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Article:

Prevalence and Impacts of *Vibrio alginolyticus* infections in Red Sea Fishes from the Coastal Waters of Hurghada, Egypt

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Abstract

This study investigated the prevalence of *V. alginolyticus* infections in two marine fish species *Siganus rivulatus* (*S. rivulatus)* and *Rhabdosargus haffara* (*R. haffara).* The overall prevalence was 18.84% among the sample examined. A significant difference in infection rates was observed between the two species, in which *R. haffara* exhibiting a higher infection rate of 61.54% compared to a lower rate of 38.46% in *S. rivulatus*. The results highlight that *V. alginolyticus* as an important pathogen for both species, especially *R. haffara* being more susceptible. The pathogen was identified through morpho-chemical tests, and experimental infection trials confirmed its pathogenicity in both species with 40 and 60% mortality rates in *S. rivulatus* and *R. haffara* respectively. Additionally, *V. alginolyticus* was highly sensitive to Tetracycline, Ofloxacin, and Chloramphenicol. Histopathological examination of infected *S. rivulatus* showed significant inflammatory and degenerative changes. These findings emphasize the importance of monitoring and controlling *Vibrio* infections to protect marine fish health and support sustainable aquaculture practices.

Keywords: Red Sea; *Vibrio alginolyticus; Siganus rivulatus; Rhabdosargus haffara;* isolation

Introduction

The Red Sea's diverse and unique array of fish species play a crucial role in maintaining ecosystem health and supporting economic activities through fisheries, tourism, and the aquarium sector [1]. *Siganus rivulatus,* commonly known as the marbled spinefoot, is a marine fish species well-adapted to variety of environmental conditions, including tolerance to a wide range of salinity levels and high-density environments [2]. It holds significant economic attention due to its high market demand and biological potential, making it an important species in both fisheries and aquaculture industries [3,4]. *Rhabdosargus haffara* is one of the prominent Sparid fish species in the Gulf of Suez and the Red Sea. This species is a commercial hot spot due to its popularity and high market price in areas like Hurghada. According to [5], Sparid fishes, including *R. haffara*, account for approximately 22% of the total revenue generated by these small-scale fisheries, highlighting their economic value to the region. *Vibrio* species are the cause of vibriosis which is considered a major bacterial disease affecting fish populations worldwide. The high incidence of *Vibrio* species (87%) recorded in Red Sea fish populations near Hurghada [6], underscores the bacteria's widespread distribution, adaptability in marine environments and its potential threat. Tetracyclines is a significant class of antibiotics characterized by broad-spectrum activity against a wide range of gram positive and negative bacteria. Their clinical safety, favorable tolerability, and availability in multiple formulations make them valuable in treating various infections with enhanced versatility in medical

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applications [7]. The present study aimed to investigate the prevalence and impact of *V. alginolyticus* infections in *S. rivulatus* and *R. haffara* fish in the Red Sea.

Material and methods

Ethics statement.

This study was conducted in accordance with the Declaration of Helsinki and approved by the research ethics committee of the Faculty of Veterinary Medicine, Sohag University, Egypt (Soh.un.vet/00079 R).

Location of study and fish sampling:

During the period from April to June 2021, a total of 276 wild *S. rivulatus* and *R. haffara* **(**138 fish for each species) with average body weight of 90 ± 10 grams were collected**.** Fish samples were transported to the Fish Diseases Unit's wet laboratory at Hurghada branch of the National Institute of Oceanography and Fisheries, where clinical and bacteriological examinations were conducted.

Fish for Pathogenicity test

In the study conducted from April to June 2022, a total 100 of apparently healthy *S. rivulatus* and *R. haffara* (75 ± 10 grams**)** were collected. These fishes were acclimated for 3 weeks and then used for pathogenicity test**.**

Aquaria

Glass aquaria (40 x 40 x 100 cm) filled with seawater and supplied with air supply were used in this study.

Clinical and post-mortem examination:

Fish samples were examined clinically for detection of the external abnormalities [8] and subsequently subjected to post-mortem (PM) examination [9]. Prior to dissection, live fish samples were euthanized using MS222 (Sigma-Aldrich) [10].

Bacterial isolation and biochemical identification

Loopfuls from the liver, kidney, and spleen were aseptically inoculated onto Tryptic Soy Agar (TSA) supplemented with 1.5 % NaCl and incubated at 28°C for 48 hours [11]. Pure bacterial colonies were preserved in Tryptic Soy Broth (TSB) with 1.5 % NaCl and 15 % glycerol and stored at -80°C for future use [12]. The expected isolates were identified based on their morpho-chemical characteristics following standard laboratory methods [9,13].

Pathogenicity test

A total of 50 *R. haffara* and 50 *S. rivulatus* fishes were further subdivided into:

1. Negative control group (10 fish): These fish were left untreated.
2. Sham control group (10 fish): These fish were intraperitoneally (I/P) injected with 0.1 mL of sterile saline.
3. Challenged groups (30 fish): This group was further divided into three replicates of 10 fish each.

Fish of the challenged groups of each species were I/P injected with 0.1 mL of 0.5 MCF (1×10^7 CFU/mL) *V. alginolyticus* suspension. All fish groups were monitored over a 14-day period to observe clinical signs, and behavioral changes, and to record the average mortality rate [14].

Antibiogram (Antibiotic Sensitivity Test)

Different types of commercially available antibiotic discs were used to determine the drug sensitivity pattern of *V. alginolyticus*. The concentrations were: Tetracycline (30µg), Ofloxacin (5µg), Chloramphenicol (30µg), Norfloxacin (10µg), Ciprofloxacin (5µg), Gentamycin (10µg), Trimethoprim (5µg), Cephalothin (30 µg), Oxolinic acid (2µg) and Cefotaxime (30µg). It was determined in vitro by using the standardized agar disc-diffusion method known as the Kirby Bauer (K-B) [15].

5) Histopathological examination

Specimens were dissected from the internal organs (liver and kidney) of infected fishes that survived the experimental challenge. The samples were preserved in 10% neutral buffered formalin (NBF) and processed for histopathological examination [16].

Results

1. Prevalence of infection

All visually examined fish samples showed no external signs or internal alterations. Based on the isolation, the prevalence of *V. alginolyticus* infections in both *S. rivulatus* and *R. haffara* was 18.84% among the total number of examined fishes. The prevalence varied between the two fish species; *R. haffara* exhibited a greater prevalence of 61.54% while *S. rivulatus* showed a lower prevalence of 38.46% **Table 1.** Additionally, the distribution of *V. alginolyticus* across the examined organs revealed variations in organ susceptibility where the liver exhibited the highest susceptibility of 53.85%, the spleen showed a moderate susceptibility of 36.54% and the kidney had the lowest susceptibility of 9.61% **Table 2 and Figure 1.**

Table (1): Shows the prevalence of *V. alginolyticus* infection

Vibrio species	No. of examined fishes	No. of isolates	Prevalence	Fish species			
				S. rivulatus		*R. haffara*	
				No. of isolates	Prevalence	No. of isolates	Prevalence
V. alginolyticus	276	52	18.84 %	20	38.46%	32	61.54%

Table (2): Shows the organ susceptibility to *V. alginolyticus*

Total No. of *V. alginolyticus* isolates	Liver		Spleen		Kidney	
	No. of isolates	%	No. of isolates	%	No. of isolates	%
52	28	53.85	19	36.54	5	9.61

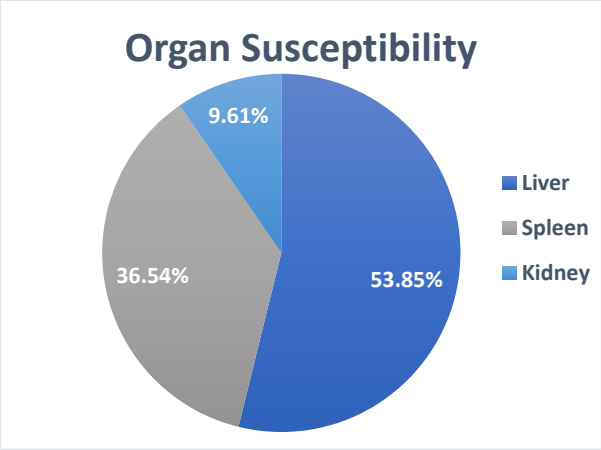

Figure (1): illustrates the organ susceptibility of the surveyed fish to *V. alginolyticus* infection.

2. Bacterial isolation and Biochemical identification

The positive TSA cultures formed round, smooth, creamy-coloured colonies that adhered to the medium. Further culturing on Thiosulfate-Citrate-Bile Salts-Sucrose (TCBS) agar resulted in the development of yellow colonies. The bacterial isolates were identified as Gram-negative, straight to slightly curved bacilli, exhibiting characteristic biochemical tests as illustrated in **Table 3**.

3. Pathogenicity test

3.1 *Siganus rivulatus* experimentally infected with *V. alginolyticus*

The experimental infection of *S. rivulatus* exhibited clear signs of compromised health, including reduced appetite, and lethargy**,** in addition to congestion in liver and spleen tissues, with a 40% average mortality rate. *V. alginolyticus* was successfully reisolated and identified from the internal organs of the infected fish, confirming its pathogenicity **Table 4** & **Figure 2**.

3.2 *Rhabdosargus haffara,* experimentally infected with *V. alginolyticus*

The experimental infection of *R. haffara* revealed an average mortality rate of 60% and clinical signs including decreased appetite, lethargy, hemorrhages at the head and mouth, and caudal fin rot. *V. alginolyticus* was successfully reisolated and identified from the internal organs of the experimentally infected fish **Table 5** & **Figure 3**.

4. Antibiogram

V. alginolyticus was highly sensitive to tetracycline, ofloxacin, and chloramphenicol, sensitive to norfloxacin, ciprofloxacin, gentamycin, trimethoprim, and cephalothin, while it was resistant to cefotaxime, and oxolinic acid **Table 6.**

5. Histopathology

The histopathological examination of the liver tissue of *S. rivulatus* experimentally infected with *V. alginolyticus* showed focal coagulative necrosis of hepatocytes, intense inflammatory cellular infiltration **Figure 4,** while kidney tissue showed proliferated melano-macrophage centrals, hyperplasia and intense hematopoietic tissue **Figure 5.**

Table (3): Shows the results of Morpho-chemical tests for *V. alginolyticus* identification

Test		*V. alginolyticus*
Colony morphology	Growth on TSA	Round, smooth, creamy colored colonies adhered to the media
	Growth on TCBS	Yellow colonies
Gram stain		Negative, Rod-shaped
KOH 3%		+
Oxidase		+
Catalase		+
Motility		+
Growth at (Nacl%)	0%	-
	2%	+
	4%	+
	6%	+
	8%	-
	10%	-
Indole production test		+
Methyl-red test		+
Voges-Proskauer test		+
Citrate utilization test		+
Vibriostatic O/129		+

Table (4): Shows average mortality rate of *S. rivulatus* experimentally infected with *V. alginolyticus*

	Dead fish per day								Total challenged fish	Total mortalities	Mortality rate
Day	1st	2nd	3rd	4th	5th	6th	7th	8th to 14th	10 fish	4	40%
S. rivulatus	0	0	2	1	1	0	0	0			

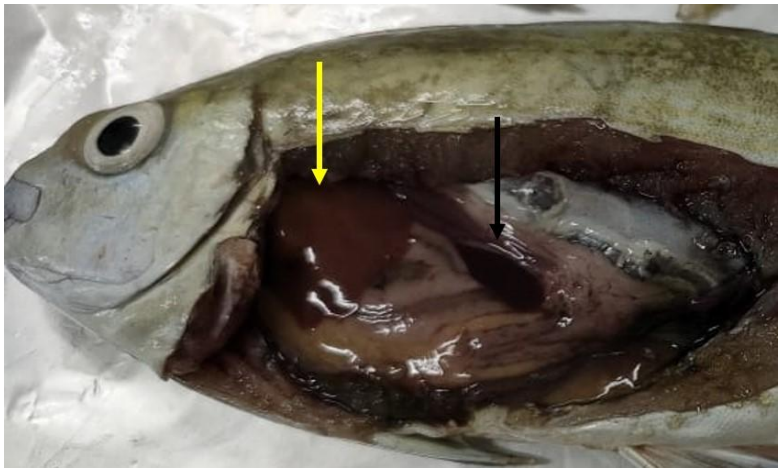

Figure (2): shows *S. rivulatus* experimentally infected with *V. alginolyticus* exhibiting congested spleen (black arrow), congested liver (yellow arrow)

Table (5): Shows average mortality rate of R. haffara experimentally infected with V. alginolyticus

	Dead fish per day								Total challenged fish	Total mortalities	Mortality rate
Days	1st	2nd	3rd	4th	5th	6th	7th	8th to 14th	10 fish	6	60%
R. Haffara	0	0	2	2	1	1	0	0			

Figure (3): Shows *R. haffara* experimentally infected with *V. alginolyticus* exhibiting hemorrhages at the head and mouth (downward arrow), caudal fin rot (upward arrow)

Table (6): shows the antibiogram of *V. alginolyticus*

Antibiotic Name and Code	Conc. (mcg)/ disc	Standard zone (mm.)			Inhibition zones of *V. alginolyticus*	Interpretation
		S	M	R		
Tetracycline (TE)	30	≥15	12-14	≤ 11	34 mm	S
Ofloxacin (OFX)	5	≥16	13-15	≤ 12	33 mm	S
Chloramphenicol (C)	30	≥18	13-17	≤ 12	35 mm	S
Norfloxacin (NOR)	10	≥17	13-16	≤ 12	29 mm	S
Ciprofloxacin (CIP)	5	≥21	16-20	≤ 15	33 mm	S
Gentamycin (CN)	10	≥15	13-14	≤ 12	20 mm	S
Trimethoprim (T)	5	≥23	14-22	≤13	29 mm	S
Cephalothin (KF)	30	≥18	15-17	≤ 14	20 mm	S
Oxolinic acid (OA)	2	≥37	29-36	≤ 28	24 mm	R
Cefotaxime (CTX)	30	≥26	23-25	≤ 22	0 mm	R

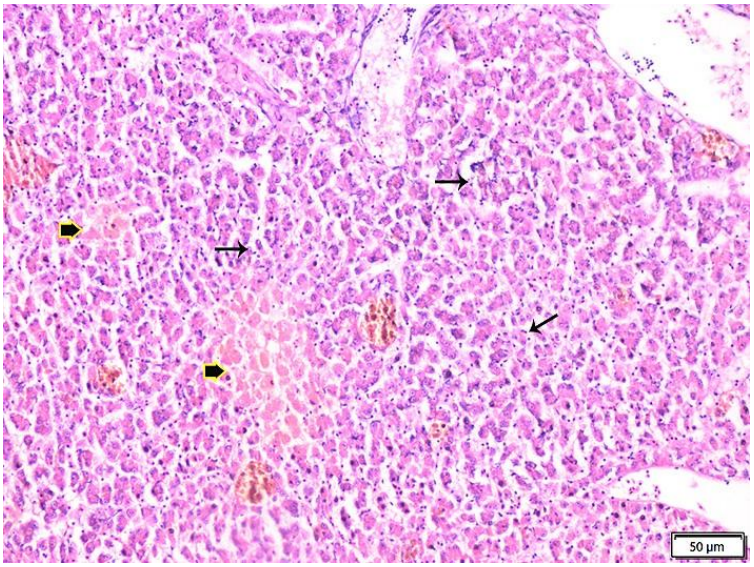

Figure (4): shows liver tissue structure from *S. rivulatus* experimentally infected with *V. alginolyticus* stained with HE stains exhibiting focal coagulative necrosis of hepatocytes (yellow arrowheads), intense inflammatory cellular infiltration (arrows) (50 µm)

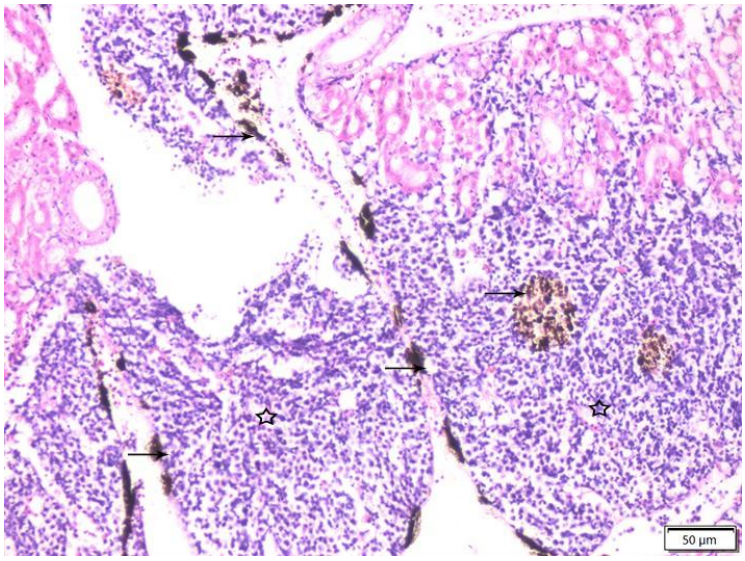

Figure (5): shows kidney tissue structure from *S. rivulatus* experimentally infected with *V. alginolyticus* stained with HE stain exhibiting proliferated melano-macrophage centrals (arrows), hyperplasia and intense hematopoietic tissue (stars) (50 µm)

Discussion

Vibrio alginolyticus is naturally found in seawater and is typically a harmless component of the normal microbial flora. However, it can become pathogenic when fish are exposed to stress or immune-compromised [17]. Nearly all marine fish species are believed to be susceptible to at least one *Vibrio* species [18]. In this study, *V. alginolyticus* showed a high prevalence in *S. rivulatus* (38.46%) and *R. haffara* (61.45%). Factors such as species-specific susceptibility, behavioral traits, and habitat preferences may contribute to this prevalence. The pathogen also appears well-suited to the environmental conditions of the Red Sea [19]. Compared to previously reported prevalence rates, *V. alginolyticus was* found in 63.3% of *S. rivulatus* and 36.7 % of *R. haffara*, the differences observed here could be attributed to variations in experimental and environmental conditions [20]. These findings highlight the role of host-specific relations and bacterial adaptability in infection dynamics among different marine fish species. The organ susceptibility analysis in *S. rivulatus* revealed the highest rate in the liver (53.85%), followed by the spleen (36.54%) and kidney (9.61%). This pattern aligns with research studies identified the liver as a primary site for *Vibrio* colonization, likely due to its high iron content, which supports bacterial growth and proliferation [6,21,22]. These results underline the importance of tissue-specific factors in bacterial colonization and infection dynamics. The morpho-chemical analysis confirmed the identity of *V. alginolyticus*. Colonies were round, smooth, and creamy on TSA but appeared yellow on TCBS. The bacteria were Gram-negative, rod-shaped, motile, grew on 2, 4, 6% NaCl and tested positive for oxidase, catalase, indole, methyl red, Voges-Proskauer and citrate. The isolates were sensitive to the vibriostatic agent O/129, consistent with the characteristics described by later research studies [9]. Experimental infections revealed distinct pathogenic effects and mortality rates in the two fish species aligning with the findings of similar studies [20,23]. *V. alginolyticus* pathogenicity could be linked to extracellular products like hydrolytic and hemolytic enzymes, which enhance bacterial invasion and proliferation [24]. Different recorded mortality rates between challenged fish species against *V. alginolyticus* infection highlights the variability in *V. alginolyticus* pathogenicity and *S. rivulatus*, and *R. haffara* fish response. When *V. alginolyticus* was tested against different antibiotics, it was highly sensitive to Tetracycline, Ofloxacin, and Chloramphenicol, and sensitive to Norfloxacin, Ciprofloxacin, Gentamycin, Trimethoprim, and Cephalothin. Resistance was noted against Cefotaxime and Oxolinic acid. These findings align with previous studies [20,25,26]. Histopathological examination of infected *S. rivulatus* showed significant inflammatory and degenerative changes. Liver tissue exhibited focal coagulative necrosis of hepatocytes, intense inflammatory cellular infiltration. The kidneys showed proliferated melano-macrophage centrals, hyperplasia and intense hematopoietic tissue. These findings are consistent with prior studies [27,28], highlighting the septicemic effects of *V. alginolyticus* and its capacity to disrupt organ structure and function [29,30]. These results emphasize the complex interplay of host, pathogen, and environmental factors in *V. alginolyticus* infections, underscoring the importance of effective monitoring and management strategies in aquaculture.

Conclusion

This study highlights the critical role of *V. alginolyticus* as an opportunistic pathogen in marine ecosystems. This bacterium was identified depending on morpho-chemical characterization. The findings demonstrate a high prevalence of infections among the studied fishes and the septicemic nature of *V. alginolyticus* infection.

Authors' Conflict of Interest

The authors declare that they have no conflict of interest.

Author's contributions:

M.AA.A., M.A.M.A., and M.H.M. involved in the conception of the idea experiments and methodology design, performed data analysis and interpretation. M.AA.A., M.A.M.A. and F.A.S contributed their scientific advice, prepared the manuscript for publication and revision. All authors read and approved the final manuscript.

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