organ showed congested kidney, congested and enlarged spleen and marbled liver (Figure 1B).

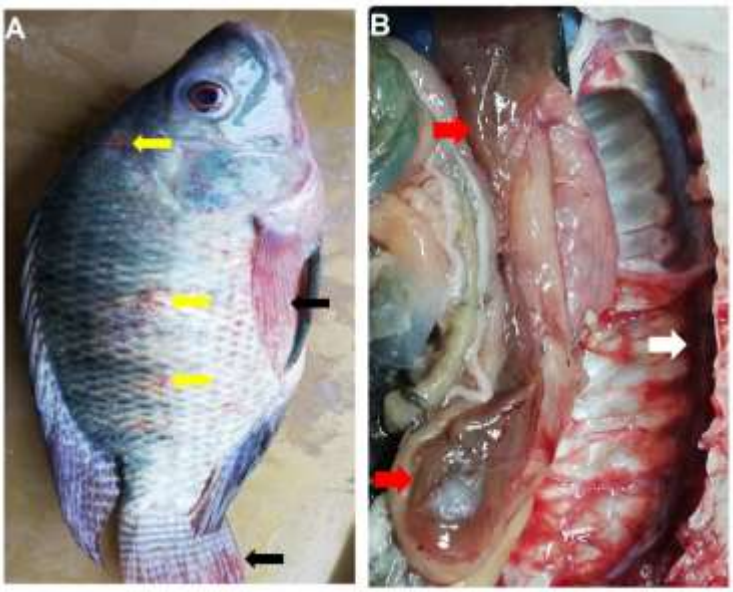

Fig.1 A) congestion in pectoral and tail fins (black arrows), loss of scales and presence of petechial hemorrhage on body surface (yellow arrows). B) congested kidney (white arrow) and marbled friable liver (red arrows).

2. Bacterial examination

Bacteriological and morphological characterization of isolates from infected *O. niloticus* revealed that, *A.hydrophila* isolates appeared colorless colonies on SS agar, *V.vulnificus* gave yellow colonies on TCBS media, *P. vulgaris* isolates produced yellow colonies in XLD media and *P.aeruginosa* isolates were greenish colonies on Pseudomonas Agar Base. All isolates were Gram-negative , motile, rods shape wile *V. vulnificus* were slightly curved rods. The Phenotypic and Biochemical characterizations of all isolates of naturally infected *O. niloticus* are summarized in table 1&2.

Table 1: Phenotypic and Biochemical characterizations of *A.hydrophila* and *V.vulnificus* using API*20NE.

Biochemical test		***A.hydrophila***	***V.vulnificus***
Colony characters onto TCBS medium		Yellow colored colonies	*Yellow colored colonies*
Colony characters onto SS		Colorless	-
NO3	Potassium nitrate	-	+
TRP	Trytophane production	-	+
GLU	Glucose fermentaion	+	+
ADH	Arginine Dihydrolase	+	-
URE	Urease	-	-
ESC	Esculin	+	+
GEL	Gelatin	+	+
PNG	Para Nitrophenyl D Galactopyranosidase B Glucosidase	+	+
GLU	Glucose assimilation	+	-
ARA	Arabinose assimilation	+	-
MNE	Mannose assimilation	+	-
MAN	Mannitol assimilation	+	-
NAG	N acetyl Glucosamine assimilation	+	-
MAL	Maltose assimilation	+	-
GNT	Potassium GlucoNate aasimilation	+	-
CAP	Capric acid assimilation	+	-
LDI	Adipic acid assimilation	-	-
MLT	Malate assimilation	+	-
CIT	Tri sodium Citrate assimilation	-	-
PAC	Phenyle acetic acid assimlation	-	-
OX	Oxidase	+	+

Table2: Phenotypic and Biochemical characterizations of *P. aeruginosa* and *Proteus vulgaris* using API*20NE.

Biochemical test		***P. aeruginosa***	***Proteus vulgaris***
Colony characters onto Pseudomonas agar base medium		Greenish colonies	-
Colony characters onto XLD medium		-	Yellow colonies
ONPG	B-galactosidase	-	-
ADH	Arginine Hydrolase	+	-
LDH	Lysine Decarboxylase	-	-
ODH	Ornithine Decarboxylase	-	-
CIT	Citrate	+	+
H_2S	H_2S production	-	+
URE	Urease	-/+	+
TDA	Tryptophane Deaminase	-	+
IND	Indole	-	+
VP	Vagous Prescour	-	-
GEL	Gelatinase	+/-	-/+
GLU	Glucose	+/-	+
MAN	Mannitol	-	-
INO	Inositol	-	-
SOR	Sorbitol	-	-
RHA	Rhaminose	-	-
SAC	Sucrose	-	+
MEL	Melobinose	-	-
AMY	Amyldain	-	-/+
ARA	Arabinose	-/+	-
OX	Cytochrome Oxidase	+	-

3 Seasonal and overall prevalence/year

Aeromonas hydrophila prevalence rate was 77.33 % in El Fayoum governorate during the year with the highest prevalence in summer season (100%) and it was 29.72 % in El Sharkia governorate with the highest prevalence in autumn season, 33.33%; *V. vulnificus* prevalence rate in El Fayoum governorate during the year was zero % and it was 16.22% in El Sharkia governorate with the highest prevalence in autumn season (33.33%), *P. vulgaris* prevalence rate was 6.67% in El Fayoum governorate with the highest prevalence in both autumn and spring seasons, 11.11%; and it was 27.03% in El Sharkia governorate with the highest prevalence in summer season (100%); while *Pseudomonas aeruginosa* prevalence rate was 20% in El Fayoum governorate with the highest prevalence in winter season, 55.56%; and it was 27.03% in El Sharkia governorate with the highest prevalence in winter season (43.75%). The prevalence rate of the isolated bacteriafrom four organs of naturally infected fish, *O. niloticus* during the four seasons starting from autumn 2018 to summer 2019 was summarized in (table 3&4).

Table 3: The prevalence rate of *A. hydrophila* and *V. vulnificus* isolated from four organs of naturally infected *O. niloticus* during the four seasons.

Place	Season and year	No. of examined fish showing clinical signs (90 fish representing 10%)			Organ	No. of isolates		Prevalence rate %	
		Isolated bacteria		Negative for gram +ve bacteria					
		A. hydrophila	*V. vulnificus*			***A.*** *hydrophila*	*V. vulnificus*	*A. hydrophila*	***V.*** *vulnificus*
El Fayoum	**Autumn, 2018**	4	0	4	Brain	0	0	88.89	0
					liver	4	0		
					kidney	3	0		
					spleen	1	0		
	Winter, 2018	4	0	1	Brain	0	0	44.44	0
					liver	2	0		
					kidney	1	0		
					spleen	1	0		
	Spring, 2019	6	0	3	Brain	0	0	77.78	0
					liver	2	0		
					kidney	3	0		
					spleen	2	0		
	Summer, 2019	1	0	9	Brain	0	0	100	0
					liver	1	0		
					kidney	1	0		
					spleen	1	0		
Total (40 examined fish)		15	0	17	**Total**	22	0	73.33	0
El Sharkia	**Autumn, 2018**	1	2	7	Brain	0	0	33.33	33.33
					liver	1	2		
					kidney	1	1		
					spleen	1	0		
	Winter, 2018	2	1	4	Brain	0	0	31.25	6.25
					liver	2	0		
					kidney	2	1		
					spleen	1	0		
	Spring, 2019	1	1	8	Brain	0	0	30	20
					liver	1	1		
					kidney	1	1		
					spleen	1	0		
	Summer, 2019	0	0	12	Brain	0	0	0	0
					liver	0	0		
					kidney	0	0		
					spleen	0	0		
Total (50 examined fish)		4	4	31	**Total**	11	6	29.72	16.22

Table 4: The prevalence rate of *Proteus vulgaris* and *Pseudomonas aeruginosa* isolated from four organs of naturally infected *O. niloticus* during the four seasons.

Place	Season and year	No. of examined fish showing clinical signs (90 fish representing 10%)			Organ	No. of isolates		Prevalence rate %	
		Isolated bacteria		Negative for gram +ve bacteria		*P. vulgaris*	*P. aeruginosa*	*P. vulgaris*	*P. aeruginosa*
		P. vulgaris	*P. aeruginosa*						
El Fayoum	Autumn, 2018	1	0	4	Brain	0	0	11.11	0
					liver	1	0		
					kidney	0	0		
					spleen	0	0		
	Winter, 2018	0	5	1	Brain	0	0	0	55.56
					liver	0	2		
					kidney	0	3		
					spleen	0	0		
	Spring, 2019	1	1	3	Brain	0	0	11.11	11.11
					liver	1	1		
					kidney	0	0		
					spleen	0	0		
	Summer, 2019	0	0	9	Brain	0	0	0	0
					liver	0	0		
					kidney	0	0		
					spleen	0	0		
Total (40 examined fish)		2	6	17	Total	2	6	6.67	20
El Sharkia	Autumn, 2018	1	1	7	Brain	0	0	22.22	11.11
					liver	1	1		
					kidney	1	0		
					spleen	0	0		
	Winter, 2018	2	3	4	Brain	0	0	18.75	43.75
					liver	2	3		
					kidney	1	4		
					spleen	0	0		
	Spring, 2019	2	1	8	Brain	0	0	30	20
					liver	1	1		
					kidney	2	1		
					spleen	0	0		
	Summer, 2019	1	0	12	Brain	0	0	100	0
					liver	1	0		
					kidney	1	0		
					spleen	0	0		
Total (50 examined fish)		6	5	31	Total	10	10	27.03	27.03

4. Antibiotic sensitivity

The antibiotic sensitivity test is demonstrated in table 5.

Table 5: antibiotic sensitivity test for of *A. hidrophila*, *V. vulnificus P. vulgaris*, and *P. aeruginosa* isolated from four organs of naturally infected *O. niloticus*.

Antibiotic Discs	*A. hydrophila*	*V. vulnificus*	*P. vulgaris*	*P. aeruginosa*
Amoxicillin (20/10mcg)	I (12 mm)	S (16 mm)	R (0 mm)	R (0 mm)
Gentamycin (10µg)	S (16 mm)	I (13 mm)	S (16 mm)	I (13 mm)
nalidixic acid (30mcg)	S (20 mm)	S (20 mm)	I (15 mm)	R (0 mm)
colistin sulphate (10µg)	R (0 mm)	S (11 mm)	R (0 mm)	S (12 mm)
Erythromycin (15 µg)	R (0 mm)	S (19 mm)	R (0 mm)	R (0 mm)
Nitrofurantoin (300µg)	S (18 mm)	S (17 mm)	R (0 mm)	R (0 mm)
sulfamethxazol/trimethoprim (25µg)	S (17 mm)	R (0 mm)	S (16 mm)	R (0 mm)
Ciprofloxacin (5µg)	S (21 mm)	S (23 mm)	I (16 mm)	I (16 mm)
O/129(150 µg)	-	S	-	-

S: Sensitive R: Resistant I: Intermediate

5. Pathological examination:

5.1. Histopathological findings of naturally infected fishes in Fayoum governorate

In histopathological examination, congestion of hepatoportal blood vessels in the liver with aggregation of eosinophilic granular cells, in addition to vacuolar degeneration in hepatocytes were commo (Fig. 2A&B). The hemosiderin pigments were deposited between the hepatocytes which positively stained by Prussian blue (Fig. 2C). The lesion in some cases was advanced and the hepatopancreatic cells showed necrobiotic changes (Fig. 2D). Thickening in wall of splenic blood vessels, depletion of white pulp and deposition of melanin pigment around some splenic blood vessels were common findings in spleen (Fig. 2E&F). In kidneys, hemorrhage was found between renal tubules with hemosiderin pigments deposition (Fig. 2G). Vacuolar degeneration in the renaltubular epithelium was also noticed together with interstitial hemorrhage and mononuclear inflammatory cells infiltration; some nuclei of the renal tubular epithelium showed pyknosis indicating necrobiotic changes in the epithelium.In such cases, deposition of melanin pigments between renal tubules and hypercelluarity in renal glomeruli were common(Fig. 2 H).

5.2. Histopathological findings of naturally infected fishes in Sharkia governorate

The liver showed congestion of hepatoportal blood vessels with necrosis in hepatocytes (Fig. 3A&B), in addition to vacuolar degeneration in some hepatocytes (Fig. 3C). Some hepatocytes showed apoptosis and hemorrhage between hepatocytes (Fig. 3D). The apoptotic bodies were positively stained by caspase 3 immunohistochemistry (Fig. 3E). Some cases showed aggregation of bacteria in the areas of necrosis; The lesion in such cases surrounded with many inflammatory cells (Fig. 3F). In spleen, lymphocytic depilation in white pulp of with melanin pigment deposition and subcapsular splenic

necrosis were common (Fig. 3G). the renal tissue showed hypercelluarity in renal glomeruli and some nuclei of renal tubular epithelium showed necrobiotic changes (Fig. 3H).

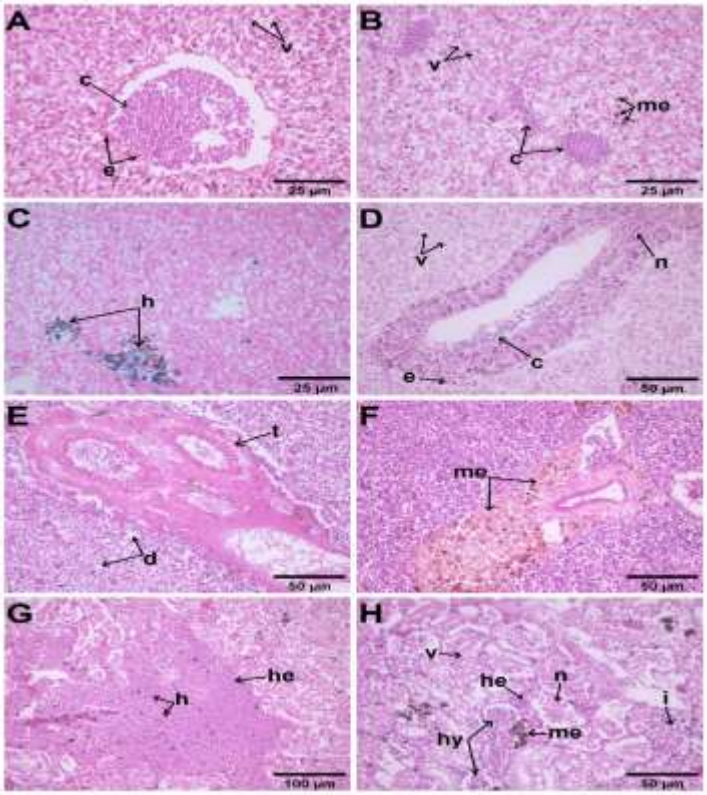

Fig.2: Histopathological sections of *Oreochromis niloticus* naturally infected with Gram negative bacteria collected from El Fayoum governorate showing; A) Congestion of hepatoportal blood vessels (c) surrounded by eosinophilic granular cells (e) and some hepatocytes showing vacuolar degeneration (v). B) hepatocytes showing vacuolar degeneration (v), congestion of hepatoportal blood vessels (c) with melanin pigment deposition between hepatocytes (me). C) Deposition of hemosiderin pigment (h) in the tissue of liver which stained positive, appear blue color, by Prussian blue. D) Necrosis in hepatopancreatic cells (n), congestion of hepatopancreatic blood vessels (c) surround by eosinophilic granular cells (e), in addition to vacuolar degeneration in hepatocytes (v). E) Thickening in wall of blood vessels of spleen (t) in addition to depletion of white pulp (d). F) melanin pigment deposition (me) around blood vessels of spleen. G) Hemorrhage (he) between renal tubules with hemosiderin deposition (h). H) Hemorrhage (he) and mononuclear cells infiltration (i) between renal tubules together with necrotic changes (n) in the epithelium, in addition to vacuolar degeneration in epithelium of renal tubules (v) and deposition of melanin pigment (me) between the renal tubules; the glomeruli showed hypercellularity (hy).

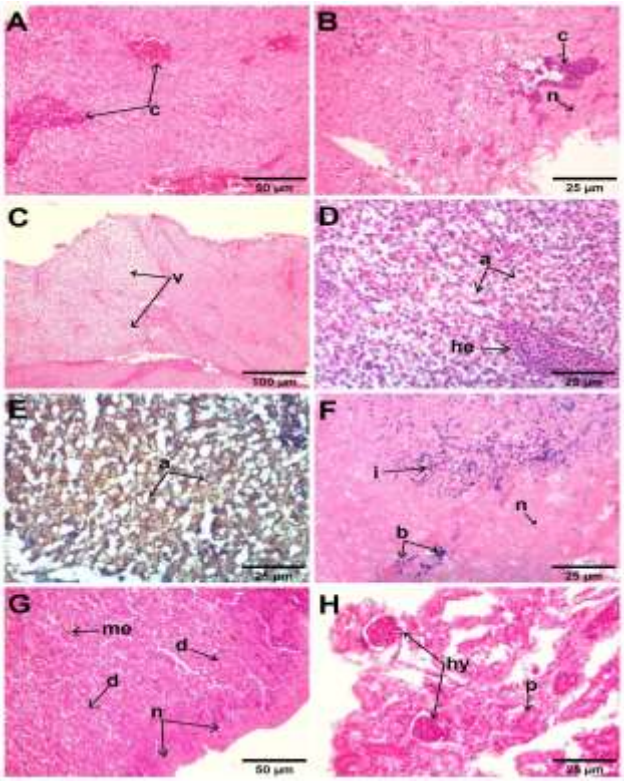

Fig. 3: Histopathological sections of *Oreochromis niloticus* naturally infected with Gram negative bacteria collected from El Sharkia governorate showing; A) congestion of hepatoportal blood vessels (c). B) Congestion of hepatoportal blood vessels (c) and some hepatocytes showing necrotic changes (n). C) Some hepatocytes showing vacuolar degeneration (v). D) Some hepatocytes showing apoptosis with formation of apoptotic bodies (a), in addition to hemorrhage (he) between hepatocytes. E) Apoptotic hepatocytes (a) positively stained by caspase 3 immunohistochemistry. F) Hepatocytes showing necrotic changes (n) with mononuclear inflammatory cells aggregation (i), in addition to presence of bacteria (b) between hepatocytes. G) Deplation (d) in white pulp with melanin pigment (me) deposition, in addition to subcapsular splenic necrosis (n). H) renal glomeruli showing hypercellularity (hy) and some nuclei of renal tubule epithelium showing pyknosis (p) with necrobiotic changes in the epithelium.

DISCUSSION

In regard to the results of clinical signs and postmortem lesions in naturally infected *O. niloticus* , the lesions in the examined tissues were correlated to the four microorganisms where these microorganisms were isolated from the same examined tissues. The lesions in infected fishes in two governorates were the same which externally including loss of scales, petechial hemorrhage on lateral part of the body with congestion of blood vessels in all body fins and internally including congested kidney, enlarged congested spleen and marbled liver. Our findings were nearly similar to external signs found by (**Ahmed, 2002; Aya, 2013; Magdy *et al*., 2014; Sumithra *et al*., 2019).**

Bacterial diseases affecting O.niloticus considered one the most important problems causing severe economic losses. In our study, the prevalence rate of *A.hydrophila* during the sampling period in El fayoum governorate was 73.33% and in El sharkia governorate was 29.72%. These finding showed variation with the finding of **Ahmed (2002)** who recorded that the prevalence rate of *A. hydrophila* was 47.3% and **Ebeed *et al.*, (2017)** who recorded that the prevalence rate of *A. hydrophila* was 14%. The prevalence rate of *V. vulnificus* in El fayoum governorate was 0% and in El sharkia governorate was 16.22% which showed variation with the finding of **Younes *et al.*, (2016)** who recorded that the prevalence rate of *V. vulnificus* was 12.5% and **Hemmat *et al.*, (2018**) who recorded that the prevalence rate of *V. vulnificus* was 4%. The prevalence rate of *P. vulgaris* in El fayoum governorate was 6.67% and in El sharkia was 27.03% which disagreed with **Aya (2013)** who recorded that prevalence rate of *P. vulgaris* was 12%. The prevalence rate of *P. aeruginosa* in El fayoum governorate was 20% and in El sharkia governorate was 27.03% which is disagreed with **Eissa *et al.*, (2010)** who recorded that the prevalence rate of Pseudomonas species was 30.83%.

From a bacteriological point of view, the the dominant isolates were from liver, kidney and spleen who agreed with (**Ahmed, 2002; Eissa *et al.*, 2010; Aya, 2013; Sumithra *et al.*, 2019)**, who isolated the *A. hydrophila, V. vulnificus*, *P. vulgaris* and *P. aeruginosa* respectively from the same three organs. The biochemical characters of *Aeromonas hydrophila* were similar to findings of **Ahmed (2002)** who isolated *Aeromonas hydrophila* from cultured tilapia. Also the biochemical characters of *V. vulnificus* were agreed with finding of **Younes *et al.*, (2016)** who isolated *V. vulnificus* Strains from Cultured *O. niloticus* around Qarun Lake, Egypt. *Proteus vulgaris* ferment sucrose which disagreed with **Aya (2013)** who isolated *Proteus vulgaris* from Nile tilapia which not ferment sucrose while the biochemical characters of *Pseudomonas aeruginosa* similar to findings of **Eissa *et al.*, (2010)** who Isolated *Pseudomonas aeruginosa* from Tilapia in Qaroun and Wadi-El-Rayan Lakes, Egypt.

In our study, *Aeromonas hydrophila* showed sensitivity to Nalidixic acid, Gentamicin and Sulfa-trimethoprime and resistant to erythromycin which is partially agreed with **Wamala *et al.*, (2018)** who found that *Aeromonas hydrophila* showed sensitivity to Nalidixic acid, Gentamicin and Sulfa-trimethoprime and intermediate resistance to erythromycin. *V. vulnificus* showed intermediate resistance to gentamicin, resistance Sulfa trimethoprime, sensitive to Nalidixic acid, colistin sulphate and ciprofloxacin which not similar to finding of **Sumithra *et al.*, *(*2019)** who showed that *V. vulnificus* resistant to colistin sulphate and sensitive to gentamicin, Sulfa trimethoprime, Nalidixic acid and ciprofloxacin. *P. aeruginosa* showed intermediate resistance to gentamicin, resistant to erythromycin and Sulfa-trimethoprime, Amoxycillin and Nalidixic which disagreed with **Eissa *et al.*, (2010)** who showed that highly sensitive to Gentamicin, Erythromycin, and Sulfa trimethoprime. In addition, showed resistance to Amoxycillin and Nalidixic acid.

The histopathological findings in our results including vacuolar degeneration in hepatocytes, congestion in hepatoportal blood vessels and hepatopancreatic blood vessels with melanin and hemosiderin pigments deposition between hepatocytes and necrosis in hepatopancreatic cells and hepatocytes were partially disagreed with (**Ahmed, 2002 ; Aya, 2013)** and agreed with **(Magdy *et al.*, 2014 ; Sumithra *et al.*, 2019)** in vacuolar degeneration and congestion of portal blood vessels. Thickening in the wall of splenic blood vessels, melanin deposition around splenic blood vessels, necrosis in subcapsular

splenic tissue with depletion of white pulp were partially agreed with **Magdy *et al.*, (2014)** who recorded depletion of white pulp together with deposition of melanin pigments and disagreed with the findings of **Sumithra *et al.*, (2019)** who mention that presence of hemosiderin pigments in splenic tissue are most common. The lesions in the kidneys including hemorrhages and mononuclear inflammatory cells aggregation between the renal tubules, deposition of melanin and hemosiderin pigments, hypercelluarity in renal glomeruli, vacuolar degeneration and necrotic changes in the epithelial lining of renal tubule could be attributed to the deieterious effect of the secreted enzymes by the isolated bacteria. In this regard, many authors have been attributed the lesions of such bacterial infection to the secretion of some bacterial enzymes; Metalloprotease enzyme which is one of protease enzymes secreted by *Vibrio vulnificus and* causing cutaneous tissues damages, hemorrhage and edema (**Jones and Oliver, 2009)**, exotoxin A secreted by *P. aeruginosa* has been incriminated as a cause of local tissue damage and tissue invasion (**Iglewski *et al.*, 1977)**. Hemolysins are another group of the virulence factors which are multifunction enzymes secreted by *A.hydrophila* , inducing multiple effects including hemolytic, cytotonic, and cytotoxic activities (**Erova *et al.*, 2012)**.

CONCLUSION

A. hydrophila, P. vulgaris, V. vulnificus and P. aeruginosa caused disease problems in some fish farms at El Fayoum and El Sharkia governorates, the diseases have seasonal variation in such locations and so, prophylactic measures against such bacteria should be considered to avoid the outbreaks in the highest prevalence seasons of the infection; Development of vaccination protocol against the locally isolated bacteria is very important as a prophylactic measure in Egyptian fish farms especially in El Fayoum and El Sharkia regions. This study could be used as a model to demonstrate and screen the bacterial infection in different seasons in other locations.

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