Egyptian Journal of Aquatic Biology & Fisheries
Zoology Department, Faculty of Science,
Ain Shams University, Cairo, Egypt.
ISSN 1110 – 6131
Vol. 28(5): 1223 – 1241 (2024)
www.ejabf.journals.ekb.eg

Parasite –Bacteria Co-Infection in the Egyptian Common Sole (*Solea solea*) by *Livoneca redmanii* and *Aeromonas veronii*

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ARTICLE INFO
Article History:
Received: Sept. 17, 2024
Accepted: Sept. 26, 2024
Online: Sep. 29, 2024

Keywords:
Qarun Lake,
Cymothoid isopod,
Aeromonas,
Brown colonies

ABSTRACT

In the present research, samples of *Solea solea* (Linnaeus) were randomly collected from Qarun Lake, Egypt, for routine parasitological, microbiological, and histopathological examinations. The parasitological analysis revealed that 6.70% of the examined samples were infested with the cymothoid isopod *Livoneca redmanii*. This parasite was isolated as females from the branchial cavity and as juveniles from the skin of the affected fish. Bacteriological examination identified *Aeromonas* species isolated from the gills, kidney, and liver of *S. solea*. Morphological, biochemical characterization, and molecular analysis confirmed the isolate as *Aeromonas veronii*. The isolated strain produced brown colonies on tryptic soy agar (TSA) supplemented with 3% NaCl. The drug susceptibility test indicated that *Aeromonas veronii* was sensitive to gentamicin, erythromycin, and trimethoprim/sulfamethoxazole, while exhibiting resistance to nalidixic acid, ampicillin, oxolinic acid, and tetracycline. Histopathological examination of the affected gills revealed edema, leukocyte infiltration, and hyperplasia of mucous cells. The infested skin exhibited sloughing and detachment of the lining epithelium, along with edema and degeneration of the muscles at the site of isopod attachment.

INTRODUCTION

Fish infections usually occur by one or more pathogens such as viruses, bacteria, fungi and parasites. Bacterial infection constitutes the most significant challenge among *Solea solea* population **(Mohd-Aris *et al.*, 2019)**. Several bacterial diseases have been identified in *S. solea*, including vibriosis, black patch necrosis, furunculosis, and red spot diseases **(Moustafa *et al.*, 2010; Abdelazeem *et al.*, 2023)**. Co-infection commonly happens in natural environments where two or more pathogens infect the same host. They compete for resources or target areas within the host. Each pathogen can amplify its effects and contribute to the overall impact when combined with other pathogens **(Bakaletz, 2004)**. Co-infections can impact the host's physiology and its ability to

interact with the surrounding environmental conditions that may result in mass mortality events in both cultured and wild fishes **(Mahmoud *et al*., 2014; Bolanle, 2023)**. *Aeromonas* species are Gram-negative bacteria of the family *Aeromonadaceae* and are known to cause severe diseases in fish **(Fernandez-Bravo & Figueras, 2020; Sherif & Kassab, 2023)**. The presence of this type of bacterium in seawater is rare compared to its occurrence in freshwater, although it can tolerate water salinity levels of up to 3.0% NaCl, classifying it as a halotolerant waterborne pathogen (**Dias *et al*., 2016**). *Aeromonas veronii* is considered a common important pathogen that infects a variety of aquatic animals. It has a wide range of hosts including *Oreochromis niloticus* **(AbdEl Latif *et al*., 2019)**, the channel catfish **(Hoai *et al*., 2019)**, the goldfish *Carassius auratus* **(Shameena *et al*., 2020)**, and the sea bass, *Lateo labrax maculatus* **(Wang *et al*., 2021)**. Infection of fish with *A. veroni* revealed deleterious impacts, such as skin ulcer, exophthalmia, visceral hemorrhage, fin rot and/or tail rot. Moreover, *A. veronii* is known to cause infections in humans, particularly in those with weakened immune systems such as the elderly and children **(Chen *et al*., 2015; Monti *et al*., 2019; Li, *et al*., 2020)**. Cymothoidae (Leach, 1818) is a parasitic isopod family infesting a wide range of marine, brackish, and freshwater fish **(Boyko *et al*., 2008; Yamauchi, 2016)**. Additionally, different parasite species including ichthyobdellid haemo-flagellates, helminthes, copepods and sopods have been observed and reported in *S. solea* throughout its entire range **(Kabata, 2003; Kayis & Ceylan, 2011; Mahmoud *et al*., 2023)**. Among the parasitic isopods infesting *S. solea*, the cymothoid species is notable. Cymothoids are protandrous hermaphrodites that attach to fish hosts at various sites, including the body surface, opercular cavity, and buccal cavity **(Smit *et al*., 2014)**. Their life cycle includes free-swimming mancae that develop into juveniles and adults, at which point their sex switches from male to female **(Williams & Williams, 1998)**. Different cymothoid isopod species have been documented to cause significant damage, potentially leading to mortality among infested fish hosts **(Kawanishi *et al*., 2016; Mahmoud *et al*., 2020)**.

Solea solea (Soleidae, Pleuronectiformes) is an economically important fish species in Egypt, inhabiting the Mediterranean Sea and Bardawil Lagoon **(Mehanna, 2014)**. It also inhabits Qarun Lake, which has suffered from cymothoid isopod invasion since 2014, resulting in the destruction of the lake's fish stock **(Mahmoud *et al*., 2023)**. Despite the synergistic effects of multiple causative agents that can lead to high fish mortality and the prevalence of co-infection in aquatic environments, relatively little attention has been given to the study of multiple pathogens infecting fish species **(Kotob *et al*., 2017)**. Therefore, the present study investigated the natural parasitic-bacterial infections detected during a survey of *Solea solea* inhabiting Qarun Lake, focusing on the synergistic impacts caused by the isolated species.

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MATERIALS AND METHODS

1. Area of investigation

Qarun Lake is situated in the Fayoum Governorate, positioned between longitudes 30°34′ and 30°49′ East and latitudes 29°25′ and 29°34′ North (Fig. 1).

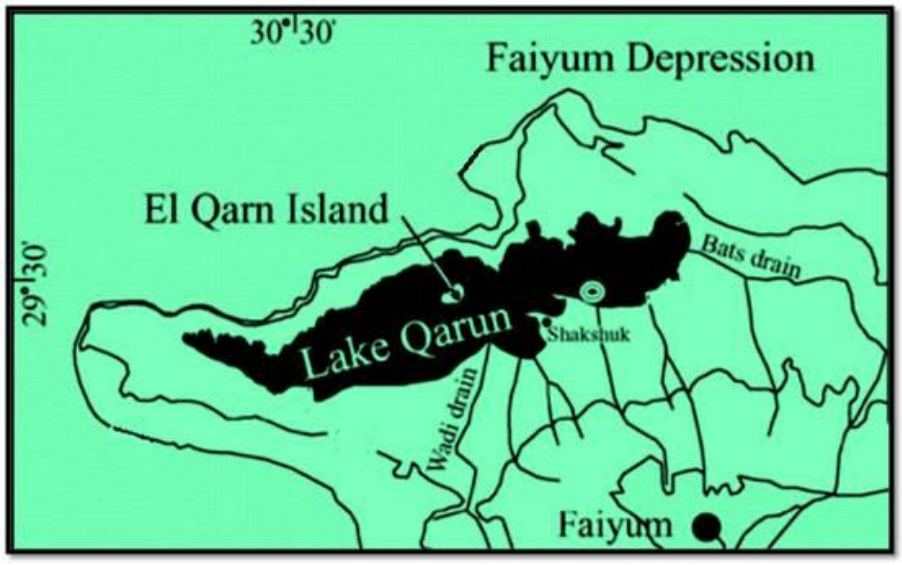

Fig. 1. Map of Lake Qarun

2. Fish sampling

During the periodic examination of *Solea solea* from Qarun Lake, 150 fish of body length 16-23cm were collected during summer 2022. It was noticed that some of the collected fish showed abnormal clinical signs and external body lesions. The affected fish were taken to the Laboratory of Parasitology, Faculty of Veterinary Medicine, Cairo University, and the wet lab of Fish Diseases Department, AHRI, Dokki, for further examination.

3. Parasitological examination

Fish samples were identified and measured to the nearest centimeter (cm) of body length then macroscopically examined for any gross lesions and /or isopod parasites on the body surface, buccal cavity, branchial cavity and fins. The observed isopods were photographed using a digital camera of 12 megapixel. The parasites were dislodged from the fish host tissues, washed in normal saline then kept in 70% ethyl alcohol according to **Hadfield *et al*. (2014)**.

4. Identification of fish and isopod species

The isolated isopods were previously identified through morphological, ultrastructure and molecular characterization as *Livoneca redmanii* by **Mahmoud *et al*. (2023)**. The examined fish species was identified as *Solea solea* (Soleidae, Pleuronectiformes),

according to **Sabatini (2018)**. The prevalence and intensity of parasites were calculated in accordance with **Margolis *et al.* (1982)**.

5. Bacteriological examination

Fish surfaces were swapped with 70% ethyl alcohol. Under aseptic condition, the specimens were dissected and loopful from gills, liver, kidney and spleen were inoculated onto tryptic soy agar TSA (Difco, USA) supplemented with 3% NaCl and incubated at 28°C/24h **(Abolghait *et al.*, 2013)**. Pure colonies were streaked up onto *Aeromonas* selective agar base (Biolife, Italy) supplemented with ampicillin. All cultures were incubated at 28°C/24h. A single bacterial colony was selected for further Phenotypic characterization according to **Bergey (1994)** and **Madigan and Martinko (2005)**. The obtained pure colonies were preserved in TSB broth with 20% sterile glycerol at -80^{o}C.

5.1. Phenotypic and biochemical identification of the isolated bacteria.

The isolated *Aeromonas veronii* was phenotypically characterized through Gram staining. For biochemical profiling, the GN24 KIT (DIAGNOSTICS sro, Hodská 68, Galanta, 924 01, SRO), a commercial system for Gram-negative bacteria, was employed. Results were recorded after adhering to the manufacturer's incubation guidelines.

5.2. Molecular identification of A. veronii

5.2.1 DNA extraction and amplification of 16S rRNA

DNA extraction from bacterial colonies was carried out using the QIAamp DNA Mini kit (Qiagen, Germany, GmbH) with modifications from the manufacturer's recommendations. Metabion (Germany) supplied the oligonucleotide primers; PCR amplification details are listed in Table (1).

Table 1. PCR amplification

Target gene	Primers sequences	Amplified segment (bp)	Primary Denaturation	Amplification (35 cycles)			Final extension	Reference
				Secondary denaturation	Annealing	Extension		
16S rRNA	CTACTTTTG CCGGCGAGC GG TGATTCCCG AAGGCACTC CC	953	94˚C / 5 min.	94˚C /30 s.	50˚C /40 s.	72˚C /50 s.	72˚C/10min.	**Gordon *et al.*,2007**

Analysis of the PCR products was performed by separating them through electrophoresis on a 1.5% agarose gel (AppliChem, Germany, GmbH).

5.2.2 DNA sequencing and phylogenetic analysis

PCR products were purified with the QI Aquick PCR Product Extraction Kit (Qiagen, Germany). Sequence reactions were performed using the BigDye Terminator V3.1 Cycle Sequencing Kit (Perkin-Elmer), and subsequent purification was done with Centrisep spin columns. DNA sequences were obtained using the applied biosystems 3130 genetic analyzer, manufactured by HITACHI in Japan. To determine sequence identity, an initial BLAST® analysis (Basic Local Alignment Search Tool) as outlined by **Altschul *et al.* (1990)** was performed against GenBank accessions. The phylogenetic tree was constructed using the MegAlign module, and phylogenetic analysis was carried out with the neighbor-joining method in MEGA X **(Kumar *et al*., 2018)**.

6. Drug sensitivity tests

A 24 hour bacterial culture was adjusted to a turbidity equivalent to McFarland 0.5 using saline. Then, 0.1mL of this suspension was spread onto Mueller-Hinton agar. After allowing the plates to dry, antibiotic disks (Oxoid), as listed in Table (3), were placed on the surface. The plates were then incubated at 25°C for 24 hours, following the agar diffusion method described by **Quinn *et al*. (2002)**. The diameters of the inhibition zones were interpreted as sensitive, intermediate, or resistant according to the guidelines of **CLSI (2010)**.

7. Histopathological examination

Tissue specimen from gills and skin were immersed in 10% neutral buffered formalin for fixation, and then underwent processing by ascending grades of ethanol for dehydration and by xylene for clearence. Tissues were embedded in paraffin, sectioned using a rotary microtome into 4µm thick tissue sections, and stained with hematoxylin and eosin as well as Giemsa stain **(Suvarna *et al,* 2012)**. Examination and photography of the tissue were conducted using a light microscope equipped with a digital camera.

RESULTS

1. Macroscopic examination

The affected *S. solea* showed hemorrhagic spots on the skin of the ventral body aspect with excessive mucus secretion, gill congestion with protrusion of the gill covers due to the presence of isopods. Adult females were isolated from the gill cavities and juveniles were from the body surface (Fig. 1A, C). Gross examination of internal organs showed severe congestion and enlargement of spleen, liver and kidney (Fig. 1B).

2. Parasitological examination

Prevalence and incidence of parasite infestation

Out of the examined 150 *S. solea* samples, 10 were found infested with the isopod L. *redmanii* (6.70%) with a mean intensity of 1.3± 0.35. The total number of isolated parasites was 13 (4 females, 9 juvenile stages).

3. Bacteriological examination

A. veronii was isolated from 41 of 150 (27.33%) of the examined *S. solea*. *A. veronii* isolate produced brown pigmented colonies on TSA (Fig. 1D) increased pigmentation producing at 4°C and produced yellow colonies on *Aermonas* base media, the pure colonies were gram-negative, rod shape, motile, and oxidase positive.

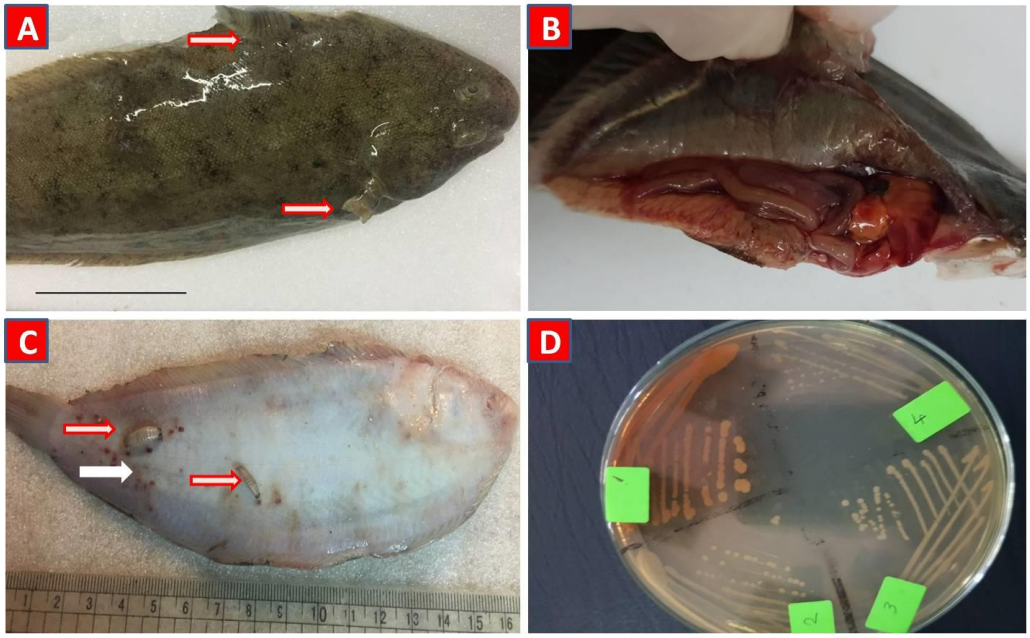

Fig. 1. (A & C) Adult *L. redmanii* females in the gill cavities and juveniles on the body surface of the affected *S. solea* with hemorrhagic spots on the skin of the ventral body aspect. **(B)** Gross examination of internal organs showed severe congestion and enlargement of spleen, liver and kidney. **(D)** *A.veronii* colonies on TSA showing No. 1: The observation showed brown pigment-producing colonies. Numbers 2, 3, and 4 displayed whitish, smooth, non-pigment-producing colonies

3.1. Biochemical characteristics of A.veronii

The results of the biochemical characteristics of *A. veronii* are listed in Table (2).

Table 2. Biochemical characterization of *A.veronii* by GN24 SRO

Characterization	Reaction	Characterization	Reaction
Urea	-	Trehalose	+
Glucose	+	Mannose	+
H2S	-	Lactose	-
Arginine dihydrolase	-	Cellouse	V
Ornithine decarboxylase	V	Maltose	-
Lysine	+	GGT	-

decarboxylase			
SCI	-	PHS	v
BGL	V	GLR	v
PHE	+	Esculin	+
Indole	+	Dulcitol	-
NAG	+	Adonitol	-
Sucrose	+	Sorbitol	-
Raffinose	-	L-rhamnose	-
Inositol	-	BGA	+
Citrate	-	Nitrate	+

3.2. Molecular characterization

The molecular characterization of *A.veronii* was confirmed through PCR at 953bp. The nucleotide sequence of the 16S rRNA genes of *A.veronii* was deposited in GenBank under accession number OL771444. Phylogenetic trees were employed to analyze the relationship between the *A. veronii* isolate and representative isolates from other fish species, as illustrated in Fig. (4) and Table (3).

Fig. 4. The phylogenetic tree exhibited the comparative analysis of 16S rRNA sequence *A. veronii* infected *S. solea*

Table 3. Nucleotide identity

	1	2	3	4	5	6	7	8		Seq
1		100%	99%	100%	100%	99%	99%	99%	1	OL771444.1 A. veronii/ Aero.ver.1
2	100%		99%	100%	100%	99%	99%	99%	2	MW831507.1 A. veronii /REI2021Aero1
3	99%	99%		99%	100%	100%	100%	99%	3	MW426312.1 A. veronii/ Aer1
4	100%	100%	99%		100%	99%	99%	99%	4	MW836109.1 A. veronii/ REE2021Aero2
5	100%	100%	100%	100%		99%	100%	100%	5	KX768735.1 A. veronii/ zy01
6	99%	99%	100%	99%	99%		99%	99%	6	MZ853939.1 A. veronii/ YL-41
7	99%	99%	100%	99%	100%	99%		100%	7	KC210792.1 A. veronii/ SA
8	99%	99%	99%	99%	100%	99%	100%		8	FJ940849.1 A. veronii/ CYJ205
	1	2	3	4	5	6	7	8		

4. Drug sensitivity testing

The *A. veronii* isolate exhibited resistance to nalidixic acid, ampicillin, oxolinic acid, and tetracycline, on the other hand, it was susceptible to other antibiotic drugs, as summarized in the Table (4).

Table 4. Antibiotic sensitivity test to *A. veronii*

Antibiotic sensitivity discs	inhibition zone mm			Result
	R	I	S	
Ampicillin (AM)10mcg	11	12-13	14	0(R)
Erythromycin(E)15 mcg	13	14-22	24	25(S)
Gentamycin(GN)10µg	12	13-14	15	18(S)
Nalidixic(NA)30 µg	13	14-18	19	0 (R)
Oxolinic(OA)2 µg	10	11-12	13	0(R)
Tetracycline(T)30 µg	14	15-18	19	0(R)
Trimethoprim/sulphamthoxazole (SXT) 25µg	10	11-15	16	24(S)

R(Resistance)-S(Senstive)

5. Histopathological findings

Microscopy of the gills of *S.solea* fish revealed edema in the primary and secondary gill lamellae in addition to few leukocytes infiltration (Fig. 5a). By Geimsa stain, the leukocytes infiltration and mucous cells were demonstrated in the edematous gill lamellae (Fig. 5b). The mucous cells in certain areas of the gills also showed hyperplasia (Fig. 5c). Positively stained aggregations of bacteria were noticed in the exudates present in between the gill lamellae (Fig. 5d).

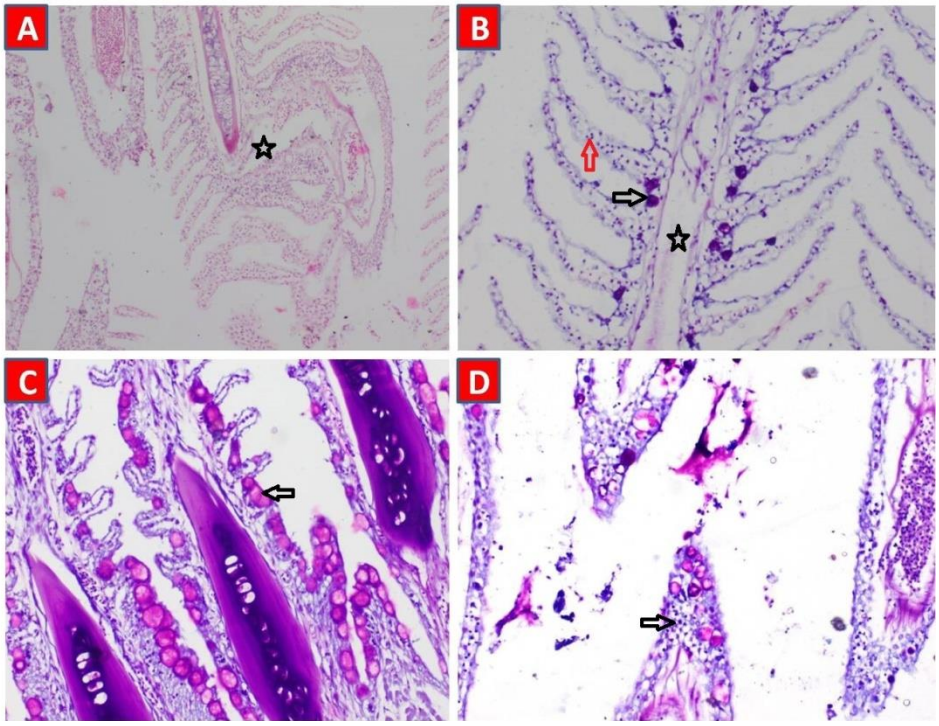

Fig. 5. Histopathology of gills in *Solea solea* fish. **(a)** Edema in the primary and secondary gill lamellae (star) (H&E stain, X400). **(b)** Few mucous cells (arrow) and edema (star) with few leukocytes infiltration in the primary and secondary gill lamellae (red arrow) (Giemsa Stain, X400). **(c)** Mucous cells hyperplasia in primary gill lamellae. (arroew) **(d)** Positively blue stained aggregation of suspected bacteria was observed in the interlamellar exudates (arrow) (Giemsa stain). (X400)

Microscopy of the skin revealed detachment and sloughing of the lining epithelium at the site of parasite leg attachment. The Pereopod parts of the isopod parasite penetrated the skin and was embedded in muscles. The underlaying muscles showed edema and degeneration (Fig. 6a, b).

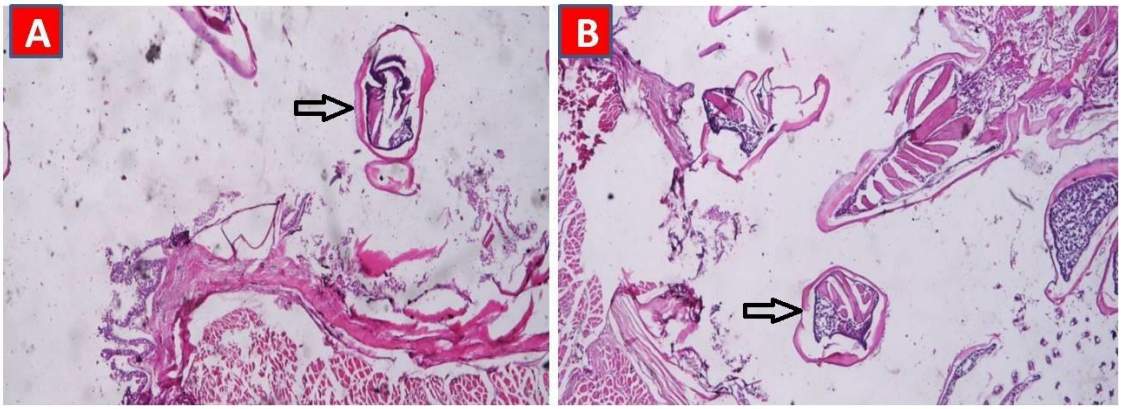

Fig. 6. Histopathology of the fish skin showing **(a)** the pereopod parts of isopod parasite attaching to the skin with detachment and sloughing of the lining epithelium, **(b)** exposure of the underlying mucles which showed edema and degeneration. H&E stain. X 400

DISCUSSION

Co-infections occur when a host is infected by two or more genetically distinct pathogens. Each pathogen is responsible for its own harmful effects and contributes to the overall damage to the host when combined with other pathogens **(Okon *et al.*, 2023)**. Most fish diseases are multifactorial **(Younes *et al.*, 2016)**. Co-infections have a substantial impact on fish health, potentially leading to significant changes in the progression and severity of various fish diseases **(Kotob *et al.*, 2017a)**. The damages caused by the parasite provided a route of entry for invading bacteria **(Xu *et al.*, 2009; Abd-ElGhany *et al.*, 2014)**.

In this research, the isolated cymothoid isopod *L.redmanii* was identified morphologically and molecularly by **Mahmoud *et al*. (2023)**. The parasite recorded an infestation rate of 6.70% among the examined *S. solea* collected from Qarun Lake. A higher incidence of the same isopod species was recorded in *Solea* spp. (20.33%) and (8.16%) by **Khalaf-Allah and Yousef (2019)** and **Mahmoud *et al*. (2023)**, respectively. In regard to the lower infestation rate reported in this study, it is clear that the rate with the isopod *L.redmanii* in Qarun lake has declined compared to the previous years. This could be due to the successful and effective implementation of the scientific strategy developed by **Mahmoud *et al*. (2019b)**, who aimed to combat the isopod and limit its spread in the lake. Furthermore, improving the water quality in Qarun Lake **(Mahmoud *et al.*, 2019a; Fahmy *et al*., 2022)**, particularly regarding salinity levels, could provide an opportunity to rebuild its fish stock and restore the environmental balance **(Mahmoud *et al.*, 2019b)**. The findings of the current study indicate that the clinical signs presented by *S. solea* infected with *A. veronii*, including cutaneous haemorrhage, gill congestion, and internal organ congestion, are similar to those exhibited by *S. aegyptica* from Qarun lakes, which were also infected with *Aeromonas* species, particularly during the summer season **(El-kabany *et al*., 2023)**. Another study by **Wang *et al*. (2021)** revealed that the sea bass (*Lateolabrax maculatus*) from freshwater farms in China, which were naturally infected with *A. veronii* showed congestion ulcerations on their body surface and haemorrhage in their internal organs. However, the different *Aeromonas* strains can cause similar or dissimilar clinical symptoms. Furthermore, the same species of *Aeromonas* may produce different clinical symptoms in different fish. For instance, the moribund crucian carp (*Carassius auratus gibelio*) from a freshwater farm in China, which were naturally infected with *A. veronii*, suffered from hemorrhagic spots on their bodies. In contrast, experimental fish infected with *A. veronii* showed grey gills and abdominal distension, as reported by **Chen *et al*. (2019)**.

This study revealed that *A. veronii* infecting *S. solea* fish recorded a prevalence rate of 27.33%. Similarly, **Algammal *et al*. (2022)** examined *Mugil seheli* fish from private farms in Suez Province, Egypt, showing that the infection rate of *A. veronii* was 22.5%. Moreover, **Uzun and Ogut (2015)** reported a higher incidence of *A. veronii* subspecies in

the sea bass (*Dicentrarchus labrax*) from the Black Sea farms in Turkey, with a rate of 65.2%.

In the current research, the bacterial colonies observed on TSA media were found to be motile, rod-shaped, and Gram-negative. These colonies had a creamy to brown color, and their surface was round and convex. The *A. veronii* isolate was examined for its growth culture and was found to produce brown pigments resembling those of several motile *Aeromonas* spp. strains, including *A. bestiarum*, *A. media*, and *A. eucrenophila*. Furthermore, the isolate exhibited the production of brown to dark brown pigments on TSA, similar to those of *A. salmonicida*, as reported by **Gibson *et al*. (1998)** and **Abbott *et al.* (2003)**.

The isolated *A. veronii* strain is similar in the biochemical characteristics to the *A. veronii* CFJY-623 strain, which was isolated from infected crucian carp (*Carassius auratus gibelio*) by **Chen *et al.* (2019)**. Both strains tested negative for urea, citrate, H2S, and arginine dihydrolase, while testing positive for glucose, sucrose, mannose, ornithine, and lysine decarboxylase. Conversely, **EL-Sharaby *et al*. (2021)** found that *A. veronii* isolates were positive for arginine and citrate utilization. Similarly, **Algammal *et al.* (2022)** reported that the *A. veronii* strain isolated from *M. seheli* was positive for citrate utilization and negative for nitrate reduction.

This study presents the molecular identification and gene sequencing of an *A. veronii* isolate (OL771444). The isolate showed 100% identity with *A.veronii*/REI2021Aero1 (MW831507) and *A. veronii*/REE2021Aero2 (MW836109), which were isolated from tilapia, Egypt, as well as *A. veronii*/zy01 (KX768735) from loach, China. Additionally, the isolate showed 99% identity with (MW426312.1) *A. veronii*/Aer1 from the Nile tilapia, Egypt, (KC210792.1) *A. veronii*/SA from Ephippus orbis, China (FJ940849.1) and *A. veronii*/CYJ205 from carp, China .

Antibiotic resistance is a growing concern in the field of aquaculture. The drug resistance results of *A. veronii* reveal that it is sensitive to Trimethoprim/sulphamthoxazole, Erythromycin and Gentamycin, but resistant to ampicillin, nalidixic acid, oxanilic acid, and tetracycline. These findings highlight the importance of choosing antibiotics carefully and using them responsibly in fish farms. **Lazado and Zilberg (2018)** found that their *A. veronii* isolate was resistant to norfloxacin oxytetracycline and neomycin, but sensitive to florfenicol and trimethoprim/sulfamethoxazole. In another study, **Abd El Latif *et al*. (2019)** reported that *A. veronii* was sensitive to sulfamethoxazole-trimethoprim and ofloxacin. These studies emphasize the critical role of antimicrobial spectrum analysis in the classification of microorganisms, as reported by **Chen *et al.* (2019)**. It is crucial to avoid the excessive use of antibiotics in aquaculture to prevent them from accumulating in agricultural and municipal waste, as highlighted by **Huddleston *et al* (2006)** and **Abdel-Aziz *et al*. (2013)**. Additionally, by using antibiotics responsibly, we can help reduce the risk of antibiotic resistance and safeguard the health and well-being of aquatic animals, as well

as humans who rely on them as a source of food. Regarding the pathological impacts, the fish gills are in constant exposure to water that may contain potential harmful biological and chemical substances, so its tissues are subjected to morphological changes and loss of functions **(Antychowicz & Matras, 2008)**. Moreover, fish health status and environmental pollution levels can be assessed by evaluating the morphology of gills **(Flores-Lopes & Thomaz, 2011)**. The histopathological alteration observed in the gills of solea fish can be attributed to the co- infection of *L.redmanii*/ *A. veronii*. The high degree of necrosis observed in the gill lamellae, accompanied by an increase in leukocytic cellular infiltrations, is consistent with findings reported by **Abd El Latif *et al.* (2019)** for *Aeromonas veronii* in *Oreochromis niloticus*. *Aeromonas veronii* has also been associated with tissue degeneration, necrosis, and varying degrees of hemorrhage in the parenchymatous organs of the sea bass **(Wang *et al.*, 2021)**. Additionally, the detected edema, leukocyte infiltration, and hyperplasia of mucous cells in the primary and secondary gill lamellae were similarly reported by **Mahmoud *et al.* (2023)** for the same isopod species.

Conversely, the lesions observed in the skin of *Solea solea* are primarily attributed to the attachment of *Livoneca redmanii*, which induces pressure atrophy in the underlying muscles, leading to detachment and sloughing of the lining epithelium and exposure of the underlying muscle tissue. These lesions have also been documented by **Mahmoud *et al.* (2023)**. Furthermore, the embedding of the parasite's pereopod parts in the skin resulted in edema, degeneration, and ulceration, exposing the underlying tissue.

CONCLUSION

The isopod *L. redmanii* is a parasitic species that invades Qaron Lake. Initially, the parasite's incidence is low, but it compromises the natural defenses of the ecosystem, making it more susceptible to the widespread pathogen *A. veronii*. Inadequate management practices and poor water quality increase the susceptibility of fish to multiple infections. Additionally, improper antibiotic use in aquaculture and untreated sewage discharge into water may lead to antibiotic-resistant bacterial strains in affected fish populations.

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