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Article:

Investigating *Vibrio harveyi* infections in *Siganus rivulatus* and *Rhabdosargus haffara* from the Red Sea, Hurghada, Egypt

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Abstract

This study investigated the prevalence of *Vibrio harveyi* infections in two marine fish species, *Siganus rivulatus* and *Rhabdosargus haffara*. The overall infection rate among the sampled fish was 11.23%. A significantly higher prevalence was observed in *R. haffara* (64.52%) compared to *S. rivulatus* (35.48%). These findings identify *V. harveyi* as a notable pathogen impacting both species, with *R. haffara* showing greater susceptibility. The identification of *V. harveyi* was conducted through morpho-chemical tests and experimental infection trials demonstrated its pathogenicity in both species with greater pathogenicity to *R. haffara* (70% mortality rate) in comparison with *S. rivulatus* (50% mortality rate). The histopathological examination of specimens from infected *S. rivulatus* revealed distinct changes in the tissue structure of the liver and kidney. The antimicrobial sensitivity test revealed that *V. harveyi* was highly sensitive to chloramphenicol, with additional sensitivity observed for tetracycline, ciprofloxacin, and ofloxacin. This underscores the critical need for monitoring and managing *Vibrio* infections to safeguard marine fish health and support sustainability in aquaculture industry.

Keywords: Isolation, Red Sea, *Rhabdosargus haffara*, *Siganus rivulatus*, *Vibrio harveyi*.

Introduction

The Red Sea is widely recognized as a biodiversity hotspot, supporting unique ecosystems that sustain a diverse range of marine life, including numerous fish species [1]. It plays a vital role in regional economic activities such as fisheries and tourism, which provide essential livelihoods for local communities and make substantial contributions to regional economies [2].

Rabbitfish, also known as spinefoot, are a notable group of marine fish species within the *Siganidae* family [3]. Among these, *S. rivulatus* holds considerable economic importance in the Eastern Mediterranean, Red Sea, and Arabian Gulf due to its increasing demand, making it highly valuable to fishermen [4]. In the Egyptian Red Sea, *S. rivulatus* is one of four species from the *Siganidae* family, prized for its commercial potential in intensive aquaculture. It is a popular seafood choice known for its excellent taste, availability, and affordability [5].

Rhabdosargus haffara, a tropical fish native to the western Indian Ocean and the Red Sea, is particularly abundant in the northern Red Sea region. This species is commonly found in shallow waters, including coral reefs and sandy or muddy seabeds [6]. It is an essential part of the *Sparidae* family's catch in the Egyptian Red Sea, where it is heavily exploited for commercial purposes and serves

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as a key target for small-scale fisheries and trawling operations [7].

Vibriosis, caused by various *Vibrio* species, is a significant bacterial disease that poses a serious threat to marine aquaculture globally. Its high prevalence, the involvement of multiple *Vibrio* species, and its economic and public health consequences make it a major challenge for aquaculture systems [8].

Chloramphenicol (CAP) is a broad-spectrum antibiotic which makes it widely used in aquaculture practices [9]. Tetracyclines also is a significant class of antibiotics characterized by broad-spectrum activity against a wide range of bacteria, clinical safety and generally favourable tolerability [10]. However, their extensive use in agriculture, often in quantities greater than other antibiotic classes, has led to concerns about the spread of resistance genes [11].

This study focuses on assessing the prevalence and impacts of *V. harveyi* infections in *S. rivulatus* and *R. haffara* within the Red Sea.

Materials and Methods

Ethics Statement

This study adhered to the principles outlined in the Declaration of Helsinki and was approved by the Research Ethics Committee of the Faculty of Veterinary Medicine at Sohag University, Egypt (Soh.un.vet/00080 R).

Study Location and Fish Sampling

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

Fish for Pathogenicity Testing

In a subsequent study conducted from April to June 2022, 50 apparently healthy individuals of each species (*S. rivulatus* and *R. haffara*) with an average weight of 75 ± 10 grams were collected. These fish were acclimated for three weeks before being used in pathogenicity tests.

Aquaria

Glass aquaria (40 x 40 x 100 cm) filled with seawater and supplied with air supply systems were used throughout the study.

Methods

Clinical and Post-Mortem Examination

Fish were clinically examined for external abnormalities following the methods of [12]. Prior to dissection, live fish were euthanized using MS222 (Sigma-Aldrich) in accordance with [13]. Post-mortem examinations were conducted to assess internal changes, following the protocols of [14].

Bacterial Isolation and Biochemical Identification

Samples from the liver, kidney, and spleen of examined fish were aseptically collected and inoculated onto Tryptic Soy Agar (TSA) supplemented with 1.5% NaCl. The cultures were incubated at 28°C for 48 hours [15]. Pure bacterial colonies were isolated through repeated streaking and identified based on morpho-chemical characterization as described by [14,16].

Pathogenicity Test

A total of 50 *R. haffara* and 50 *S. rivulatus* were divided into five groups (10 fish per group):

1.**Negative Control Group**: Untreated fish.

2.**Sham Control Group**: Fish injected intraperitoneally (I/P) with 0.1 mL of sterile saline.

3.**Challenged Groups**: Fish were divided into three replicates, then I/P injected with 0.1 mL of 0.5 MCF *V. harveyi* bacterial suspension.

Fish were closely monitored for 14 days to record clinical signs, behavioral changes, and average mortality rates [17].

Antibiotic Sensitivity Test

The antibiotic sensitivity pattern of *V. harveyi* was assessed using commercially available antibiotic discs. The concentrations of antibiotics tested were as follows: tetracycline (30 µg), ofloxacin (5 µg), ciprofloxacin (5 µg), chloramphenicol (30 µg), erythromycin (15 µg), cefotaxime (30 µg), oxolinic acid (2 µg), cephalothin (30 µg), gentamicin (10 µg), amikacin (30 µg), and streptomycin (10 µg). The analysis was conducted *in vitro* using the Kirby-Bauer standardized agar disc-diffusion method, as described by [18].

Histopathological Examination

At the conclusion of the experiment, tissue specimens from the liver and kidney of infected fish that survived the challenge were collected. Samples were preserved in 10% neutral buffered formalin (NBF) and processed for histopathological analysis following [19].

Results

Prevalence of infection

None of the examined fish displayed any external abnormalities or internal changes. Isolation results indicated that the overall prevalence of *Vibrio harveyi* infections across all examined fishes was 11.23% (**Table 1**). However, the prevalence varied between species, with *R. haffara* showing a significant higher prevalence of 64.52%, compared to 35.48% in *S. rivulatus* (**Table 1**). Furthermore, organ-specific susceptibility to *V. harveyi* varied, with the liver being the most affected (51.61%), followed by the spleen (35.48%), and the kidney showing the lowest susceptibility (12.91%) (**Table 2**).

Bacterial isolation and Biochemical identification

On Tryptone Soy Agar (TSA), the bacterial isolates produced round, smooth, creamy-colored colonies that adhered firmly to the medium. When cultured on Thiosulfate-Citrate-Bile Salts-Sucrose (TCBS) agar, the colonies appeared yellow. The isolates were identified as Gram-negative rods and exhibited distinct biochemical characteristics, as summarized in Table (3).

Pathogenicity test

Siganus rivulatus, experimentally infected with *V. harveyi*

The experimental infection of *S. rivulatus* with 0.1 mL of a 0.5 MCF suspension of *V. harveyi* resulted in an average mortality rate of 50%. Infected fish displayed notable clinical signs, including lethargy, diminished appetite and head, and mouth ulceration. *V. harveyi* was successfully reisolated and identified from the internal organs of the deceased fish, confirming its involvement in the observed mortality (**Table 4**).

Rhabdosargus haffara, experimentally infected with *V. harveyi*

The experimental infection of *R. haffara* via I/P injection of 0.1 mL of a 0.5 MCF *V. harveyi* suspension resulted in a notable average mortality rate of 70%. Affected fish displayed various clinical signs, including lethargy, reduced appetite, scale loss and tail rot. *V. harveyi* was successfully reisolated and identified from the internal organs of the experimentally infected fish (**Table 5** and **Figure 2**).

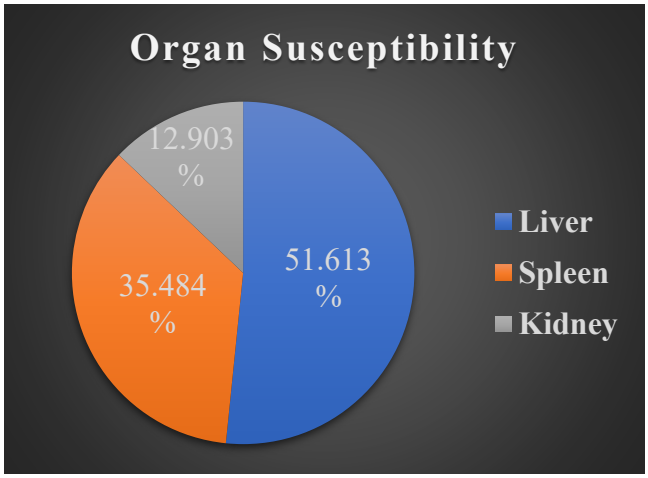

Figure 1: *illustrates the organ susceptibility of the examined fish to V. harveyi infection.*

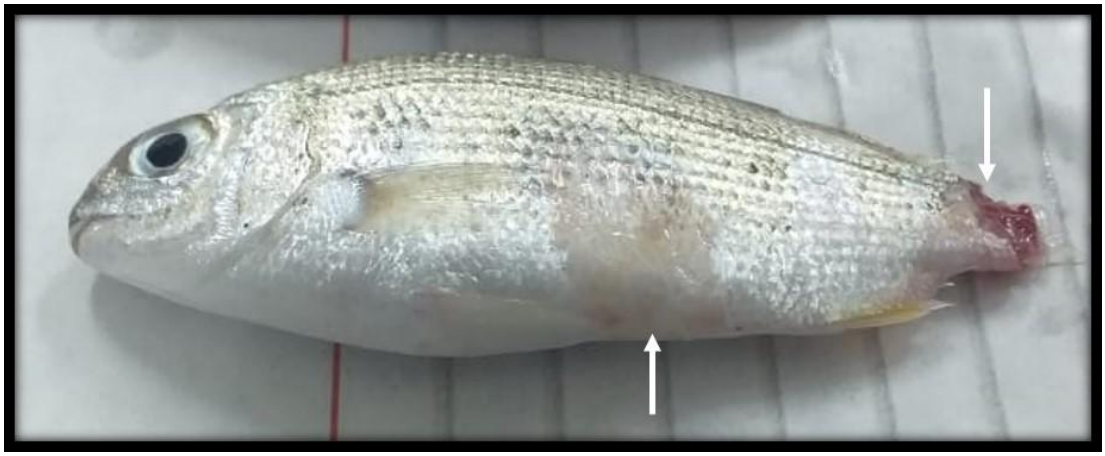

Figure 2: shows R. haffara experimentally infected with V. harveyi exhibiting scale loss (upward arrow) and tail rot (downward arrow)

Antibiotic Sensitivity Test

Vibrio harveyi was highly sensitive to Chloramphenicol, sensitive to Tetracycline, Ciprofloxacin, and Ofloxacin, with medium resistance to Erythromycin, while it was resistant to Cefotaxime, Cephalothin, Amikacin, Oxolinic acid, Streptomycin, and Gentamycin (**Table 6**).

Histopathology

The histopathological examination of kidney tissue from *S. rivulatus* experimentally infected with *V. harveyi* revealed hydropic degeneration in the collecting tubules, accompanied by intense inflammatory cell infiltration, glomerular atrophy, melano-macrophage centers, hyperplasia, and significant hematopoietic tissue. In the liver tissue, dilated and congested portal venules was observed alongside with thick-walled dilated bile ductules **(Figures 3 and 4)**.

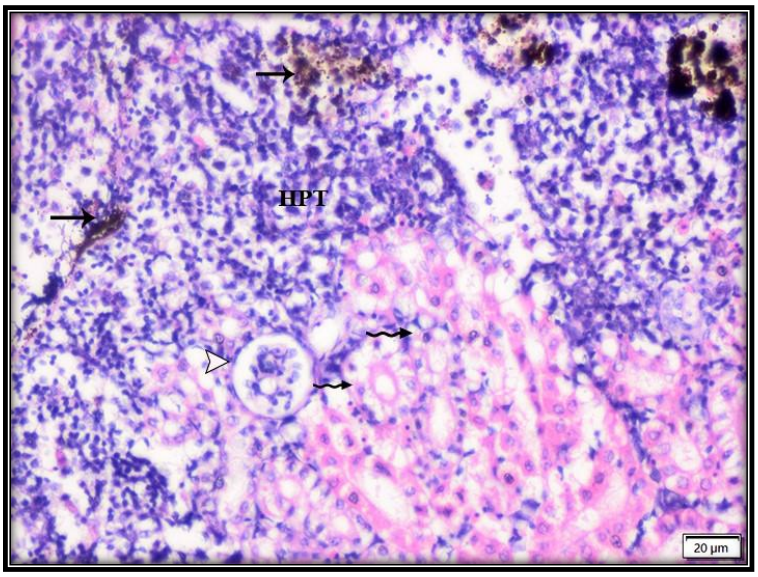

Figure 3: *shows kidney tissue structure from S. rivulatus infected with V. harveyi stained with HE stain exhibiting atrophy glomerulus (arrowheads), hydropic degeneration in collecting tubules with intense inflammatory cellular infiltration (zigzag arrows), melano-macrophage centrals (arrows), hyperplasia and intense hematopoietic tissue (HPT).*

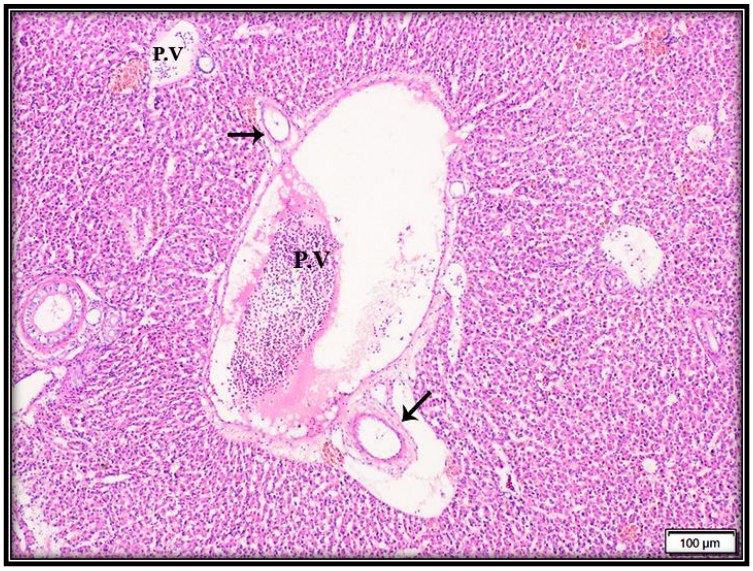

Figure 4: *shows liver tissue structure from S. rivulatus infected with V. harveyi stained with HE stains exhibiting dilated and congested portal venules (P.V), thick-walled dilated bile ductulus (arrows)*

Table 1 : Prevalence of V. harveyi 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. harveyi	276	31	11.23%	11	35.48%	20	64.52%

Table 2: Organ susceptibility to V. harveyi

Total No. of ***V. harveyi*** isolates	Liver		Spleen		Kidney	
	No. of isolates	%	No. of isolates	%	No. of isolates	%
31	16	51.613	11	35.484	4	12.903

Table 3: Morpho-chemical tests results for V. harveyi identification

Test		***V. harveyi***
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: Mortality rate of S. rivulatus experimentally infected with V. harveyi

Idiom	Dead fish per day								Total challenged fish	Total mortalities	Mortality rate
Day	1st	2nd	3rd	4th	5th	6th	7th	8th to 14th	10 fish	5	50%
S. rivulatus	0	0	2	1	1	0	1	0			

Table 5: Mortality rate of R. haffara experimentally infected with V. harveyi

Idiom	Dead fish per day								Total challenged fish	Total mortalities	Mortality rate
Days	1st	2nd	3rd	4th	5th	6th	7th	8th to 14th			
R. Haffara	0	1	2	2	1	0	1	0	10 fish	7	70%

Table 6: Antibiotic sensitivity test of V. harveyi

Antibiotic					Inhibition zones of *V. harveyi*	Interpretation
Name and code	Conc. (mcg)/ disc	Standard zone (mm.)				
		S	M	R		
Chloramphenicol (C)	30	≥18	13-17	≤ 12	44 mm	S
Tetracycline (TE)	30	≥15	12-14	≤ 11	22 mm	S
Ofloxacin (O)	5	≥16	13-15	≤ 12	20 mm	S
Ciprofloxacin (CIP)	5	≥21	16-20	≤ 15	25 mm	S
Erythromycin (E)	15	≥23	14-22	≤ 13	22 mm	M
Gentamycin (CN)	10	≥15	13-14	≤ 12	11 mm	R
Streptomycin (S)	10	≥15	12-14	≤ 11	10 mm	R
Oxolinic acid (OA)	2	≥37	29-36	≤ 28	17 mm	R
Amikacin (AK)	30	≥17	15-16	≤ 14	2 mm	R
Cephalothin (KF)	30	≥18	15-17	≤ 14	0 mm	R
Cefotaxime (CTX)	30	≥26	23-25	≤ 22	0 mm	R

Discussion

Among bacterial diseases affecting fish, *Vibrio* species belong to the leading contributors to economic losses in aquaculture. These bacteria are naturally found in marine environments, and outbreaks are frequently triggered by stress factors that compromise the fish health and facilitate bacterial proliferation [20]. Stressors associated with *Vibrio* outbreaks include poor water quality, inadequate nutrient levels, and excessive stocking densities, all of which can increase bacterial loads on fish surfaces [21].

This study reveals that *S. rivulatus* exhibits a lower susceptibility (35.48%) to *V. harveyi* infection compared to *R. haffara* (64.52%). These findings are consistent with previous research by [22], who reported a 22.5% infection rate in *S. rivulatus*, and are comparable to [23],

who observed a 36.7% infection rate in *R. haffara*. The reduced susceptibility in *S. rivulatus* may be attributed to its more robust immune defenses. For instance, [24] highlighted elevated phagocytic and lysozyme activity in *S. rivulatus*, while [25] noted lower tissue iron concentrations in this species. Since iron is essential for bacterial growth [26]. The reduced iron levels in *S. rivulatus* tissues may inhibit bacterial proliferation. These results underscore the importance of species-specific physiological and immune factors in influencing the prevalence of *Vibrio* infections in marine fish populations.

Analysis of organ-specific susceptibility to *V. harveyi* revealed a significant presence in the liver (51.61%), followed by the spleen (35.48%) and kidney (12.91%). This pattern aligns with [27], who identified the liver as the primary site for *Vibrio* colonization, with subsequent involvement of the spleen and kidney. The liver's heightened vulnerability is likely due to its high iron content, which supports bacterial growth [25,28]. This organ-specific susceptibility highlights the role of iron availability and other tissue-specific factors in bacterial colonization and infection dynamics.

Vibrio harveyi demonstrated high sensitivity to Chloramphenicol, sensitivity to Tetracycline, Ciprofloxacin, and Ofloxacin, and intermediate resistance to Erythromycin while it was resistant to Cefotaxime, Cephalothin, Amikacin, Oxolinic acid, Streptomycin, and Gentamycin. These results agreed with the research conducted by [15,29] both of which reported *V. harveyi* sensitivity to Chloramphenicol and Ciprofloxacin. This alignment across studies highlights the importance of these antibiotics in controlling *Vibrio* infections while underscoring the specific resistances that may pose challenges in treatment efforts of these infections. Continuous monitoring of antibiotic susceptibility is essential for effective therapeutic programs in aquaculture settings to mitigate the impacts of *V. harveyi* on marine fish health.

Experimental infection of *S. rivulatus* with *V. harveyi* resulted in observable clinical signs, including lethargy, diminished appetite and head, and mouth ulceration, with an average mortality rate of 50%. Our observations are consistent with those of [22], who recorded similar findings on *S. rivulatus* infected with *vibrio*. In contrast, *R. haffara* displayed higher susceptibility, when infected with *V. harveyi*, the fish exhibited lethargy, reduced appetite with scale loss with extensive haemorrhages and tail rot leading to a 70% average mortality rate which is close to 80% mortality recorded by [23] in *R. haffara*. These findings highlight the species-specific susceptibility of the fish to *Vibrio* infections, indicating *R. haffara* with a higher degree of vulnerability than *S. rivulatus.* The varying clinical responses and mortality rates suggest differences in immune response mechanisms, habitat conditions, and adaptive strategies between the two species, which play a crucial role in their ability to withstand *Vibrio* infections [23,25].

Histopathological examination of *S. rivulatus* infected with *V. harveyi* revealed distinct pathological changes in kidney and liver tissues. Kidney alterations included glomerular atrophy, hydropic degeneration in collecting tubules, the presence of melano-macrophage centers, dense inflammatory cell infiltration, hyperplasia, and intense hematopoietic activity. Liver tissue exhibited dilated and congested portal venules and thick-walled dilated bile ductulus. These observations are consistent with previous studies, such as [30,31], which documented similar inflammatory responses in fish infected with *Vibrio* species. Furthermore, [32] reported hepatocyte necrosis and the presence of melano-macrophage centers in infected fish. Reports by [30,31,33,34] also described glomerulonephritis, tubular degeneration, and leukocyte infiltration in the kidneys of fish infected with *V. harveyi*. These consistent histopathological changes underscore the severe pathogenic impact of *Vibrio* infections in marine fish, characterized by significant tissue damage and inflammatory responses.

Conclusion

This study underscores the important role of *V. harveyi* as an opportunistic pathogen in marine ecosystems. The bacterium was identified through morphological and chemical characterization. The findings reveal a high prevalence of infections among the examined fish species and confirm the septicemic nature of *V. harveyi* infection. The results underscore the urgent need for effective monitoring and management strategies to mitigate the impacts of *V. harveyi* on marine fish population.

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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