
Studies on Vibriosis among Some Marine Fishes in Lake Tamsah

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Abstract

During the current study, a total of 160 marine fishes of two different species represented as: seabass (*Dicentrarchus labrax*) and *Tilapia zillii* randomly collected from Lake Tamsah at Ismailia governorate from August 2015 to July 2016. Seventy-six of fishes found to be infected with both *V. anguillarum* and *V. parahaemolyticus*, as Gram negative, short rod bacilli as comma shaped and motile bacteria. *D. labrax* was the most infected fish spp 41 %, pursued by *Tilapia zillii* 35 %. The highest prevalence of vibriosis among naturally infected marine fishes was recorded during summer 67.5 %, pursued by spring 55 %, then autumn 40 %, while the minimal prevalence 27.5 % was registered during winter. Molecular investigation of pure isolates using sets of both universal and specific primers gave PCR products with clear band of 120 and 519 bp lengths, respectively. Experimentally infected fish subjected to a dose (10^7) of *V. anguillarum* presented higher morbidity and mortality rate up to 80 %. Most of infected fish showed skin ulceration in the ocular side, others displayed redness and scattered hemorrhagic spots on the abdomen with fin erosion. The histopathology of naturally infected marine fishes exhibited severe inflammatory reactions manifested by leukocytes infiltration with activation of melanomacrophage centers as well as tissue necrosis.

Keywords: Marine fishes, *Vibrio* spp, Lake Tamsah, Seasonal prevalence, Experimental infection.

Introduction

Aquaculture mainly pisciculture represented one of the most effective solutions for our nutritive problems and promising hope to cover the gap in food consumption. In the future, there are many worth due to excessive use of water resources that will be the most limiting factor to be considered in

aquaculture development, especially freshwater resources. Therefore, seawater is the immediate alternative sources for mariculture. Aquatic diseases are contributed one of the most threatening problems facing fish industries particularly, during fish rearing and husbandry. Like others, marine fish spontaneously exposed to several

diseases, especially induced by bacteria. About 10% of annual fish losses is due to diseases; more than 50% of these losses are due to bacterial pathogens (*Freund et al., 1990*). *Vibrio* species are considered one of the most prominent pathogen frequently affecting a wide range of fish species (*Lee et al., 2002; Alcaide, 2003*). Vibrionaceae constitutes 60% of the heterotrophic bacteria population that widely distributed in the coastal seawaters and /or brackish ones. As aquaculture production and the consumption of aquaculture products increase, the possibility of contracting zoonotic infections also increases. The zoonotic transmission of vibrio was through the consumption of raw or undercooked shellfish or via eroded skin and wound during exposure to warm seawater (*Levine et al., 1993; Wachsmuth et al., 1994; Gomathi et al., 2013*).

Nowadays, serious attempts for further detection of vibriosis were considered not only for its economic impact but also for its public health importance. Using the advanced techniques for proper diagnosis still not fully elucidated. Therefore, the current study aimed to demonstrate the most characteristic clinical picture of vibriosis in some naturally infected marine fishes in Suez Canal area at Ismailia Governorate, isolate the causative agent using plating techniques and molecular approach, evaluate the total and seasonal

prevalence of such disease, and finally, clarifying the histopathological alterations associated with that disease.

Material and Methods

1- Sampling and processing

160 marine fishes of two different marine species represented as 80 seabass (*Dicentrarchus labrax*) and 80 *Tilapia zillii* of different average body weight and lengths were collected randomly and seasonally. They were examined freshly from Lake Tamsah in Ismailia Governorate from August 2015 to July 2016. For further investigation and bacteriological assays, specimens of either live or freshly moribund fishes were kept in a strong plastic bags supplied with compressed air, sealed in a large ice box and were rapidly transferred to the laboratory of Fish Diseases and Management Department at Faculty of Veterinary Medicine, Suez Canal University. For experimental trials, acclimated *Tilapia zillii* weighing 60 ± 6 g were randomly distributed by two closed seawater systems comprised by two flat-bottomed aquaria (6.5 L). Each aquarium contained 10 fish with aerated seawater and were left to acclimate for one week prior to bacterial challenge. The dissolved oxygen was maintained around 5 mg/L, water temperature at 22 ± 1 °C, and a 12 hr light/12 hr dark photoperiod was adopted. The fishes were fed twice-daily to apparent satiety with commercial pellets. Ammonia and

nitrite levels were measured twice a week and never exceeded 0.025 and 0.3 mg/L, respectively. Only apparently healthy fish, as indicated by their activity and external appearance were used in the experiments.

2- Bacteriological assay

Fresh specimens were examined immediately following collection. The samples were taken aseptically from the lesions, in the external body surface, liver, kidneys and spleen. The initial isolation was done on tryptic soy agar (TSA) supplied with different concentrations of sodium chloride (1.5-8 %). After that, the inoculum streaked on specific medium (TCBS) and left incubated for 24 hr at 30 ± 1 °C. The recovered suspected colonies were picked up and purified for further analysis. The morphology, culture and biochemical characteristics of all isolates were performed according to the criteria of Bergey's Manual of Determinative Bacteriology (Baumann and Furniss, 1994) and confirmed using commercial API-20E strips following manufacture procedures.

3- Molecular approach

For molecular diagnosis, the DNA of purified adjusted bacterial suspension was extracted using DNA zole (Invitrogen, USA) following manufacturer's instructions. To ensure that the isolates were belong to genus vibrio spp., four sets of either universal (567 F, 680 R) or specific primers

(rpoN-ang5' F, rpoN-ang3' R) were selected according to *Thompson et al. (2004) and Gonzalez et al. (2003)*, respectively. PCR reaction mixtures (25 µl) were simultaneously amplified in T Gradient Thermo cycler (Biometra) and Mastercycler personal (Eppendorf) apparatus using a commercial kit of Green Master Mix (NZYtech). All samples were then subjected to initial denaturation at 95 °C for 3 min, followed by 30 cycles of denaturing at 95 °C for 1 min, annealing 65 °C for 1 min, extension and acquiring signal at 72 °C for 30 s. DNA from pure cultures of *V. anguillarum* was included as a positive control, whereas molecular biology water was used as a negative control. Amplified products were detected by horizontal 1% (w/v) agarose gel electrophoresis for 45 min at 100 V in TAE 1× (0.04 M Tris, 0.0001 M EDTA, pH 8.0) electrophoresis buffer, visualized Using 15 µl of DNA gel stain (Invitrogen) and photographed under UV light and computer digitized (Gel Doc 100, Bio-Rad). A 100 bp ladder (SOL BIODYNE) was used as a molecular mass marker.

4- Experimental trial

It was performed following the Universal Directive on the protection of animals used for scientific purposes. Two groups of ten *T. zillii* each was randomly distributed in two identical water systems filled with 8 L of aerated seawater. Thereafter, fish were left

to acclimate for five days prior to bacterial challenge. Subsequently, fish in one of those systems (1 tank, n = 10) were intraperitoneal challenged with *V. anguillarum* according to *Avci et al. (2012)* with 0.1mL of 5.5×10^8 cfu/mL bacterial suspension in NaCl 0.15M (bacterial load: 5.5×10^7 cfu). Fish in the other system were challenged with a same volume of sterile marine broth (MB) and served as controls. Challenged fish were kept under observation for 7 days. Specific mortalities were recorded by plating the aseptic samples of skin lesions; kidneys and gills from the moribund fish on TCBS agar. Cumulative mortalities including the pathognomonic lesions were compared to those obtained following natural infection to specify the pathogen virulence.

5- Histopathological examination

Specimens for histopathological techniques were freshly collected from affected organs and tissues of naturally and experimentally infected fishes. Specimens were then trimmed, fixed in 10% phosphate buffered formalin, rinsed under tap water for 24 h, dehydrated in different concentrations of alcohol, cleared in xylol, and finally embedded in paraffin wax. After that, samples were desiccated into thin sections of 5-micron thickness and finally stained with H&E stain for microscopic examination according to *Roberts (2001)*.

Results

1- Clinical signs and Postmortem inspection

Most of naturally infected marine fishes displayed hemorrhages on the external body surface particularly, at caudal and anal fins. Others showed abdominal distention, red fins, ulceration close to the gill cover and behind the mouth, eroded gill, and corneal opacity (Photo. 1, 2). The necropsy findings revealed pale liver, engorged spleen, and necrotized gill. Some cases in advanced stage showed severe congested gills with marbling appearance, distended abdomen with serous fluids and partially stricken liver (Photo 3-5).

2- Bacteriological assay

The colonies on TSA were medium sized (2-3 mm in diameter) and creamy whitish in color; whereas on TCBS showed swarming activity and displayed two different colors either yellow or green. For salt tolerance assessment, all isolates showed visible turbidity following inoculation into tryptone broth supplemented with different concentrations of NaCl (0, 3, 8 %). Moreover, all isolates grew well at 20, 30 and 40°C following inoculation into tryptic soya broth (TSB), and often showed a visible turbidity. All isolates were resistant to vibriostat O/129-10µg and sensitive to vibriostat O/129-150 µg. On blood agar, the bacteria showed a clear zone of hemolysis after 18 hr of incubation. Microscopically, isolates were Gram- negative, comma shaped

scattered in arrangement and motile. The Biochemical analysis using API 20 E strips showed 87% similarity to *V. parahemolyticus* with serial code 4367104. Molecular diagnosis of all pure isolates using universal and specific primers gave PCR products with clear band of expected 120 and 519 bp lengths (Fig. 1,2), respectively.

3- Prevalence of vibriosis among naturally infected marine fishes

The total prevalence of vibrios among naturally infected was found 47.5 %. The highest seasonal prevalence was recorded in summer 67.5 %, pursued by spring and autumn 55 and 40%, respectively; whereas the lowest prevalence was recorded during winter season 27.5 %, (Table. 1). It was observed that the majority of infection during summer, autumn and winter seasons was due to *V. anguillarum* 66.6, 62.5, and 54.5%, respectively while in spring was due to *V. parahemolyticus* 63.6%, (Table. 2). Regarding fish species, the highest prevalence was recorded in seabass (*D. labrax*) 51.3 % while the lowest one was recorded in *T. Zillii* 43.7 %, (Table. 3). The majority of infection in both fish species was mediated by *V. anguillarum* 73.2, 60%, respectively, (Table. 4).

The seasonal prevalence of vibrios in naturally infected seabass (*D. labrax*) was 75 % in summer perused by spring 60%, then autumn and winter 40 , 30 %, respectively while in case of *T. zillii* species it was 60, 50, 40, and 25%,

respectively, Tables (5, 6). Concerning the intensities of *Vibrio* in different organs, the highest value was recorded in kidney 39.4 %, followed by liver 30.7 % then spleen 21.2% and gills 8.6%. In both kidney and liver the higher percentage was due to *V. anguillarum* 62.5, 75.6%, respectively while in case of spleen and gills was due to *V. parahemolyticus* 63.6, 66.7%, respectively, (Table. 7).

4- Experimental challenge

The morbidity and mortality of the experimentally infected *T. zillii* was assessed for 2 weeks post inoculation. All fish infected with the higher dose (10^7) presented higher morbidity and mortality rate (up to 80%). Most of infected fish showed skin ulceration in the ocular side (Photo. 6), others displayed redness and scattered hemorrhagic spots on the abdomen with fin erosion (Photo. 7). Internally, all fishes exhibited hemorrhagic gills, necrotized livers and severely congested kidneys (Photo. 8).

5- Histopathological findings

The gill lamellae revealed either hyperplasia or sloughing in the secondary gill lamellae with leukocytic infiltration and edema as well as congestion in the primary gill lamellae (Photo. 9). The gill arch showed vacuolar degeneration in the epithelial covering, hyaline and/or Zenker's necrosis in the muscular layer and edema as well as congestion together with mononuclear cells

infiltration (Photo. 10). Liver showed vacuolar degeneration and/or coagulative necrosis of hepatocytes. The hepatic vessels were dilated and the majority of erythrocytes were hemolyzed with marked hemosiderosis (Photo. 11). The pancreas exhibited congestion and marked degeneration (Photo. 12). Kidneys displayed tubular nephrosis mainly vacuolar degeneration and/or necrosis in the renal epithelium. Focal depletion of hematopoietic tissue was evident

(Photo. 13). The melanomacrophages showed either proliferation as well as activation or atrophy and necrosis (Photo. 14). Spleen exhibited depletion of lymphoid follicles where the lymphocytes were scarce and necrotic. Hemolysis of erythrocytes was evident with marked parenchymal hemosiderosis. The melanomacrophages centers (MMCs) showed atrophy with necrosis of melanomacrophage cells (Photo. 15).

Table (1): Showing the total prevalence of vibriosis among the examined seabass *D. labrax*, and *Tilapia T. zillii*

Season	No. of specimens	No. of infected fish	Percentage (%)
Summer	40	27	67.5
Autumn	40	16	40
Winter	40	11	27.5
Spring	40	22	55
Total	160	76	47.5

Table (2): Showing the total prevalence of vibrio isolates among naturally infected seabass *D. labrax*, and *Tilapia T. zillii*

Season	<i>V. anguillarum</i>		<i>V. parahemolyticus</i>	
	No	(%)	No	(%)
Summer	18	66.6	9	33.3
Autumn	10	62.5	6	37.5
Winter	6	54.5	5	45.5
Spring	8	36.4	14	63.6
Total	42	55.3	34	44.7

Table (3): Showing the prevalence of vibriosis among the examined seabass *D. labrax*, and *Tilapia T. zillii*

Fish species	No. of examined fish	No. of infected fish	Percentage (%)
<i>D. labrax</i>	80	41	51.3
<i>T. zillii</i>	80	35	43.7
Total	160	76	47.5

Table (4): Showing the prevalence of vibrio isolates among naturally infected seabass *D. labrax*, and Tilapia *T. zillii*

Fish species	<i>V. anguillarum</i>		<i>V. parahemolyticus</i>	
	No	(%)	No	(%)
<i>D. labrax</i>	30	73.2	11	26.8
<i>T. zillii</i>	21	60	14	40
Total	51	67.1	25	32.9

Table (5): Showing the seasonal prevalence of vibriosis among the examined seabass *D. labrax*

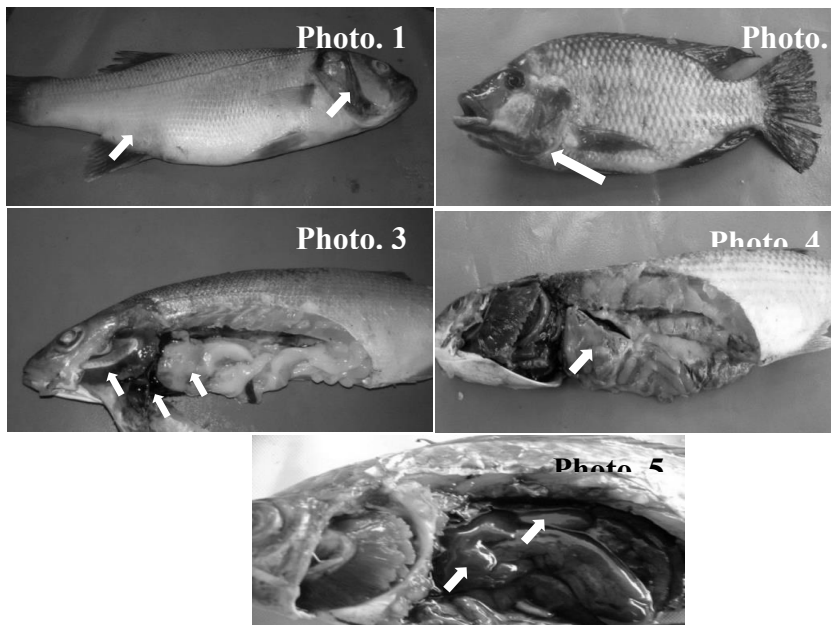
Season	No. of specimens	No. of infected fish	Percentage (%)
Summer	20	15	75
Autumn	20	8	40
Winter	20	6	30
Spring	20	12	60
Total	80	41	51

Table (6): Showing the seasonal prevalence of vibriosis among the examined Tilapia. *T. zillii*

Season	No. of specimens	No. of infected fish	Percentage (%)
Summer	20	12	60
Autumn	20	8	40
Winter	20	5	25
Spring	20	10	50
Total	80	35	44

Table (7): Showing the intensities of Vibrio isolates in different organs of naturally infected seabass *D. labrax*, and Tilapia *T. zillii*.

Organs	Total isolates	Total percentage (%)	Bacterial isolates			
			<i>V. anguillarum</i>		<i>V. parahemolyticus</i>	
			No	%	No	%
Liver	32	30.8	20	62.5	12	37.5
Kidneys	41	39.4	31	75.6	10	24.4
Spleen	22	21.2	8	36.4	14	63.6
Gills	9	8.6	3	33.3	6	66.7
Total	104	100	62	59.6	42	40.4



Photos (1-5): Naturally infected seabass *D. labrax* with *Vibrio* spp. showing abdominal distention, red fins, eroded gill cover, and corneal opacity (Photo.1), pale liver, congested spleen, and necrotized gill (Photo. 3), severe congested gills, and partially stricken liver (Photo.4). Naturally infected *T. zillii* with *Vibrio* spp. showing skin ulceration close to the gill cover and under the mouth (Photo. 2), congested liver and serous fluids in the abdominal cavity (Photo. 5).

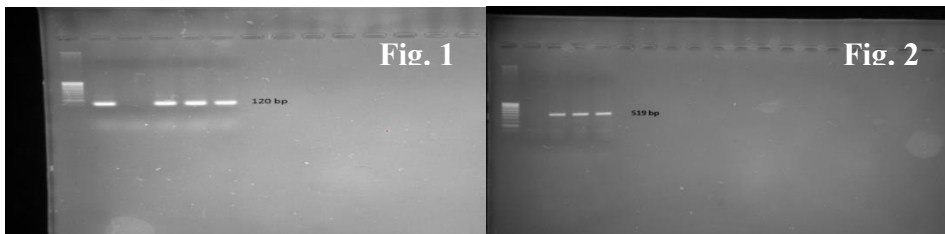


Fig. 1: PCR product obtained from pure isolates of *Vibrio* spp. using two sets of universal primers (567F and 680R) described by *Thompson et al. (2004)*. Lane 1, molecular weight ladder; lane 2, a positive control; lane 3 a negative control; lanes 4-6, the specific DNA product of about 120 base pairs (bp) amplified from isolates.

Fig. 2: PCR product obtained from pure *V. anguillarum* isolates using two sets of specific primers (rpoN-ang5' F and rpoN-ang3' R) described by *Gonzalez et al. (2003)*. Lane 1, molecular weight ladder; lane 2 a negative

control ; lane 3 positive control; lanes 4-5, the specific DNA product of about 519 base pairs (bp) amplified from isolates.

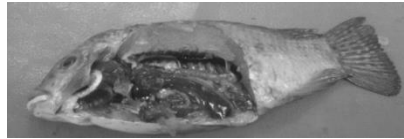
Photo. 6



Photo. 7



Photo. 8



Photos (6-8): Experimentally infected *T. zillii* with *V. anguillarum* showing skin ulceration in the body side (Photo. 6), redness and scattered hemorrhagic spots on the abdomen with fin erosion (Photo. 7), hemorrhagic gills, necrotized livers and severely congested kidney (Photo. 8).

Photo 9

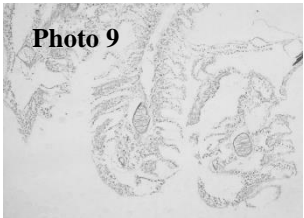


Photo 10

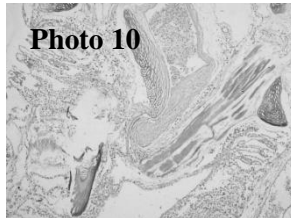


Photo 11

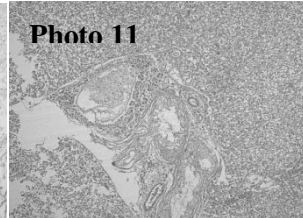


Photo 12

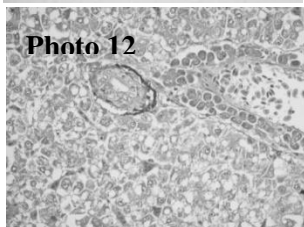


Photo 13

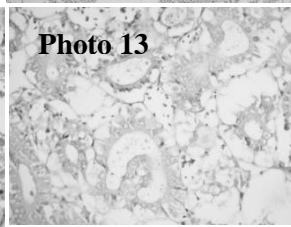


Photo 14

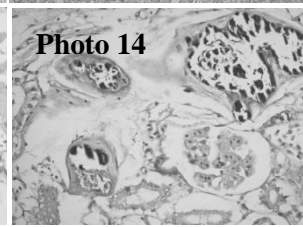
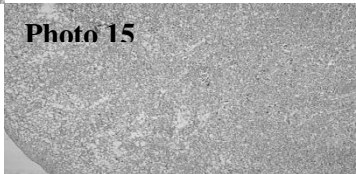


Photo 15



Photos (9-15): Naturally infected *T. zillii* with *Vibrio*. Spp, showing sloughing in the secondary gill lamellae with leukocyte infiltration and edema as well as congestion in the primary gill lamellae (Photo. 9), hyaline and/or zenkers necrosis in the muscular layer with edema, congestion and mononuclear cells infiltration (Photo. 10), vacuolar degeneration and coagulative necrosis of hepatocytes with congestion and marked

hemosiderosis (Photo. 11), congestion and vacuolar degeneration and necrosis in both hepatocytes with activation of melanomacrophage cells (Photo. 12), tubular nephrosis mainly vacuolar degeneration and coagulative necrosis in the renal epithelium with focal depletion of hematopoietic tissue (Photo. 13), proliferation as well as atrophy and necrosis of melanomacrophages center (Photo. 14), and depletion of lymphoid follicles, scarce lymphocytes, marked parenchymal hemosiderosis and melanomacrophage cells necrosis (Photo. 15).

Discussion

The current study revealed that the total prevalence among the examined species was 48%. Those values were extremely high and to some extent exceeded our expectation as we deal with not only wild fishes but also with seawater, which itself was considered a predisposing factor for the existence and the viability of numerous microorganisms including vibrio. Furthermore, it reflects the degree of pollution expelled from ships in addition to the industrial and domestic effluents that continually travel through the Lake. These results is in agreement with *Doukas et al. (1998)* who explained the hazardous effect of waste water drainage as a stressful factors that potentiate the opportunistic bacterial pathogens among fish population.

Concerning the clinical picture, most of naturally infected fish displayed hemorrhages on the external body surface, abdominal distention, red fins, eroded gill cover, and corneal opacity. Gills sometimes are hemorrhagic or pale as a consequence result of disease progression. The post mortem

inspection revealed pale liver, congested spleen, and necrotized gill. Abdomen was distended and filled with bloody fluid. These results nearly similar to those observed by *Golomazou et al. (2006); Robert and Moeller (2012), and Eissa et al. (2013)*. All signs of hemorrhagic septicemia were attributed to bacterial haemolysins that lysate host erythrocytes (*Zhang and Austin 2005*). *V. anguillarum* for instance has several extracellular haemolysins (VAH 1–5), associating with the haemolytic activity of the pathogen (*Rodkhum et al., 2005*). Moreover, *Li et al. (2008)* recognized repeat in toxin (rtx) operon which also contributed to the hemolytic activity of the strain. Furthermore, *V. parahaemolyticus* produced thermostable direct hemolysin (TDH) (*Nishibuchi et al., 1992*). Their biological activities including erythrocytes hemolysis, cytotoxicity, lethality, and altered vascular permeability were previously evaluated in several species (*Honda et al., 1988*).

Concerning the morphological characters of *Vibrio* spp, the present study revealed that they were gram-

negative, motile straight to slightly curved rods grow on marine agar and TCBS following incubation at 25°C for 24 -48 hours. Suspected colonies were creamy whitish medium sized on TSA; whereas on TCBS showed swarming activity and displayed two different colors either yellow or green. These results are in concordance with those obtained by *Stephen and Jones (2002)*; *Sabir et al. (2013)*. All isolates under discussion were motile, sensitive to O/129 vibriostatic disc and Novobiocin. They were positive in respect to, TDA, LDC, ODC Aesculin, Indole, VP, Catalase, Gelatin, and gave variable results for citrate test, while they were negative for ONPG, ADH, URE and H₂S production. They produced acid only from glucose and mannitol. These results are nearly similar to those obtained by *Roberts (2001)*, and *Zorrilla (2003)*. It was known that 66% of *V. anguillarum* isolate exhibited false negative or positive reactions for CIT, GEL, SOR, RHA and AMY, and was identified as *A. hydrophila (Santos et al., 1993)*. Based on this argument, the suspected *V. anguillarum* isolates of yellow colored colonies was not subjected to the test. Only *V. parahaemolyticus* isolates of green colony were evaluated and the code number on API20E strips was 4367104. Similar results were recorded by *Martinez-Urtaza et al. (2006)*.

Using conventional methods as a tool for pathogen recognition may fall short when a bacterium exhibit unusual phenotypic profile, beside the fact that they require long time to peruse. Recently, nucleic acid-based methods broadly used to identify the most threaten pathogens in aquaculture (*Osorio et al., 1999, Cepeda and Santos, 2000*). Detection of *V. anguillarum* using PCR technique was first reported by *Hirono et al. (1996)*, based on a hemolysin gene of this bacterium. *Gonzales et al. (2003)* developed other technique based on primers derived from rpoN gene. The detection limit was one cell per reaction in case of pure culture of the bacteria, or 10-100 bacterial cells per reaction in case of fish tissue contaminated samples, which corresponds to 2000 - 20000 bacterial cell per gram of fish tissue. This technique is highly sensitive and required 5 hours of work. Based on this argument, the evaluation of all pure isolates were conducted using two sets of universal and specific primers described by *Thompson et al. (2004)* and *Gonzalez et al. (2003)*, respectively. The end result gave PCR products with clear band of expected 120 and 519 bp lengths. Regarding the total prevalence of vibrio septicemia, the current result reported that 47.5 % of the infected fish were positive for vibrio septicemia. These results are in compatible with those observed by *Khan et al. (1981)* who attributed

the mortalities (28%) among captive *Gadus morhus* L. during fin rot outbreaks to vibrio spp. It was observed that the majority of infection during summer, autumn and winter seasons was due to *V. anguillarum* while in spring was due to *V. parahemolyticus*. Additionally, the majority of infection in both examined fish species was mediated by *V. anguillarum*. Thus, explained the ubiquitous of *V. anguillarum* in marine coastal waters, and it represented as an opportunistic pathogen for various marine fishes (**Muroga, 2001**).

Regarding the seasonal prevalence, the highest prevalence was recorded in summer season; whereas the lowest prevalence was recorded in winter season. Our results were in agreement with (**Roberts et al., 2001 and Moustafa et al., 2010**). The highest summer prevalence proposing that the pathogen could survive in marine sediment and reintroduced to the water column when temperatures increased. Thus, was attributed to the ability of *V. anguillarum* to utilize skin mucus which massively increase during summer as a chemoattractant and thereby enhanced the entry of that pathogen into the host (**O'toole et al., 1999**). This argument was confirmed by **Austin and Austin (2007)** who mentioned that vibrios outbreak frequently occur when water temperature exceeds 15 °C. Moreover, **Su and Liu (2007)** mentioned that in winter season

when water temperatures are unfavorable, *V. parahemolyticus* may be undetectable. On the other hand, **Margaret (1987)** correlated the higher incidence of vibriosis among salmonid populations to the high water temperature. The negative effect of raised temperature besides the decrease of DO level could create a stressful condition which intern decreased the host resistance and gave the chance for opportunistic bacterial pathogen to be established into the host (**Gilles et al., 1998**).

Concerning the intensities of vibrio in different organs of naturally infected fish, the highest value was recorded in kidney, followed by liver then spleen and gills. In both kidney and liver the higher percentage was due to *V. anguillarum* while in case of spleen and gills were due to *V. parahemolyticus*. Thus, explained that the spleen, liver and kidney are the main target organs of *V. anguillarum*. These results are in agreement with those observed by **Moustafa et al. (2010) and Abou ElGeit et al. (2013)**. Regarding the experimental infection, it was observed that apparently healthy *T. zillii* subjected to high concentration of *V. anguillarum* (10^7) cells mL⁻¹ showed nearly the same clinical signs and postmortem lesions found in naturally infected fish. Mortality rate was 80 % and most infected fish died within one-week post infection. Thus, was attributed to the lethal and toxic effect of the

bacteria proteases and extracellular which in turn induce tissue or cell damage (*Nottage and Birkbeck, 1987*). The fish of control group showed no mortalities until the end of the trial, indicating that the mortalities recorded in the treated fish were only attributed to the experimental challenge. These results were in agreement with those obtained by *Moustafa et al. (1990)*, and *Badran and Eissa (1991)*.

Concerning the histopathological alterations associated with vibrios, the current study showed vacuolar degeneration and/or coagulative necrosis of hepatocytes and pancreatic cells. The portal area exhibited marked degeneration with mononuclear cell infiltration. Eosinophilic infiltration, together with degranulation of mononuclear cell suggested the phagocytic activity of that cells and their role in inflammatory constricture (*Roberts, 2001*). Kidneys displayed tubular nephrosis mainly vacuolar degeneration and/or necrosis in the renal epithelium. Focal depletion of hematopoietic tissue was evident. The melanomacrophages showed either proliferation as well as activation or atrophy and necrosis. Haemosiderin pigment deposition was also noticed inside the renal tissues. The pigment deposition indicates further haemoglobin dissociation influenced by hemolysins produced by the bacterium (*Zhang and Austin, 2005*). Spleen exhibited depletion of

lymphoid follicles where the lymphocytes were scarce and necrotic. Thus could either attributed to the influence of bacteria extracellular products that induced necrosis in the haemopoietic elements *Ghoneum et al. (1986)* or, to the immunosuppressive action of corticosteroids that released in response to any stressful condition. Hemolysis of erythrocytes was evident with marked parenchymal haemosiderosis. The melanomacrophages centers showed atrophy with necrosis of melanomacrophage cells. The depletion and fragmentation of melanomacrophage cells was a sequential process of MMCs hyperactivity, which reflect their major role in chronic inflammation (*Hjeltnes and Roberts, 1993*). MMCs hyperactivity was due to rapid elimination of bacteria from peripheral circulation that subsequently settle in the haemopoietic tissues. Similarly, *Roberts (1975)* explained briefly the main function of MMCs in the deposition of resistant pathogens. Gills showed mononuclear cell infiltrations in the gill arch and gill lamellae. The gill arch showed vacuolar degeneration in the epithelial covering, hyaline and/or zenkers necrosis in the muscular layer and edema as well as congestion together with mononuclear cells infiltration. The gill lamellae revealed either hyperplasia or sloughing in the

secondary gill lamellae with leukocyte infiltration and edema as well as congestion in the primary gill lamellae. Thus could be a spontaneous response from the host to altered environmental conditions following exposure to bacterial infections (*Villamil et al., 2003*). Gill damage has a passive effect on ionic exchanges (*Morrison et al., 2001*) which in turn facilitate pathogen entry and disease progression (*Moraes and Martins, 2004*). All these results were in agreement with those obtained by *Korun and Timur (2008)*. These alterations were attributed to the extracellular enzymes especially, proteases, and other toxins produced by *Vibrio* isolates that in turn induced tissue and cell damage (*Howard and Buckley, 1985; Li et al., 2008*).

In conclusion, the plentitude of vibrios in coastal environments has been linked to water temperature, molecular approach act as a fundamental tool for proper and fast recognition of vibrio isolates.

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ملخص عربي

تم تجميع 160 عينة من الأسماك البحرية من نوعين مختلفين تمثلت في أسماك القاروص والبلطي الأخضر بطريقه عشوائية وموسميه من بحيرة التمساح فرع محافظة الاسماعيلية في المواسم المختلفة ابتداء من اغسطس 2015 وحتى يوليو 2016. وقد تم تسجيل معظم العلامات المرضية التي ظهرت على هذه الأسماك فوجد ان حوالي 76 من هذه الأسماك مصابه بنوعين من بكتيريا الفيبريو وهي سالبه الجرام عصويه الشكل تشبه حرف الضمة ومتحركة. أظهرت النتائج أن أعلى إصابة بمرض الفيبريو في الأسماك التي تم فحصها كانت في أسماك القاروص بنسبة 51% ويليه أسماك البلطي الأخضر بنسبة 44%. كما اثبتت الدراسة ان أعلى نسبة إصابة موسمية سجلت في فصل الصيف بنسبة 68% يتبعها الربيع بنسبة 55% ويليه الخريف 40% ثم فصل الشتاء بنسبة 28%. أيضا تم تقييم جميع العزلات النقية باستخدام بادئات عالمية ومحددة خاصه بعزلات الفيبريو وأوضحت النتائج ظهور اختبار تفاعل البلمرة المتسلسل بطول جزئي 120 و 519 بيز بير. كما اوضحت اختبارات الإصابة المعملية ان هذا الميكروب قادر علي احداث معدلات اعتلال ونفوق بنسبة تصل الي 80%. وقد كشفت الدراسات الهستوباثولوجيه لبعض من هذه الأسماك المصابة وجود زيادة في بعض الخلايا المناعية داخل انسجتها مع وجود بعض الخلايا التالفة والميته.