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Concurrent natural infection of *Acanthocephalan* species and *Euclinostomum heterostomum* synergistically increase Motile *Aeromonas septicemia* and *Vibriosis* in the Nile tilapia (*Oreochomis niloticus*)

Maather El-lamie[1], Mona Ismail [1], Salah M. Aly [2], Eman Youssef [3], Esraa Abdalla[1] and Hassnaa Elsheshtawy [1]*

1. Fish Diseases and Management Dept., Fac. of Vet. Medicine, Suez Canal University, Egypt.
2. Pathology Dept., Fac. of Vet. Medicine, Suez Canal University, Egypt.
3. Parasitology Dept., Fac. Of Vet. Medicine, Suez Canal University, Egypt

***Corresponding Author: Hassnaa_elshshtawy@vet.suez.edu.eg**

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ABSTRACT

A seasonal study was performed on the wild *Oreochomis niloticus* collected seasonally from Suez channel, branched of the River Nile in Fayed region, Ismailia Governorate in Egypt. Specimens were addressed to investigate the role of natural infestation with parasites in secondary infection with *Aeromonas* and *Vibrio* species in a combination with unfavorable environmental condition. *Acanthocephalans* (*Acanthocentis tilapiae* and *Neoechinorhynchus* sp.) and trematode/ digenean *Euclinostomum heterostomum* were isolated from the intestine and kidneys of *O. niloticus*, respectively. *Aeromonas hydrophila*, *Vibrio alginolyticus*, and *Vibrio parahaemolyticus* were isolated from different organs of some examined tilapias and identified biochemically by VITEK®2 compac system and by PCR. The water quality parameters were significantly elevated over the permissible levels, whereas an annual average elevation was detected in nitrite (0.06 ±0.08 mg/L) and un-ionized ammonia (0.31 ±0.29 mg/L). The prevalence of co-infections of Motile Aeromonas Septicemia (MAS) with *acanthocephalan* was the highest in summer season (33.3%). Additionally, the vibriosis with *acanthocephalan* infestation were the highest in summer (66.7%) and the lowest in winter (10.0%) in both cases. While co-infection of MAS with *Euclinostomum heterostomum* infestation together with the vibriosis were recorded the highest in summer with 50% and 100%, respectively, and the lowest was in winter (00.0%) in both cases. Histopathological alterations were recorded in different organs as gills, liver, kidney and spleen in affected fish. It could be concluded that bad water quality and parasitism may depress immunity and stress fish facilitating *Aeromonas* and *Vibrio* infections.

INTRODUCTION

Egypt is the highest African country in tilapia production, mostly derived from either semi-extensive or semi-intensive pond systems using fresh and low saline brackish water **(Soliman & Yacout, 2016)**. The Nile tilapia, *O. niloticus*, is the most common and cheapest fish species available for Egyptians in the Nile River. In addition, it is one of the

most important cultured tilapias worldwide (**Lovshin, 1997**). The tilapia adapt itself easily to changes in salinity levels and oxygen availability, and can feed at different trophic levels, and addingly under certain circumstances, it can tolerate overcrowding. Thus, it is an excellent species for aquaculture (**Coward & Little, 2001**). Aquaculture problems can be summarized in 2 main factors, fish diseases and water pollution that are complementary to each other. Fish diseases are the result of an interaction between 3 major variables in the aquatic environments; fish (as a host; resistance, age and predisposed), pathogen (virulence and environmental requirements) and water environment (temperature, pH, O2 level) (**Austin & Austin, 2007**) . Fish -microorganism interactions are usually harmless if the immune system of fish is not compromised by a stressor. However, fish diseases frequently occur after fish are subjected to stressful conditions (**Harper & Wolf, 2009**) . Fish diseases can arise from either infectious or non-infectious causes. Infectious causes include viruses, bacteria, fungi and parasites. Disorders in aquaculture conditions lead to changes in the parasite/host equilibrium, causing diseases and mortalities. Parasitism can cause mechanical damage (gill lamellae fusion and skin damage), physiological damage (cell proliferation and immunomodulation), and reproductive capacity damage (**Iwanowicz, 2011**). Remarkably, bacterial diseases are the most important causes of economic losses in the tilapia culture, including bacterial haemorrhagic septicaemia caused by several aeromonads including *Aeromonas hydrophila* (**Austin *et al.*, 2012**) .Additionally, *Vibrio* spp. were isolated from cultured tilapia after mass mortalities in farms around Qarun lake in Egypt (**Younes *et al.*, 2016**). The *Aeromonas* spp. are common pathogens causing Motile Aeromonas Septicemia (MAS) and result in high mortalities and economic losses **(Zaman & Khalequzzaman, 2013)**.Vibriosis severity depends on *Vibrio* species, which cause massive economic losses and clinical disease outbreaks **(Abdel-Aziz *et al.*, 2013)**. Parasitic infestations are considered as a stress factor; reducing fish resistance to secondary bacterial infections and act as a vehicle; transmitting bacterial pathogens by their damaging effects and opening a portal of entry with increased mortalities **(Kotob *et al.*, 2017)** or harboring bacteria and act as a source of infection for their hosts while feeding **(Bowers *et al.*, 2000).** Mixed infections are common in *O. niloticus* intensive aquaculture **(Xu *et al.*, 2007)**. Moreover, they are common in free-living tilapias but are found at high rates in aquaculture. Due to the restriction measures in fish farms, some parasitic affections such as metacercarial infection are more common in free-living tilapias **(El-Sayed *et al.*, 2019)**. Metacercarial infections appeared on fish in excessive mucus secretions, scale loss, respiratory distress, and colored spots or nodules on the affected organs resulting in atrophy of the affected organ **(Aly *et al.*, 2005)**. The deterioration of the physical and chemical properties of water such as temperature, alkalinity, un-ionized ammonia (NH3), dissolved oxygen (DO), nitrite (NO2), pH value and total hardness has a strong influence on the fish health status as it reduces their immune response and increases their susceptibility to the invading bacterial and parasitic

pathogens (**Hossain *et al.*, 2007**). Therefore, in the present study, the occurrence of natural co-infection was examined with some parasites and MAS or vibriosis among *O. niloticus*. Moreover, this work aimed to assess the clinical picture of the affected fishes and its relationship with the deteriorated water quality parameters and the prevalence of mixed infections while highlighting the histopathological alterations due to these co-infections.

MATERIALS AND METHODS

1. Fish collection and sampling

A total of 121 wild *O. niloticus* (moribund and/or freshly dead), with weights ≥70 - ≥99g, were collected seasonally from Suez channel; branched of Nile river in Fayed region, Ismailia Governorate, Egypt from September 2018 to August 2019. The sampling patterns during the different seasons were as follows: 26 in winter, 35 in spring, 28 in summer, and 32 in autumn. The collected samples were transferred immediately to the laboratory of Fish Diseases and Management, Faculty of Veterinary Medicine, Suez Canal University for further investigation

2. Ethical considerations

This work contains a surveillance study that aimed to determine the possible cause of the fish mortalities and does not need ethical approval. Fish before examination were euthanized by pithing according to **Barker *et al.*** (**2002**)

3. Clinical and postmortem examinations

Fish samples were clinically examined for any abnormal clinical signs and gross lesions. Clinical and postmortem examinations were carried out according to **Noga** (**2010**).

4. Water quality parameters

Throughout the survey period (from September 2018 to August 2019), 10 water samples / season were collected from different sites in the lake and were put in sterile 500 ml glass bottles according to the standard methods of **APHA** (**1981**).Water parameters; namely, temperature, dissolved oxygen and the pH were measured on spot using alcoholic thermometer, oxygen metre and pH metre, respectively. Kits were utilized for measuring the levels of un-ionized ammonia and nitrite found in the water (USA, Virginia Company, lot. No. 201134).

5. Parasitological assay

Internal organs were dissected out and examined under a stereozoom microscope (Zeiss- Germany). Live parasites were examined; their morphological details were recorded for further taxonomic determination. Small pieces from internal organs were cut, compressed between two slides, and examined microscopically for the presence of encysted metacercariae (EMC). Their morphological details were addressed, then samples were fixed and mounted. *Acanthocephalans* were fixed, stained and mounted following the method of **Noga** (**2010**).

6. Bacteriological assay

It was performed according to the method of **Buller (2014)**. A loopful of hepatopancreas, kidney, gills, and spleen were inoculated in nutrient broth (NB) (LAB M) and incubated at 25°C for 24h. Positive turbid cultured broths were sub-cultured on nutrient agar (NA, LAB M). The recovered colonies were inoculated onto specific media; Thiosulfate citrate bile salt sucrose agar (TCBS) and Aeromonas base agar (LAB M), and left incubated at 24°C for 24 h. The suspected colonies of *Vibrio* and *Aeromonas* species were identified biochemically using standard methods, and were confirmed by VITEK®2 compac systems (Biomerieux). All isolates were stored at -80°C in Brain Heart Infusion Broth (BHIB) with 3% NaCl and 20% glycerol according to **Gauthier *et al.* (1995)**.

7. Molecular characterization of bacterial species

7.1. Bacterial DNA extraction

Genomic DNA was extracted by the boiling centrifugation method **(Soumet *et al.*, 1994)**. Following the technique of **Mendes-Marques *et al.* (2013)**, the conventional and Duplex PCR technique was used to detect *Aeromonas hydrophila* and *Vibrio alginolyticus* and *Vibrio parahaemolyticus*, respectively.

7.2. Oligonucleotide designs for collagenase, toxR, and 16SrRNA genes amplification

As shown in Table (1), the nucleotide sequence of collagenase gene, partial toxR gene, and 16SrRNA gene primers were obtained according to **Kim *et al.*** (**1999**), **Gordon *et al.*** (**2007**) and **Abu-Elala *et al.*** (**2016**), respectively. They were used for the identification of *Vibrio alginolyticus (V. alginolyticus)*, *Vibrio parahaemolyticus (V. parahaemolyticus)*, and *Aeromonas hydrophila (A. hydrophila)* strains.

Table 1: Oligonucleotide primer sequences for *Vibrio* spp. and genus *Aeromonas* and *A. hydrophila*

Target	Sequence (5'-3')	Amplified product	Reference
V. alginolyticus *Collagenase*	CGAGTACAGTCACTTGAAAGCC CACAACAGAACTCGCGTTACC	737 bp	**Abu-Elala *et al.*, 2016**
V. parahaemolyticus *toxR*	GTCTTCTGACGCAATCGTTG ATACGAGTGGTTGCTGTCATG	368 bp	**Kim *et al*., 1999**
Aeromonas genus 16S rRNA	CTACTTTTGCCGGCGAGCGG TGATTCCCGAAGGCACTCCC	953 bp	**Gordon *et al.*, 2007**
Aeromonas hydrophila 16S rRNA	GAAAGGTTGATGCCTAATACGTA CGTGCTGGCAACAAAGGACAG	625 bp	

7.3. PCR amplification and agarose gel electrophoresis

The PCR reaction was run in a thermal cycler (Techne, England). A typical mixture of 5 µl of the extracted DNA, 12.5 µl PCR master mix (2X DreamTaq Green mastermix kit), 1 µl of forward and reverse primers in a volume of 25 µl. was prepared. The DNA denaturation was carried out at 94°C for 5 min, and then a total of 35 PCR cycles were run under the following conditions: DNA denaturation at 94°C for 30 s, primer annealing at 50°C for 40 s and DNA polymerization at 72°C for 45 s. After the final cycle, reactions were terminated at 72°C for 10 min as a final extension step for the two genes. Agarose gel electrophoresis was performed by the addition of 6 µl of PCR product for each sample in 1.5% agarose containing ethidium bromide stain (0.5 µg/ml). A 100 bp DNA ladder (NZYDNA Ladder V) was used as a molecular weight marker according to **Sambrook and Fritscgh** (**1989**). PCR products were compared using the SynGene Gel Documentation System. *V. alginolyticus* give bands at ~737 bp, *V. parahaemlyticus* at ~368 bp, Aeromonas species at ~953bp, and *A. hydrophila* at ~625 bp.

8. Histopathological examination

Small pieces of infected organs (liver, kidney, spleen and gills) with visible lesions were taken and preserved in 10% neutral buffered formalin, dehydrated by ascending grades of ethyl alcohol (70, 80, 90 and 100%), cleared in 50% alcohol-xylol mixture, embedded in paraffin wax, and then sectioned at 5 µm thickness, mounted on a glass slide and stained with H&E according to **Korun and Timur** (**2005**).

RESULTS

1. Clinical signs and postmortem lesions

Infected fish showed emaciated body and respiratory manifestations. Hemorrhages all over the body surface or black discoloration of the skin were also observed. Loss of scales were recorded in some cases. Other cases showed exophthalmia (Fig. 1.A.), ascites, and hyperemic protruded vent with an emaciated body. Gills had marbling appearance with excessive mucus secretions. Internally, there was accumulated serous, cloudy, or bloody fluid in the abdominal cavity. Hepatopancreas showed congestion with mottled appearance or yellowish discoloration. In some cases, the spleen revealed congestion and enlargement. While others, showing congested stomach and intestine with thick intestinal wall and excessive mucus secretions in their lumens, were observed (Fig. 1.B.). Kidneys were congested and enlarged; sometimes had attached yellow-white cyst(s) (3mm × 5mm) (Fig. 1.C.). Some cases showed gas bubbles in the intestinal lumen, and others appeared with attached parasitic helminthes to the inner wall of the intestine.

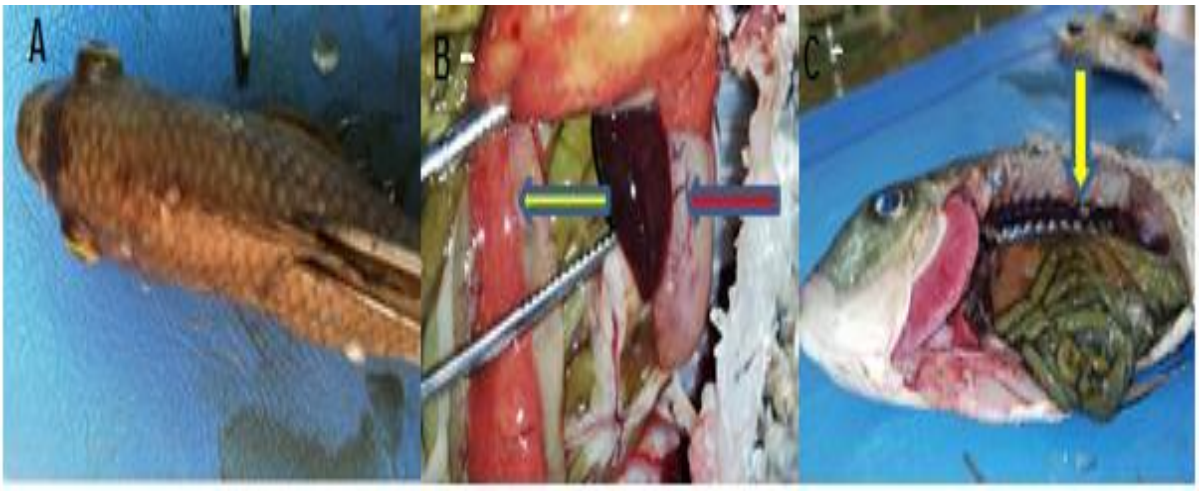

Fig. 1: Photographs of mixed infected *O. niloticus* showing **(A)** Bilateral exophthalmia, **(B)** Congested and enlarged spleen, congested stomach (red arrow) and intestines (yellow arrow) with thick intestinal wall and excessive mucus secretions in their lumens and **(C)** Congested kidneys with (3mm×5mm) attached yellow-white cyst (arrow).

2. Physiochemical parameters of water samples

The physical and chemical properties of canal water were illustrated in (Table 2) during different seasons of this study, whereas the annual average of DO was (6.2 ±1.58 mg/L), the pH value was (7.9 ± 0.20), and the water temperature was (25.2±5.1°C). In comparison with the standard values of **WHO** (**1993**), an elevation was spotted in the nitrite (NO2) (0.06 ±0.08 mg/L) and the un-ionized ammonia (NH3) (0.31 ±0.29mg/L).

Table 2: The mean values of seasonal variations of physical and chemical properties of examined water during the study and their relation to the standard permissible limits (mean ± standard deviation

Season	**Dissolved oxygen (DO) (mg/L)**	**Temprature (°C)**	**pH value**	**NH3 (mg/L)**	**NO2 (mg/L)**
Winter	6.5 ±0.7	17.90 ± 6.4	8.04 ± 2.9	0.2± 0.3	0.01±0.1
Spring	8.60 ±0.4	28.03 ± 5.9	7.95 ± 0.5	0.071±0.04	0.02±0.2
Summer	4.28 ±0.4	31.5 ± 6.7	7.58 ± 0.5	0.8 ± 0.6	0.2±0.3
Autumn	5.5 ±0.6	23.40 ± 6.2	8.11 ±.0.7	0.15±0.02	0.015±0.2
Annual average	6.22 ±1.58	25.20 ±5.1	7.92 ±0.20	0.31±0.29	0.06±0.08
Permissible limits (**WHO, 1993**)	5-6		8-8.5	0.01	0.01

3. Parasitological examination

Microscopic examination revealed two *acanthocephalan* species from the intestine with a club-shaped and elongated body with a retractable proboscis. The first *acanthocephalan* species, after staining with Semichon's acetocarmine, thee rows of six hooks were observed curving posteriorly in both sexes. Tegument had alternative folds and pores. Lemnisci were two mononucleated, unequal blind and compact. The reproductive system in the posterior half of the trunk extended to the posterior end of the bursa. Morphologically, it is related to Order Gyracanthocephala Family: Quadrigyridae, Genus: *Acanthogyrus,* Species: *Acanthosentis tilapiae* according to **Baylis (1947)** as presented in Fig. (2.D.). The second *acanthocephalan* is characterized by females being twice as large as males. All other shared structures were of similar size and shape in both sexes. The trunk was arched, long, and slender. The epidermal surface was porous. Proboscis was as long as wide. Proboscis hooks were equal in each circle, and circles of hooks were evenly spaced. Proboscis receptacle had a large pyramid-shaped cephalic ganglion at its base. Lemnisci were of comparable sizes in both sexes, much longer than the receptacle. Morphologically, it is related to Class: Eoacanthocephala Order: Neoechinorhynchida Family: Neochinorhynchidae, Genus: *Neochinorhynchinus* Species *Neochinorhynchinus* species according to **Stiles and Hassall** (**1905**), and the data are showed in Fig. (2.E.). Gross examination revealed a well-defined yellowish-white visible cyst (3mm × 5mm) attached to and/or embedded in kidneys. After excystation, staining, and microscopic examination, a characteristic black and tree-like branched intestine was monitored with the presence of oral and ventral suckers. Morphologically, this EMC is related to Family: Clinostomatidae, Genus: *Euclinostomum,* Species: *Euclinostomum heterostomum (E. heterostomum)* according to **Dönges** (**1974**) (Fig. 2.F.).

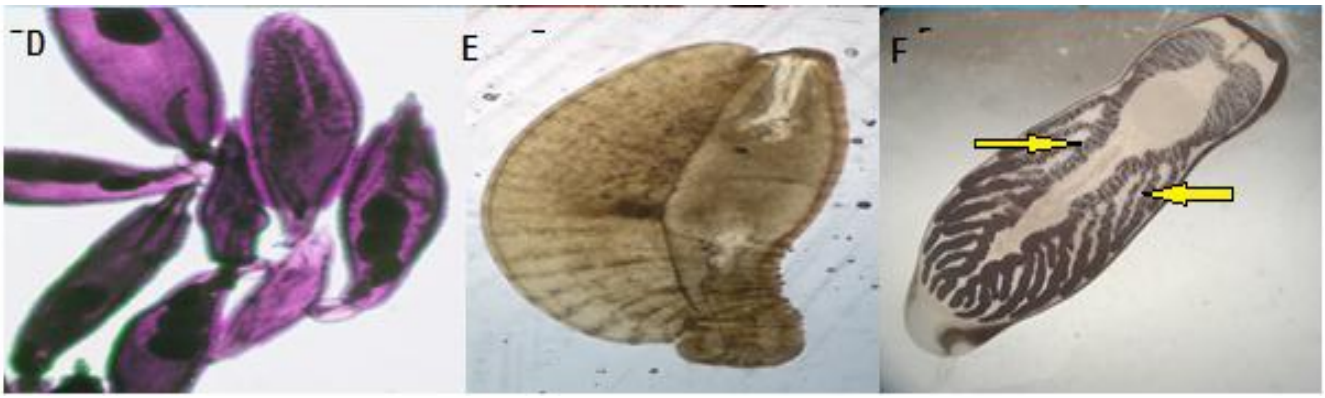

Fig. 2: Photomicrographs showing **(D)** Heavy infestation with Semichon's acetocarmine stained *Acanthocentis tilapiae* (male and female), **(E)** Unstained *Neoechinorhynchus* sp., and **(F)** Unstained excysted *E. heterostomum* with black and branched intestine (arrows).

3. Bacteriological examination

The isolated colonies of *V. alginolyticus* and *V. parahaemolyticus* on nutrient agar were medium-sized (2-3 mm in diameter) and creamy in color, while *A. hydrophila* appeared as grey-white translucent colonies. On Thiosulphate citrate bile salt agar (TCBS), colonies of *V. alginolyticus* were swarming and yellow-colored, *V. parahaemolyticus* were swarming, green-colored with yellow pigmentation, and *A. hydrophila* appeared as homogenous yellow colonies and cannot be picked as a separate colony. On Aeromonas base agar medium, *Vibrio* species did not grow while *A. hydrophila* colonies appeared as dark green, opaque with darker center with a diameter of 0.5-1.5 mm. Microscopic examination revealed that *Vibrio* species were gram-negative, comma or rod-shaped, motile bacteria, and scattered in arrangement. They were oxidase, and catalase tests positive. The *Aeromonas hydrophila* was gram-negative, rod-shaped bacilli with rounded ends, motile, catalase and oxidase tests positive. Results of the phenotypic characters and biochemical test of *V. alginolyticus, V. parahaemolyticus* and *A. hydrophila* by traditional methods are listed in Table (3). VITEK®2 compac system confirmed that the isolates were *V. alginolyticus, V. parahaemolyticus* and *A. hydrophila* with probability of 95%, 96%, and 86%, respectively

Table 3: The morphological and biochemical characteristics of the isolated bacterial species

Test	***V. alginolyticus***	***V. parahaemolyticus***	***A. hydrophila***
Gram staining	(-ve) short, coma shaped bacilli	(-ve) short, coma shaped bacilli	(-ve) rod-shaped with rounded ends
TCBS	Yellow-colored colonies	Scattered green with yellow pigmentation colonies	Homogeneous yellow colonies
Aeromonas base agar media	-	-	dark green, opaque with a darker center
Hemolysis	+	+	B hemolysis
Oxidase	+	+	+
Catalase	+	+	+
Growth on medium with NaCl%			
0%	-	-	+
3%	+	+	++
6%	+	+	-
8%	+	+	-
10%	+	+	-
Growth temperature, ^{o}C			
4 ^{o}C	-	-	+

25 & 37 °C	+	+	+
42 °C	+	+	-
Arginine dihydrolase production	-	-	+
Lysine decarboxylase production	+	+	+
Ornithine decarboxylase production	±	±	-
Citrate utilization	+	+	+
H_2S production (H_2S)	-	-	+
Urease production	±	±	-
Tryptophane deaminase production (TDA)	-	-	-
Indol production	±	±	+
Acetoin production	-	±	+
Gelatinase production GEL)	+	+	+
Acid from glucose	+	+	+
Acid from mannitol (MAN)	+	+	+
Acid from inositol (INO)	-	-	-
Acid from Sorbitol (SOR)	-	-	-
Acid from sucrose	-	+	+
Acid from arabinose (ARA)	±	±	Variable

4. Molecular identification of bacterial strains

It was yielded amplification products of expected molecular size at 737bp and 368bp specific for *V. alginolyticus* and *V. parahaemolyticus* strains, respectively, as shown in Fig. (3). Detection of *Aeromonas* species using 16SrRNA amplicon represented genus *Aeromonas* at 953bp and *A. hydrophila* at 652 bp as shown in Fig. (4).

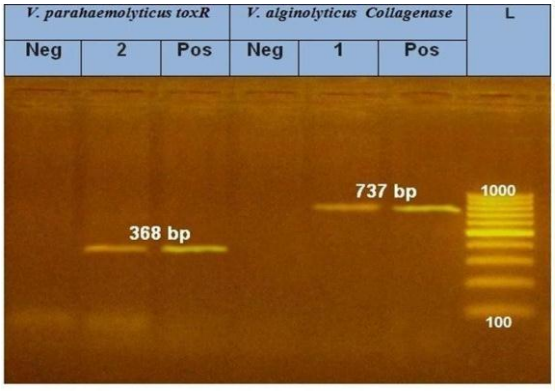

Fig. 3: A photograph showing 737bp and 368bp amplicons representing collagenase and toxR genes in *V. alginolyticus* and *V.parahemolyticus*, respectively. Neg.: Negative, Pos Positive, L: 100 bp DNA ladder, 1 sample 1, 2: sample 2

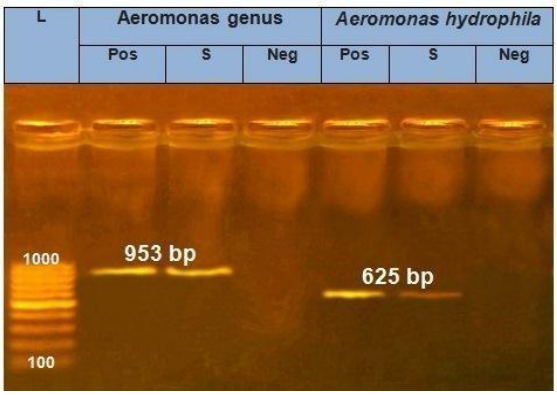

Fig. 4: 16S rRNA amplicon representing genus *Aeromonas* at 53bp and *Aeromonas hydrophila* at 652bp. Neg.: Negative, Pos: Positive, L: 100 bp DNA ladder, s: sample

5. Prevalence of infections

Prevalence of *acanthocephalan* infestation was higher than that of the *E. heterostomum*. The highest prevalence for *acanthocephalan* were recorded in summer (57.1%) and the lowest were in winter (31.3%). While for *E. heterostomum* infestations, the highest was in autumn (15.4%) and the lowest in winter (3.1%) (Fig. 5). The total prevalence of Motile Aeromonas Septicemia (MAS) and vibriosis were 19.8% and 20.7%, respectively with the highest prevalence in summer (28.6%) ,(35.7%) and the lowest in spring (11.4%) and winter (11.5%), respectively (Table 4). Prevalence of MAS in fishes infested with *acanthocephalan* and *E. heterostomum* EMC were (53.3%) and (30.8%), respectively. The MAS recorded the highest prevalence in summer (33.3%) and (50%) and the lowest in winter (10%) and (0%) in infested *acanthocephalan* and *E. heterostomum* fish, respectively. Prevalence of vibriosis in infested fishes with *acanthocephalan* and *E. heterostomum* were (56.6%) and (46.2%), respectively. It recorded the highest prevalence of 66.7% and 100% in summer, and the lowest prevalence were in winter (10%) and (0%) in *acanthocephalan* and *E. heterostomum* infested fish, respectively (Fig. 6).

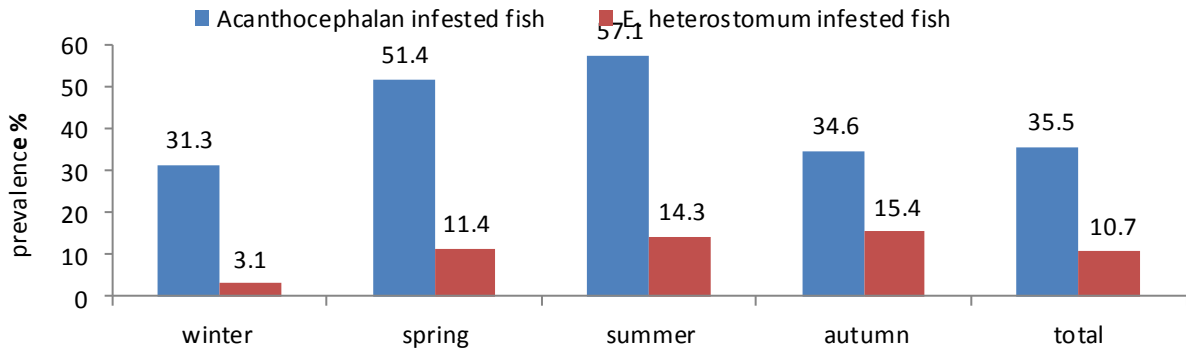

Fig. 5: Total and seasonal prevalence of *O. niloticus* infested with different parasites

Table 4: Total and seasonal prevalence of MAS, vibriosis and mixed bacterial infection in wild *O. niloticus*

	Winter n=26	Spring n=35	Summer n=28	Autumn n=32	Total n=121
Type of infection in *O. niloticus*					
MAS	5 (19.2%)	4 (11.4%)	8(28.6%)	7 (21.9%)	24 (19.8%)
Vibriosis	3 (11.5%)	8 (22.9%)	10 (35.7%)	4 (12.5%)	25 (20.7%)
Mixed (MAS + vibriosis)	8 (30.8%)	10 (28.6%)	9 (32.1%)	7 (21.9%)	37 (30.6%)
Total number of infected fish	8 (30.8%)	12 (34.3%)	18 (64.3%)	11 (34.4%)	49 (40.5%)

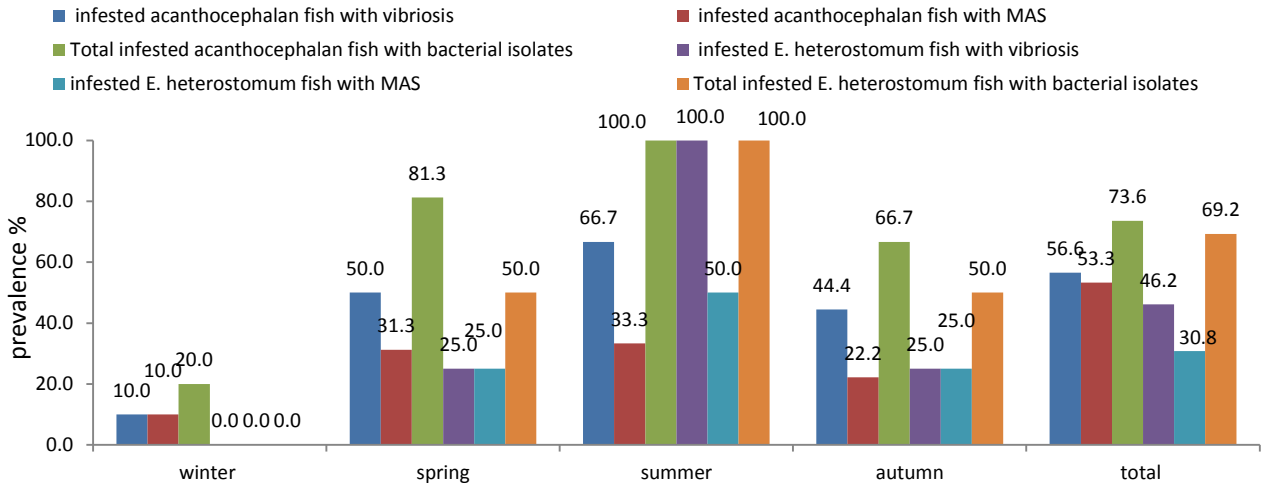

Fig. 6: Total and seasonal prevalences of mixed infected *O.niloticus* (MAS and vibriosis with each parasite)

6. Histopathological examination

The Nile tilapia infected with MAS and vibriosis in the presence of parasitic infestations revealed degeneration and necrosis of secondary lamellae with congestion. The kidney showed vacuolar degeneration and necrosis of renal tubules and focal leukocytic infiltration. The spleen showed severe destruction of hematopoietic tissue and mild hyperplasia of melanomachrophage cells. Hepatopancreas revealed advanced vacuolar degeneration in hepatocytes. Some hepatic cells were necrotic and they lacked nuclei. The pancreatic acini showed degeneration and necrosis of the pancreatic cells and congested pancreatic vessels (Fig. 7).

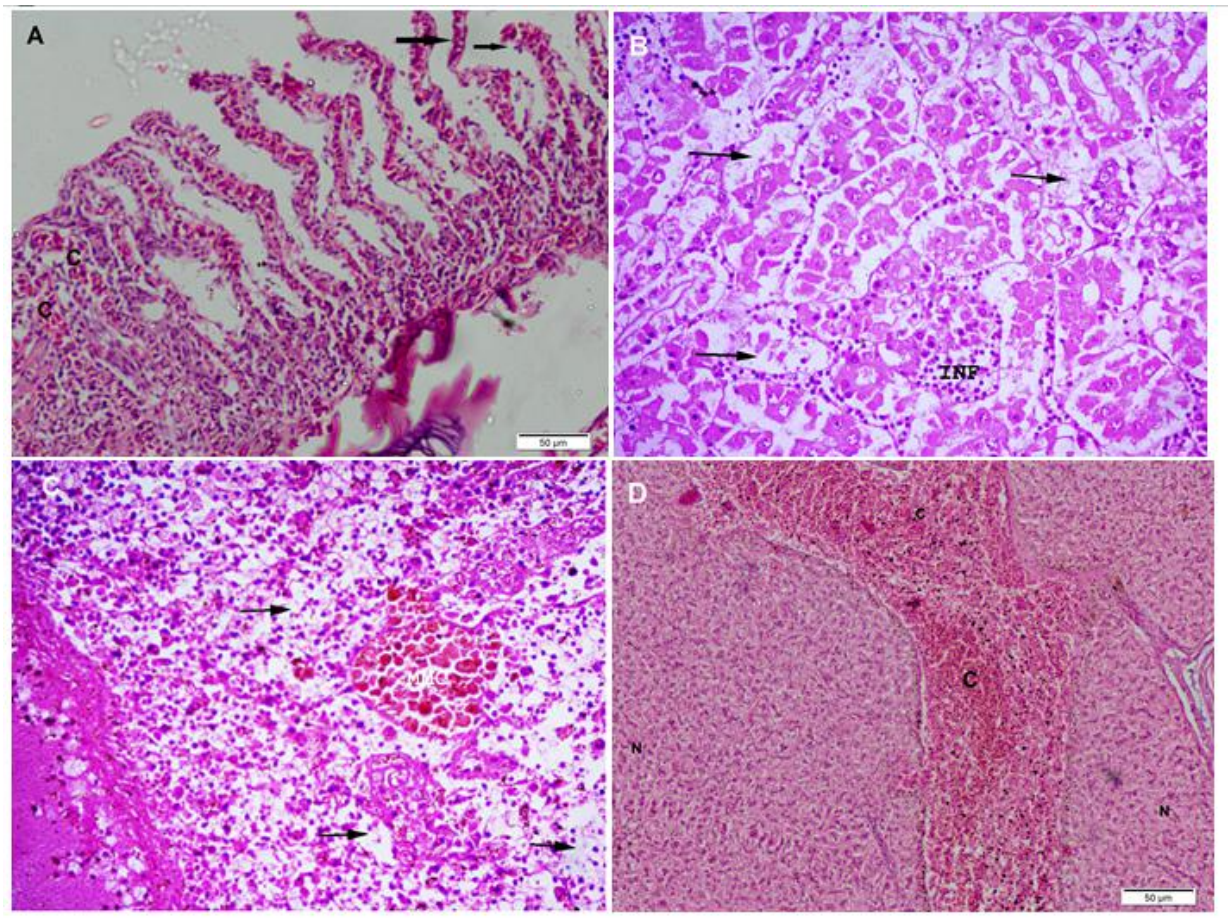

Fig. 7: Photomicrographs of histopathological examination of naturally mixed infected *O. niloticus:* (A) Gills showing degeneration and necrosis of secondary lamellae (arrow) with congestion (C) (B) Anterior kidney showing vacuolar degeneration and necrosis of renal tubules (arrows) and focal leukocytic infiltration (INF). (C) Spleen showing severe destruction of hematopoietic tissue (arrows) and mild hyperplasia of melanomachrophage cells (MMC). (D) Hepatopancreas showing advanced vacuolar degeneration (N) in hepatocytes. Some hepatic cells were necrotic and lacked nuclei. The pancreatic acini showed degeneration and necrosis of the pancreatic cells and congested pancreatic vessels (C) (arrows) .

DISCUSSION

Fish diseases are stress related with a multifactorial nature (**Austin & Austin, 1993**). Concurrent infections are common in aquatic ecosystems due to the presence of multifactors affecting fish health. Parasitic infestations act as a stress factor increasing secondary bacterial infections by different methods (**Kotob *et al.*, 2017**) or harboring bacteria and act as a source of infection while feeding (**Bowers *et al.*, 2000**). In this study, the clinical signs and post-mortem lesions of the diseased tilapia showed emaciated body and typical signs of septicaemia that included exophthalmia, ascites, hemorrhages

all over the body surface or as black discoloration of the skin and hyperemic protruded vent. The current results are in concordance with the findings reported by **Plumb** (**1999**), **Diggles *et al.*** (**2000**) and **Eissa** (**2002**). Additionally, congested gills, liver spleen and kidney were detected with accumulation of bloody tinged in the abdominal cavity and thickening of intestinal wall with excessive mucus secretions in their lumens. These findings concur with that reported by **Lunder *et al.*** (**2000**), **El-Ashram** (**2002**) , **Korun and Timur** (**2008**), **Sarkar and Rashid** (**2012**) and **Dahdouh *et al.*** (**2016**).The gross lesions and P.M findings could be attributed to various factors; among which is the pathogenic characters of the bacteria including invasion, multiplication and colonization. The action of the bacterial exotoxins (toxic extracellular metabolites) of *A. hydrophila* and *V. alginolyticus* and *V.paraheamolyticus* also played an important role in the formation of the haemolysin, aerolysin and cytotoxic toxins due to their haemolytic, cytolytic and enterotoxic activities (**Stintzi & Raymond, 2000**; **Eissa *et al.*, 2013)** Another facor that is worthy mentioning is the synergistic, parasitic and bacterial interactions which increase the disease occurrence (**Kotob *et al.*, 2017**). Furthermore, the *acanthocephalan*s' role in competing for nutrients and decreasing protein ratio in musculature (**Wanstall & Terry, 1984**) together with their presence in the intestine resulted in impaired nutrient absorption (**Nickol, 2006**).

In the present study, water quality parameters revealed elevations of non ionized ammonia and nitrite that surpassed the permissible limits in a way that may multiply the disease. This could happen because the deterioration of physicochemical water quality parameters may act as potential environmental stressors, and thus predispose fish for infectious agents (**Evans *et al.*, 2006**). The results of poor water quality were associated with the infection of the Nile tilapia with bacterial and parasitic diseases. This may be attributed to the deteriorated water quality parameters that have significantly increased the susceptibility to various pathogenic bacteria and parasites and led to disease outbreaks (**Glibert *et al.*, 2002**).

Based on the convention of morphological, biochemical identification and VITEK®2 Compac system characterization of the bacterial isolates, the identification of the *A. hydrophila* strains was achieved. These findings are in agreement with those of **Abbott *et al.*** (**2003**), **Garrity** (**2005**) and **Cai *et al.*** (**2012**)**.** On the other hand**,** the bacterial isolates of V. *alginolyticus* and *V. parahaemolyticus* coincide with those of **Elamparithi and Ramanathan** (**2011**), **Lee *et al.*** (**2015**) and **Eissa *et al.*** (**2019**) **.**

In this study, the sequence of the universal 16SrRNA gene was used to identify *A. hydrophila* with a typical homology at 625 pb **(Gordon *et al.*, 2007**). Additionally, the PCR analysis for the detection of virulence genes revealed that, all bacterial isolates of *V. alginolyticus* and *V. parahemolyticus* strains contained collagenase and toxR genes at their specific segment sizes 737bp (**Abu-Elala *et al.*, 2016**) and 368 bp (**Kim *et al.*, 1999**) , respectively. This findings could explain the detected haemorrhagic, ulcerative and septicaemic signs of the infected fish in the current study.

Notably, the parasitological examination revealed the existence of two *acanthocephalan* parasites isolated from the intestine: *Acanthosentis tilapiae* (**Bayoumy *et al.*, 2006**) and *Neochinorhincus* sp. (**de la Cruz *et al.*, 2013**). The number and arrangement of hooks were the basis for identification to genus level. Additionally, the existence of the *E. heterostomum* excysted metacercaria resembles that detected in the study of **Taher (2009**). Total prevalence of *acanthocephalan* and vibriosis coinfection (56.6%) were more than *acanthocephalan* and MAS coinfection (53.3%) as many *Vibrio* spp. prefer entrance at foregut (**Chen *et al.*, 2008**); the favorable site for *acanthocephala*n parasites (**Ebtsam *et al.*, 2017**). *Acanthocephalan*s facilitate entrance of opportunistic bacteria through the damaged intestinal villi **(El-Mansy, 2011),** and they attract more local immunity (**Dezfuli *et al.*, 2015**) that leads to a decrease in the external surface defense mechanism of the fish. Besides, the leakage of blood protein, due to their feeding behavior (**Szalai & Dick, 1987**), may affect the serum protein level used for the production of immunoglobulins which opsonizes pathogens (**Janeway *et al.*, 2004**).Thus, the pathogenicity and the incidence of vibriosis was higher than Motile Aeromonas Septicemia. The prevalence of *E. heterostomum* coinfections with Motile Aeromonas Septicemia was 30.8%, while together with vibriosis, the percentage was 46.2%. These results may be attributed to the fact that, the mechanical injures resulting from cercarial penetration are related to the transmission of opportunistic bacteria through the dermal route (**Abdelaziz *et al.*, 2017**). *E. heterostomum* EMC mostly affects kidneys and hepatopancreas (**Caffara *et al.*, 2016**), which are hematopoietic organs responsible for immunity and detoxification. Hence, it gives a chance for more opportunistic bacterial infections (**Yildiz *et al.*, 2005**). Regarding seasonal prevalence, MAS and vibriosis in *O. niloticus* infested with *acanthocephalan* parasites, results revealed 53.3% and 56.6% in summer. This may be due to the high *acanthocephalan* infestation rate in this season (57.1%). Vibriosis increased by increasing intestinal *acanthocephalans* opening more portals for entry (**Abram *et al.*, 2017**). Besides, the increase of temperature during summer (**Austin and Austin, 2007**) causes an imbalance of physicochemical water quality parameters that may act as potential environmental stressors. Hence, the aforementioned condition would predispose fish to infectious agents; either bacterial or parasitic (**Plumb *et al.*, 1976**). Winter was the lowest season of coinfection (10%) between MAS and vibriosis in fish infested with *acanthocephalan* parasites, this may be attributed to the good immunity of fish at 15°C (**Abbas *et al.*, 2008**), but vibriosis is able to infect fish though its favorable site (intestinal tract) (**Chen *et al.*, 2008**). In this study, Motile Aeromonas Septicemia and vibriosis in *E. heterostomum* infested *O. niloticus* were the highest prevalence 50% and 100% were recorded in summer season respectively, and 0% were the lowest prevalence recorded in winter season; this may be due to the availability of intermediate hosts of these parasites at these seasons and increase the feeding activity in warm temperature (**Shehata *et al.*, 2018**).

Concerning the histopathological findings, the liver, kidney and spleen are known for their affinity to trap the circulating pathogens, and so, they act as the target organs for many diseases (**Agius and Roberts, 2003**). In the current study, several histopathological changes were revealed degeneration and necrosis of secondary lamellae of infected Nile Tilapia gills with congestion in some areas, these agreed with the findings of . Kidney showing vacuolar degeneration and necrosis of renal tubules and focal leukocytic infiltration. Spleen showed severe destruction of hematopoietic tissue and mild hyperplasia of melanomachrophage cells.. Our results were much like the findings found in other fish species in previous investigations (**Mahdy *et al.*, 2017**) and (**Eissa *et al.*, 2019**) in *Sparus auratus* and *Mugil cephalus* infected with vibriosis and *Euclinostomum* spp. The hepatopancreas revealed advanced vacuolar degeneration in hepatocytes. Some hepatic cells were necrotic and lack nuclei. The pancreatic acini showed degeneration and necrosis of the pancreatic cells and congested pancreatic vessels. These findings were in concordance with that reported by **Ismail *et al.*** (**2011**), who recorded the same results but in *Mugil capito* infected with vibriosis. These lesions could be attributed to the lack of oxygen resulting from gills degeneration (**Yardımcı and Aydın, 2011**) and bacterial toxins. Moreover, the activation and hyperplasia of melanomacrophage cells were evident in the spleen of infected fish. These pigmented cells may appear in the form of a cluster where it indicates the positive presence of neutral carbohydrate and melanin (**Agius and Roberts, 2003**) .

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