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Natural Parasitic and Bacterial Coinfection in Some Fish Species in Egypt

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ABSTRACT

The present study surveyed the natural concurrent parasitic-bacterial infection among some fish species from different aquatic environments in Egypt. A total of 379 moribund freshly dead fish with different body weights were collected in 2019; 259 (*Oreochromis niloticus* and *Tilapia zilli*) freshwater fish were seasonally taken from the Nile River in Giza, while 120 (*T. zilli* and *Solea aegyptiaca*) marine water fish were collected in summer from Manzala and Qaroun Lakes at Fayoum province. Fish were examined for parasites. Bacteria were isolated from infested fish and identified at the genus level. Examination of freshwater fish exposed the presence of *Clinostomum* and/ or *Euclinostomum* spp., while only *Pseudomonas* was isolated. Between seasons, summer's both total infestation and total concurrent infection prevalences of 43.59% and 73%, respectively, were significantly the highest. Within parasite, the prevalence of *Clinostomum* infestation alone with 77.27%, 70.31% and 76.09% for winter, spring and autumn, respectively, was higher than summer with only 47.06%. Conversely, the prevalence of concurrent infection in summer for *Clinostomum* alone (63%) was significantly higher than in winter (18%). For both of parasites together, infestation prevalence in summer with 26.47% was significantly the highest. Examination of marine water fish revealed the presence of at least one isopod, and *Vibrio*, *Pseudomonas* and *Aeromonas* were isolated. The prevalence of concurrent *Vibrio* infection (64.0%) was significantly higher than 26.0% and 52.0% for *Pseudomonas* and *Aeromonas*, respectively. Total infestation prevalence in freshwater (90.35%) was significantly higher than in marine water (41.7%). Alternatively, the total prevalence of concurrent infection in marine water (96.0%) was significantly higher than in fresh water (56.41%). In conclusion, the light was spotted on the effect of increasing temperature and the high burden of natural parasitic and bacterial concurrent infection. Further investigation is required to refine the relationship between parasites and bacteria for more epidemiological knowledge that aids in disease prevention and control.

INTRODUCTION

Global climate change is one of the most serious environmental threats facing the world's ecosystems. Temperature plays a key role in the occurrence and transmission of aquatic parasitic diseases that primarily take place during the warm summer period **(Karvonen *et al.*, 2010)**. Increasing temperature can increase the seasonal abundance,

virulence, timing and transmission efficiency of pathogens, leading to higher total prevalence of disease and more widespread of epidemics **(Karvonen *et al.*, 2010; Chiaramonte *et al*., 2016)**.

The presence of numerous pathogens in fish can pose a significant problem for both cultured and wild fish, particularly in subtropical countries such as Egypt **(Elamie, 2001)**. Pathogenic microorganisms in aquatic habitats can cause problems to economically important fishes as a consequence of their secondary invasion on the fish's body, which have been primarily infested with parasites **(Ravichandran *et al.*, 2016)**. A number of pathogenic bacteria can cause infection in fish, including *Pseudomonas* spp., *Aeromonas* spp. and *Vibrio* spp **(Ayoub *et al.*, 2021).**

The majority of fish diseases research and studies focus on one cause and neglect other factors though most fish diseases are multifactorial **(Younes *et al*., 2016)**. The synergistic effect caused by parasites reduce the immunity of fish resulting in increasing mortality in parasitized/ bacteria co-infected fish **(Salama & Yousef, 2020)**. Many studies have experimentally demonstrated this parasitized/ bacteria co-infection **(Bandilla *et al.*, 2006; Xu *et al.*, 2007; Zhang *et al.*, 2015)**. However, limited studies have documented natural co-infection. Therefore, the present study was conducted to survey the natural concurrent parasitic-bacterial infections among some fish species from different aquatic environments in Egypt.

MATERIALS AND METHODS

1. Fish sampling

Total of 379 moribund freshly dead fish of different body weights were collected from different localities in Egypt from January to December 2019; 259 (131 *Oreochromis niloticus* and 128 *Tilapia zilli*) fish were seasonally taken from the freshwater environment of the Nile River at Al Bahr Al Aazam, Giza, while 120 (44 *T. zilli* and 76 *Solea aegyptiaca*) fish individuals were collected during summer from marine environment of Manzla Lake and Lake Qarun in Fayoum province. Samples were transported in an ice-box with minimum delay to the laboratory for examination**.**

2. Parasitological examination

Fish samples were macroscopically examined for parasites. Skin, gills, fins, buccal cavities and kidneys were inspected for *Clinostomum* and *Euclinostomum* metacercarial cysts according to **Mahdy *et al.* (2022)**. For isopod, fish samples were examined for any gross lesions and/or isopod parasites on the body surface, buccal cavity, branchial cavity and fins as described in the study of **Mahmoud *et al*. (2016)**. Located parasites were dislodged from the host tissues, washed three times using phosphate buffer saline then kept in 70% ethyl alcohol according to **Hanson and Ow (1982)**.

3. Bacterial isolation

Fish surfaces were swapped with 70% ethyl alcohol. A triangular incision technique was applied for opening the fish specimens. Swabs were aseptically taken from each organ (liver, kidney, spleen and gills), cultured onto brain heart infusion broth (BHIB; HIMEDIA, India) and incubated at 28°C for 24h. A loopful of the obtained broth culture was streaked on the selective media Thiosulfate-citrate- bile salt- Sucrose agar (Vibrio selective media) (TCBS; HIMEDIA, India), *Aeromonas* Isolation Medium Base, supplemented with rehydrated ampicillin (HIMEDIA, India), and *Pseudomonas* Agar Base, supplemented with cetrinix supplement (HIMEDIA, India) (HIMEDIA, India). For marine isolates, 2% NaCl was added to media. The inoculated plates were incubated at 27±1°C for up to 24hr. Morphologically, similar and dominant colonies were selected and streaked onto nutrient agar plates for 24h at 27±1°C. Pure colonies were transferred to nutrient agar slants, and the purified strains were stored in BHI + 15% (vol/ vol) glycerol at - 20°C.

Purified isolates were identified based on colony growth characteristics. Gram staining, motility, cytochrome oxidase and catalase test of each isolate were used as preliminary microbiological approaches. The bacteria isolates were identified according to schemes of biochemical tests provided by **Holt *et al.* (1994)** and **(Buller, 2004**).

The extraction of bacterial genomic DNA was performed using boiling technique according to **Devi *et al*. (2009)**. PCR reaction was performed using genus-specific primers (Table 1). The reaction mixture was performed using 6.5µl RNase-free water, 0.5µl of each set of primers, 12.5µl 2x PCR master mix and 5µl genomic DNA to reach the final volume of 25µl. Reaction cycles were performed using TC-25/H thermal cycle. Table (2) shows the cycling conditions. The amplicons were electrophoresed on 1.5% agarose gel stained with ethidium bromide (0.5µg/ ml), and the sizes of the amplified product were determined by a 100- bp DNA Ladder (GeneDirex). The resulting fragments were visualized by UV transillumination.

Table 1. Primers sequences

Species	*Oligonucleotide sequences (5'-3')*	*product*	*Reference*
Vibrio species	*F: CAGGCCTAACACATGCAAGTC*	*700 bp*	**(Montieri *et al.*, 2010)**
	R:GCATCTGAGTGTCAGTATCTGTCC		
Pseudomonas species	F:GACGGGTGAGTAATGCCTA	618 *bp*	**(Spilker *et al.*, 2004)**
	R: CACTGGTGTTCCTTCCTATA		
Aeromonas species	F: GAAAGGTTGATGCCTAATACGTA	625 *bp*	**(Gordon *et al.*, 2007)**
	R: CGTGCTGGCAACAAAGGACAG		

Table 2. Cycling conditions of the different primers during cPCR

Gene	**Initial denaturation**	**Amplification (30 cycle)**			**Final extension**
		Denaturation	**Annealing**	**Extension**	
Vibrio 16SrRNA	95 °C for 10 min	95 °C for 1min	55 °C for 1 min	72 °C for 1.5min	72°C for 5 min
Pseudomonas 16SrDNA	94˚C for 5 min	94˚C for 30 sec	57 °C for 1 min	72 °C for 1 min	72°C for 10 min
Aeromonas	94˚C for 5 min	94˚C for 30 sec	50°C for 40 sec	72°C for 50 sec	72 °C for 10 min

4. Statistical analysis

Analyses were performed using SPSS software® version 20 (IBM Corp, USA). The Chi-square or Fisher's exact test was used to determine differences in parasite prevalence between and within seasons and the prevalence of concurrent infection. $P< 0.05$ was considered significant.

RESULTS

1. Clinical examination

Gross examination of freshwater fish (*O. niloticus* and *T. zilli*) collected from Bahr Azam, Giza revealed skin darkness, detached scales, excessive secretion of mucus, cloudiness of eyes, destructed dorsal fin, distended abdomen and the presence of hemorrhagic lesions on skin, fins and gills. For internal organs' examination, some appeared pale and anemic, while others were enlarged and congested. Intestine was filled with yellowish exudate. Macroscopic examination exposed the presence of *Clinostomum*

and/or *Euclinostomum* spp. encysted metacercariae (EMC). *Clinostomum* EMC were found in the buccal cavity as yellowish-white nodules or orange pea- like cysts (Fig. 1), while *Euclinostomum* EMC were embedded in kidney tissues as greyish black spherical cysts. The excysted metacercaria of *Clinostomum* were elongated and whitish yellow in color, while that of *Euclinostomum* appeared large leaf- like (Fig. 2).

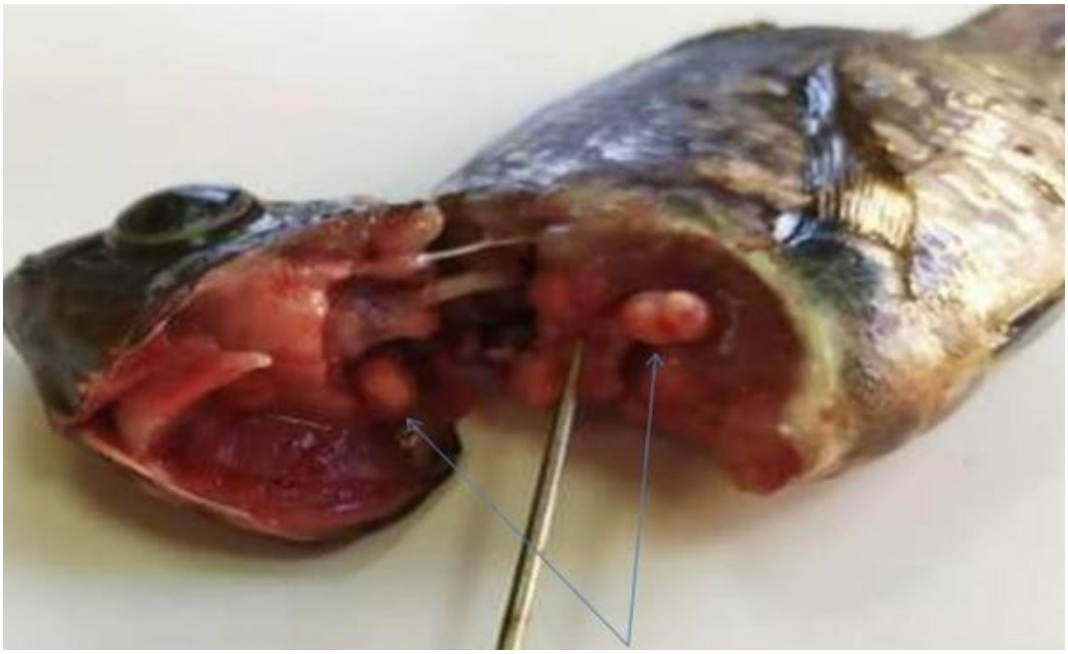

Fig. 1. *Clinostomum* EMC appeared in the buccal cavity of *T. zilli* as yellowish-white nodules (arrows)

Gross examination of marine water fish (*T. zilli* and *S. aegyptica* collected from Manzla and Qaroun Lakes, respectively) showed hemorrhages in different areas of the body (fins, gills, around mouth and anus) exophthalmia, corneal opacity, separated scales and ulcers. Gills were congested and swollen spleen, liver and kidney also showed congestion and enlargement. In other advanced cases, liver appeared pale. Macroscopic examination revealed the presence of at least one isopod in gill chamber (unilateral or bilateral) or on the body surface attached to skin, with slight protrusion of gill cover (Fig. 3).

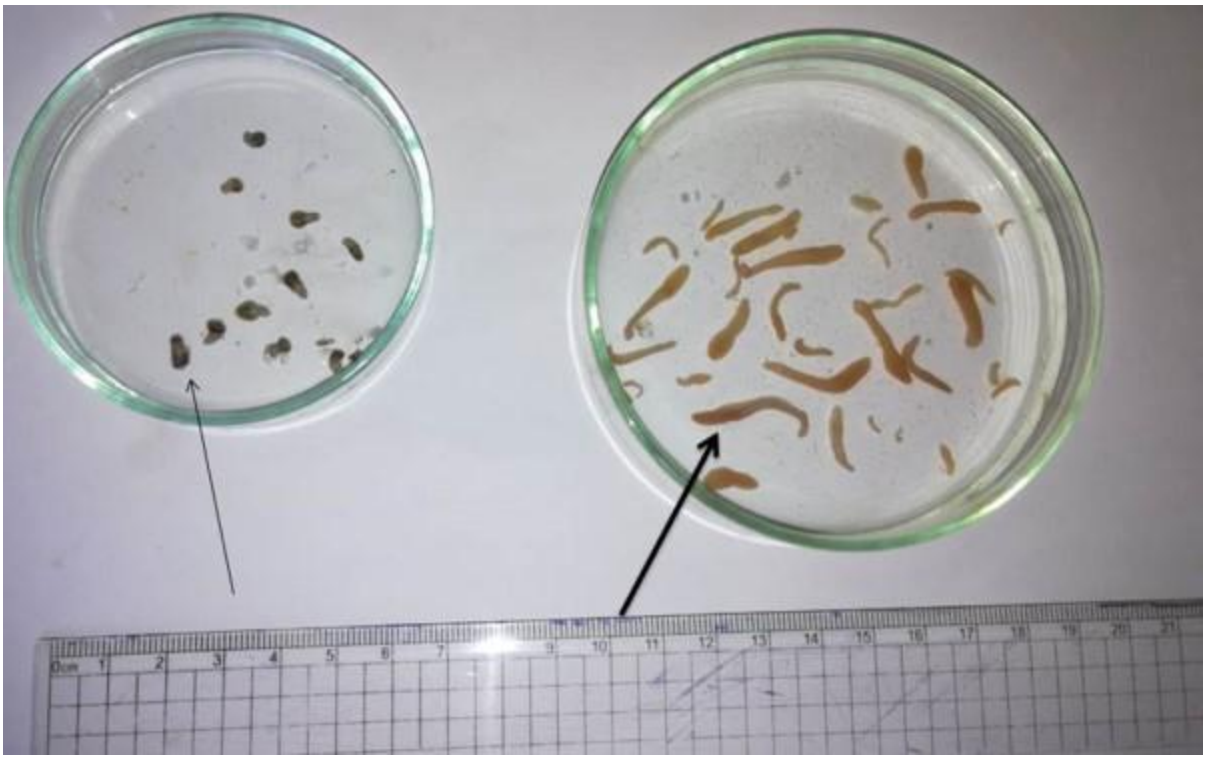

Fig. 2. Excysted metacercaria of *Clinostomum* (Thick black arrow) and excysted metacercaria of *Euclinostomum* (Thin black arrow)

Fig. 3. Infested *T. zilli* showing bilateral isopod in gill chambers, darkness of skin, emaciation and hemorrhage all over the body.

2. Bacteriological examination

Vibrio, Aeromonas and *Pseudomonas* species were isolated from the infested fish samples. On the TCBS media, *Vibrio* colonies were large yellow and sticky while on *Aeromonas* isolation base medium. *Aeromonas* colonies were dark green and rounded. on *Pseudomonas* base media, and *Pseudomonas* colonies were yellow. All isolates were

motile, oxidase and catalase positive. *Vibrio* species were indol and methyl red positive but citrate negative, whereas *Aeromonas* species were indol and citrate positive but methyl red negative. *Pseudomonas* species were indol and vogus-proskour negative.

Based on phenotypic identification, the 16SrDNA universal *Vibrio* primers produce positive 700bp amplicons in all isolated *Vibrio* species, while the presence of *Pseudomonas* species was confirmed by targeting the genus specific 16SrDNA at 618bp. The presence of *Aeromonas* species was confirmed by the detection of partial *AH* gene with 625 bp.

3. Prevalence of parasitic infestation and bacterial concurrent infection

From freshwater environment, only *Pseudomonas* was isolated. Table (3) shows the prevalence of *Clinostmum* and/or *Euclinostomum* species infestation and its concurrent infection with *pseudomonas* spp. in *O. niloticus* and *T. zilli* fishes in different seasons of the year.

For *O. niloticus*, total infestation prevalence over the year was 100% (131/131), while the natural concurrent *Pseudomonas* infection was 64.89% (85/131). Between seasons, summer's both total infestation and total concurrent infection prevalence of 45% and 86%, respectively, were significantly the highest compared to winter, spring and autumn, with 9.16%, 25.19% and 20.61%, respectively, for total infestation prevalence and 33%, 64% and 33%, respectively, for total concurrent *Pseudomonas* infection. During a single season, the prevalence of *Clinostmum* infestation was detected alone; covering all seasons; its prevalence was higher than that recorded for *Euclinostomum* alone or both parasites together. For a parasite throughout different seasons, the prevalence of *Clinostomum* infestation alone was recorded with 83.33%, 69.7% and 74.07% in winter, spring and autumn, respectively. Estimates were higher than those recorded in summer (42.37%). On the other hand, the prevalence of concurrent infection in summer for *Clinostomum* alone (76%) was significantly higher than that recorded in winter (20%). For *Euclinostomum* alone or both parasites together, no significant differences were detected either for infestation or concurrent infection prevalence.

For *T. zilli*, total infestation prevalence over the year was 80.47% (103/128), while the natural concurrent *Pseudomonas* infection was 45.63% (47/103). Between seasons, summer's total infestation of 41.75% was significantly higher than winter, spring and autumn, with 9.71%, 30.1% and 18.45%, respectively. No significant difference was observed between seasons in the total concurrent infection prevalence that recorded 30%, 48%, 53% and 32% for winter, spring, summer and autumn, respectively. During a single season, no significant difference was observed in the prevalence of any parasite considering all the seasons. Within parasite for different seasons, again no significant difference was observed in the prevalence of any parasite. Alternatively, for *Clinostomum* alone (48%). it was significantly higher than that of winter (14%). For *Euclinostomum* alone, no significant difference was seen in concurrent infection prevalence between

seasons. For the two parasites together, documented prevalence of 100% concurrent infection in winter and autumn was significantly different than that recorded during spring and summer, with 75% and 60%, respectively.

For the freshwater environment, total infestation prevalence over the year was 90.35% (234/259), while the natural concurrent *Pseudomonas* infection was 56.41% (132/234). Between seasons, summer's both total infestation and total concurrent infection prevalence of 43.59% and 73%, respectively, were significantly the highest compared to winter, spring and autumn, with 9.4%, 27.35% and 19.66%, respectively, for total infestation prevalence and 32%, 56% and 33%, respectively, for total concurrent *Pseudomonas* infection. Total infestation prevalence for *Clinostomum* was 72.2% (187/259), while for *Euclinostomum*, it was 34.36% (89/259). Within a season, the prevalence of *Clinostmum* infestation alone in all seasons was higher than that of *Euclinostomum* alone or both together. With respect to parasite in different seasons, the prevalence of *Clinostomum* infestation alone with 77.27%, 70.31% and 76.09% for winter, spring and autumn, respectively, was higher than that of summer with only 47.06%. Conversely, prevalence of concurrent infection in summer for *Clinostomum* alone (63%) was significantly higher than that recorded in winter (18%). Within *Euclinostomum* alone, no significant differences were seen either for infestation or concurrent infection prevalence. For both parasites together, the prevalence of infestation in summer (26.47%) was higher than that registered in winter, spring and autumn, with 9.09%, 14.06% and 8.69%, respectively. However, no significant difference was seen in concurrent infection prevalence of both parasites between seasons.

From marine water environment, *Vibrio*, *Pseudomonas* and *Aeromonas* were isolated. In addition, mixed infection of different combinations of two or the three species was recorded. Table (4) shows the prevalence of isopod species infestation and its concurrent infection in *T. Zilli* from Manzla Lake and *S. aegyptica* from Qaroun Lake.

In Manzla Lake, infestation prevalence of *T.Zilli* was 47.7% (21/44). The prevalence of concurrent *Vibrio* infection (85.7%) was significantly higher than 61.9% for *Aeromonas*. The prevalence of both *Vibrio* and *Aeromonas* was 52.4% (11/21)

Table 3. Prevalence of *Clinostmum* and/or *Euclinostomum* species infestation and its concurrent infection with *Pseudomonas spp* in *O.niloticus* and *T.zilli* fishes in different seasons of the year from the Nile River at Bahr Azam, Giza.

Season	Parasite	*O.niloticus*			*T.zill*			Total		
		Infested n (%)	Infected n	Concurrent infection%	Infested n (%)	Infected n	Concurrent infection%	Infested n (%)	Infected n	Concurrent infection%
Winter	*Clino*	10 (83.33)[a] *	2	20[b]	7 (70)	1	14[b]	17 (77.27) [a *]	3	18 [b]
	Euclino	1 (8.33)	1	100	2 (20)	1	50	3 (13.64)	2	67
	Both	1 (8.33)	1	100	1 (10)	1	100*	2 (9.09)	2	100
	Total	12 (9.16)	4	33	10 (9.71)	3	30	22 (9.4)	7	32
Spring	*Clino*	23 (69.70) [a] *	11	48	22 (70.97)	9	41	45 (70.31) [a *]	20	44
	Euclino	5 (15.15)	5	100	5 (16.13)	3	60	10 (15.63)	8	80
	Both	5 (15.15)	5	100	4 (12.90)	3	75	9 (14.06)	8	89
	Total	33 (25.19)	21	64	31 (30.1)	15	48	64 (27.35)	36	56
Summer	*Clino*	25 (42.37) [b] *	19	76[a]	23 (53.48)	11	48[a]	48 (47.06) [b *]	30	63 [a]
	Euclin	17(28.81)	16	94	10 (23.26)	6	60	27 (26.47)	22	81
	Both	17(28.81)	16	94	10 (23.26)	6	60	27 (26.47) [a]	22	81
	Total	59 (45.0)**	51	86**	43 (41.75)**	23	53	102 (43.59)**	74	73**
Autumn	*Clino*	20 (74.07) [a] *	5	25	15 (78.95)	4	27	35 (76.09) [a *]	9	26
	Euclino	4 (14.81)	2	50	3 (15.79)	1	33	7 (15.22)	3	43
	Both	3 (11.11)	2	67	1 (5.26)	1	100*	4 (8.69)	3	75
	Total	27 (20.61)	9	33	19 (18.45)	6	32	46 (19.66)	15	33
Total		131	85	64.89	103	47	45.63	234	132	56.41

Table 4. Bacterial spp. isolated from isopod infested fish in Manzla and Qaroun Lakes

	T.Zilli (Manzla lake)	*S. aegyptica* (Qaroun lake)	Total
No. of examined fish	44	76	120
No. of infested fish	21 (47.7%)	29 (38.2%)	50 (41.7%)
Vibrio spp. concurrent infection	18 (85.7%)*	14 (48.3%)	32 (64.0%)*
Pseudomonas spp. concurrent infection	0 (0%)	13 (44.8%)	13 (26.0%)
Aeromonas spp. concurrent infection	13 (61.9%)	13 (44.8%)	26 (52.0%)

Note: mixed pathogens' infection was noticed. * indicates significant difference between infections.

In Qaroun Lake, infestation prevalence for *S. aegyptica* was 38.2% (29/76). No significant difference was seen between the prevalence of concurrent infection of *Vibrio*, *Pseudomonas* or *Aeromonas* infection (48.3%, 44.8% and 44.8%, respectively). Mixed infections of two or three pathogens were observed in 10 out of 29 infested fish (34.5%)

For the marine water environment, total infestation prevalence was 41.7% (50/120), while total concurrent infection was 96% (48/50). Prevalence of concurrent *Vibrio* infection (64.0%) was significantly higher than 26.0% and 52.0% for *Pseudomonas* and *Aeromonas*, respectively.

In comparing freshwater and marine water environments, total infestation prevalence in freshwater (90.35%) was significantly higher than marine water (41.7%). On the contrary, total prevalence of concurrent infection in marine water (96.0%) was significantly higher than freshwater (56.41%).

DISCUSSION

In addition to *Vibrio*, *Pseudomonas* and *Aeromonas* are considered the most important stress- related- bacterial fish diseases. Predisposing factors such as presence of ectoparasites influence the disease or mortality in fish populations **(Moraes and Martins, 2004)**. In this study, we surveyed the natural parasitic bacterial coinfection of these pathogens in some fish species and environments.

In Egypt, Clinostomatid metacercariae are very common parasites of *Tilapia* sp. **(Abou-Eisha *et al.*, 2008; Eissa *et al.*, 2011).** In our study, examination of freshwater fish (*O. niloticus* and *T. zilli*), collected from the Nile River at Bahr Azam, Giza, showed its infestation with *Clinostomum* and/or *Euclinostomum* spp. EMC. The description of *Clinostomum* spp. agreed with **(Abdel-Latif, 2007) and (Shaheen *et al.*, 2014),** while picture of *Euclinostomum* spp. matched with **(Hassan *et al.*, 2012) and (Shaheen *et al.*, 2014).** Total infestation prevalence over the year was 90.35% (234/259). Total infestation prevalence for *Clinostomum* was 72.2% (187/259), while for *Euclinostomum* was 34.36% (89/259). Our recorded prevalence is higher than **(Ahmed *et al.*, 2018)** who reported total infestation prevalence of 43.3% (52/120), *Clinostmum* infestation prevalence of 39.6% and *Euclinostmum* infestation prevalence of 15% in *O. niloticus* fish caught from

the River Nile at El-Minia district. The difference in total infestation prevalence between Giza and El El-Minia districts may be attributed to pollution level of the River Nile as nearly the same prevalence were recorded by (**Simon-Oke, 2017)** who compared the prevalence in the unpolluted and polluted ends of the river in Nigeria and stated that "The fish hosts in the polluted end of the river harboured the highest percentage of parasites (71.18%) against (43.50%) parasites recovered from the unpolluted end". Also, Our results of *Clinostmum* infestation was higher than 62.25% reported by Taher, (2009)**.** For *Euclinostomum* infestation, this study prevalence was also higher than 18.3% and (25.88 ± 5.00) reported by **Shaheen *et al.*, (2014)** and **(Mahdy *et al.*, 2022),** respectively. The difference in prevalence is probably due to different study place, sample size, water quality and/or abundance of aquatic snails (intermediate hosts) and aquatic birds, which are responsible for completing some digenetic trematodes' life cycles.

Concerning seasonal prevalence, Summer was significantly the highest with 43.59% infestation prevalence against 9.4%, 27.35% and 19.66% for Winter, Spring and Autumn respectively. These results were in complete accordance with **(Shaheen *et al.*, 2014)**. This may be due to increasing the release rate of temperature-dependent cercariae from snail hosts and successful transmission to fish **(Elsheikha and Elshazly, 2008;Ibrahim and Soliman, 2010).** The prevalence and intensity of EMC decreased during winter and cold seasons due to death of the temperature dependent cercariae/metacercariae **(Taher, 2009).** Within season, prevalence of *Clinostmum* infestation alone, in all seasons, was higher than that of *Euclinostomum* alone or both EMC together. Within parasite for different seasons, prevalence of *Clinostomum* infestation alone with 77.27%, 70.31% and 76.09% for Winter, Spring and Autumn, respectively was higher than summer with only 47.06%. This comparable prevalence of *Clinostomum* infestation alone in Summer with the rest of seasons is due to the increased infestation of *Euclinostomum* EMC alone and both EMC together which resulted in lowered percentage of *Clinostomum* infestation in Summer.

Pseudomonads spp. regarded as serious fish pathogens that induce ulcerative syndrome and hemorrhagic septicemia **(Eissa *et al.*, 2010)**. In the present study, total prevalence of *pseudomonas* spp. among freshwater fish was 50.97% (132/259), while its concurrent infection was 56.41% (132/234). This percentage was higher than **(Abd El Tawab *et al.*, 2016)** who recorded 17% prevalence among Nile tilapia fish in Kaliobia Governorate. Between seasons, Summer's concurrent infection prevalence of 73% was significantly the highest over Winter, Spring and Autumn. These results agreed with **(Enany *et al.*2019)**.

Cymothoid isopods are blood feeders crustacean parasites, settling in the gill chambers or the surface of the fish leading to sever destruction of fish**(Eissa *et al.*, 2012)**. About infestation of marine fish, isopod infested fish showed sever emaciation, slight protrusion of gill cover (operculum), atrophy, hemorrhage at site of attachment and

hemorrhages all over the body. This result in agreement with those recorded by **(Eissa *et al*., 2012), (Younes *et al*.,2016) and (Rashed *et al.*, 2021)**.

Regarding isopod infestation prevalence, our study found that total prevalence of infested marine fish with isopod species was 41.7%. This percentage was higher than 20.30% recorded by **(Khalaf-allah and Yousef, 2019)** among *S. solea* fish that infested with *Livoneca Redmanii* in Qaroun lake. Also, it was higher than 32.66% prevalence reported by **(Mahmoud *et al.*, 2016)** among *D. Labrax, S. vulgaris* and *T. zilli* fish species infested with isopod species in Qaroun lake. The rate was lower than that recorded by **(Ali and Aboyadak, 2018)** as it was 53.3%. These variations in prevalence may be linked our small sample size and short investigation period which was limited to summer season only. We recorded high isopod Infestation during summer which was in accordance with **(Shaheen *et al.*, 2017)** who also noticed highest infestation of isopod (90%) among *S. solea* in summer. Increased water temperature and salinity during summer may facilitate isopod infestation **(Aneesh *et al.*, 2013; Shaheen *et al.*, 2017)**.

For the prevalence of concurrent bacterial infection from marine water, *Vibrio spp.* was the most significant representing 64 % of infested fish followed by *Aeromonas* with 52.0% and *Pseudomonas* spp. with the lowest frequency of 26%**.** These results were higher than **(El-Dakroury *et al*., 2020)** who recorded that percentages of *Vibrio*, *Pseudomonas*, and *Aeromonas* among marine fish in Damietta governorate was (28.1%) (16.1%), (22.1%), respectively. The significant higher prevalence of *Vibrio* may be related to the supportive stress factors of isopod infestation, high salinity, high temperature and mechanical injuries of fish **(Abdelaziz *et al.*, 2017)**.

In comparing freshwater and marine water environments, total infestation prevalence in freshwater (90.35%) was significantly higher than marine water (41.7%). The lower total infestation level of Manzla and Qaroun lakes could be related to the size of the parasite which makes isopood infestation a visible problem that already attracts continuous efforts of the state to eradicate it and conserve the ecosystem of the lakes. On the other hand, the higher infestation rate in the Nile River may be the result of high pollution level at Giza due to high population density and different anthropogenic activities along the banks of the river.

On the contrary, total prevalence of concurrent infection in marine water (96.0%) was significantly higher than freshwater (56.41%). This could be attributed to the type of water body. The benefit of closed water body of the lakes prevents continuous pollution as in the open water of the Nile River. However, considered a drawback in getting rid of introduced pathogens from the place by continuous dilution of running water.

In conclusion, this study has spotted a light on the effect of increasing temperature and the high burden of natural parasitic and bacterial concurrent infection in both fresh and marine water environments. Further investigation is required to refine the relationship between parasite and bacteria for more epidemiological knowledge that aids in disease prevention and control.

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