

Clinical, Microbiological, and Hematological Assessment of *Vibrio* Infection in Flathead Grey Mullet (*Mugil cephalus*) Near the Port Benghazi, Libya

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ABSTRACT

The present study aimed to address the impact of *Vibrio* bacteria on the health status of *Mugil Cephalus* inhabiting the coastal area of Benghazi port. A total of 20 samples of (*M. Cephalus*) were collected at random from the Benghazi Harbour throughout the period from March 2025 to August 2025, All the specimen from the fish were examined outwardly and inwardly and lesions were registered. Bacterial isolation from the liver and kidney was done using the appropriate culture media. The infection rate was 30% . The external lesions during the spring and summer seasons were hemorrhages at the base of the pectoral fin and around the operculum, congestion of caudal and anal fins. While internal lesions were splenomegaly, congested liver, kidney, and swim bladder. Bacteriological identification was confirmed based on the cultural characteristics and biochemical activities of isolates, On adjusted TCBS medium the recovered colonies showed as circular yellow or green colonies ranging characteristic of *Vibrio spp.* , In regards to the hematological parameters associated with diseased and apparently healthy *Mugil Cephalus*. The total protein, and globulin were lower in diseased than apparently healthy *Mugil Cephalus* fish, Data were statistically analyzed using T-test to compare healthy and infected fish, and differences were considered significant at ($p < 0.05$).

1. INTRODUCTION

Benghazi harbour is the biggest Libyan port in terms of area, covering approximately of 4.4 million square meters (Abmdas & Demirel, 2018). Contamination of water bodies with heavy metals and bacteria may affect aquatic animals, such as fish, directly or indirectly (Kılıç, 2021), fish has become a very essential source of protein in the human's food in recent years because it contains all essential amino acids and it is simpler to digest than animal protein (Balami et al., 2019).

The flathead grey mullet (*Mugil Cephalus*) is regarded as one of the most important fish species in the Mediterranean Sea (Whitfield et al., 2012). In recent year, flathead grey mullet populations in the Mediterranean, especially along the coastline, have experienced recurrent disease outbreaks. These accidents often associated with bacterial and parasitic infections, have led to significant fish mortalities, raising concerns about aquaculture sustainability (FAO, 2020; Oshebi & El-Ehfifi, 2017; Shaltami et al., 2019). Globally, there is a rise in diseases associated with *Vibrio*, which has a significant impact on both fish and human health (Roux et al., 2015), *Vibrio* species are aquatic bacteria found in the marine environment, where they cause *Vibriosis* in aquatic animals and humans (de Souza Valente & Wan, 2021). *Vibriosis* considered a serious global problem, causing substantial economic losses in aquaculture. The characterized of *Vibriosis* in fish are External skin lesions and hemorrhages, Reddening around the fins, petechial hemorrhages in the muscle wall, and fluid accumulation in the air bladder (El-Sharaby et al., 2018). *Vibriosis* is recognized as a significant zoonotic disease. In humans, *vibrio* infections are associated with clinical symptoms like gastroenteritis or primary septicemia due to the consumption of raw or undercooked contaminated seafood. The disease can lead to high mortality in aquatic animals and humans (Helmi et al., 2020). *Vibrio* is a halophilic, facultative aerobic, Gram-negative bacterium that is powerfully correlated by great salinity (30–35 ppt), significant temperature, parasitic infestation and mechanical injuries. These elements inhibit the immunity and increase the susceptibility of fish to *vibriosis* (Abdelaziz et al., 2017).

Objectives of the Study:

The present study was conducted with the following objectives:-

1. To study the prevalence of *Vibrio* infection in flathead grey mullet (*Mugil cephalus*) living in coastal waters and the port of Benghazi.
2. To identify clinical signs and pathological lesions (external and internal) associated with *Vibrio* infection in mullet.
3. To isolate and identify *Vibrio* bacteria from infected fish tissue using selective culture media and biochemical tests (including API 20E).
4. To assess hematological parameters (total protein, albumin, and globulin) in diseased and apparently healthy grey mullet to evaluate the systemic impact of *Vibrio* infection.
5. To evaluate potential environmental factors contributing to the occurrence of *Vibrio* infection in the coastal ecosystem of Benghazi.

2. MATERIALS AND METHOD

2.1. Place of study:

The present study was conducted at the Benghazi Sea Port (Figure 1), located on the northeastern coast of Libya, along the Mediterranean Sea (32°12'54"N, 20°09'16"E). The port serves as a vital hub for commercial shipping, fishing activities, and oil transport, making it one of the most strategically important harbors in the region. However, the area is subject to significant environmental pressures due to urbanization, industrial activities, and inadequate waste management, Seaports are a temporary environment for mullet fish, which feed on organic waste and marine algae

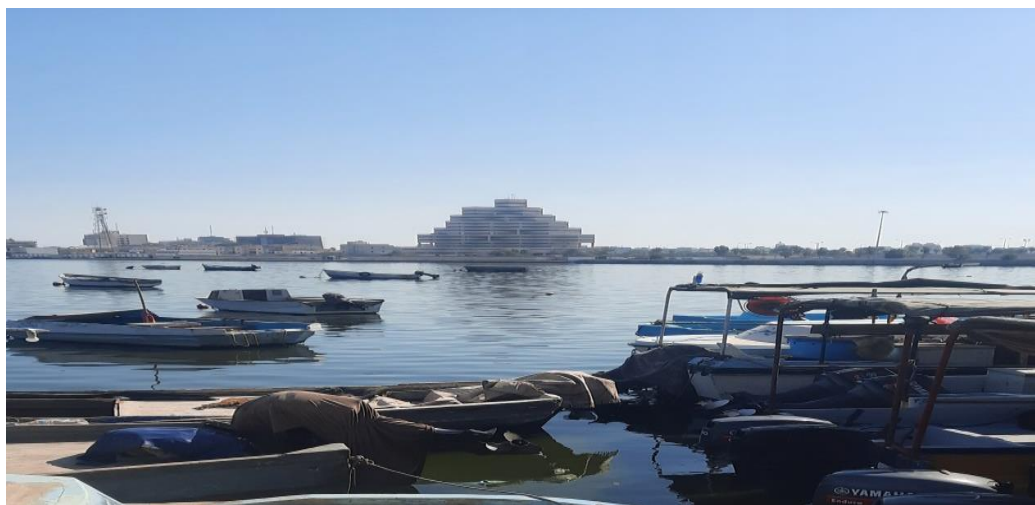


Figure (1) : Benghazi port .

2.2. Fish specimens :

A total of twenty (20) wild (*Mugil Cephalus*) (Figure 2) were collected randomly in the period from March 2025 to August 2025 from Benghazi Harbour, , 10 live fish were collected directly from the fisherman on the boat after catching by using a cast-net, and 10 fish were moribund collected from Bankina fish market. At the fishing site , sterile swabs are taken from the skin and gills of the live fish for bacteriology test , and each swab is placed in a tube containing a carrier medium APW (Alkaline Peptone Water) and all the Fish samples were kept in ice box and transported to the laboratory for Clinical and postmortem examination , External and internal examination of fish samples were performed to record clinical abnormalities according to(Austin and Austin,2016).



Figure (2) : wild (*Mugil Cephalus*) catching by using a cast-net .

2.3. Sample handling:

Fish were wiped by ethanol 70% to dispose of external contaminants. Using the three-line technique (Triangular incision) fish were cut open from the left side to expose internal organs. Fish were externally and internally examined for any possible lesions.

2.4. Bacterial isolation:

The modified protocol by Austin et al. (2016) was used for bacterial isolation. After clinical examination of the samples, liver, and kidney materials were inoculated onto alkaline peptone water (APW) tubes supplemented with 2% NaCl and incubated at 25°C–27°C for 24 hours. Aliquots from inoculated APW tubes were streaked onto modified Thiosulfate Citrate Bile Salt Sucrose Agar (TCBS). Each media was supplemented with 1%–2% NaCl. The inoculated plates were then incubated at 25°C–27°C for 24–72 hours at maximum. The plates were inspected for any possible colonial growth.

2.5. Biochemical examination: -

The biochemical characters of the isolates were determined by using by using API (Analytical Profile index) 20E system:

According to the API 20E User's Guide, the identification of *Vibrio* species using the API 20E strip is acceptable when the oxidase reaction is positive and the colony color on TCBS agar is green or yellow, although further confirmatory testing is recommended (bioMérieux,2022).

The API20E system (BioMérieux) containing 20 microtubes containing dried substrates. The bacterial inoculum was prepared by overnight culture. The microtubes were inoculated with a bacterial suspension, which reconstituted the medium. API20E plates were used according to the manufacturer's instructions, The incubation temperature for the strips was maintained at 22°C, and the incubation period ranged from 24 to 72 h. A 1.5% saline suspension was used as the inoculum, while sterile mineral oil was used to seal the cups for fermentation of sugars.

Biochemical assays examined included: beta-galactosidase (ONPG), arginine dehydrolase (ADH), lysine decarboxylase (LDC), ornithine decarboxylase (ODC), citrate utilization (CIT), hydrogen sulfide production (H₂S), urease (URE), tryptophan deaminase (TDA), indole production (IND), Voges-Proskauer (VP), gelatinase (GEL), glucose (GLU), mannitol (MAN), inositol (INO), sorbitol (SOR), rhamnose (RHA), sucrose (SAC), melibiose (MEL), amygdalin (AMY), arabinose (ARA), and cytochrome oxidase (OX).

2.6. Blood tests: -

Blood samples were collected from the caudal vein with an insulin syringe. The blood samples were allowed to clot at 4 °C for 4 h, centrifuged (10,000× g, 5 min, 4 °C) and stored at -20 °C until use. Furthermore, the body weight and length of each fish was measured (Lied et al., 1975). Serum total protein was determined according to (Doumas et al., 1981) using commercial kits produced by Pasteur Lab. Serum albumin was determined according to (Reinhold, 1953) using commercially available kits. Serum globulin was determined by subtracts the total serum albumin from total serum protein according to (Globulin = Protein – Albumin) (Kumar et al., 2005).

2.7. Statistical Analysis: -

All data were expressed as mean ± standard deviation (SD). Statistical analyses were performed using Student's t-test (Welch's correction for unequal variances) to compare between diseased and apparently healthy *Mugil cephalus*. A probability level of $p < 0.05$ was considered statistically significant, while $p < 0.001$ was considered highly significant. Data were analyzed using standard statistical software.

3. RESULTS

3.1. Clinical findings:

Most fish examined showed typical macroscopic signs of septicemia, including hemorrhagic spots on various surface areas and fin bases, around opercula, and congestion of internal organs. On average, 30% of affected fish showed at least one lesion. External lesions during the fall and summer included hemorrhages at the pectoral fin base and around the gill cover, and congestion of the pelvic, caudal, and anal fins. Internal lesions included splenomegaly, liver pallor, liver congestion, and hemorrhage in the liver, kidneys, and swim bladder.

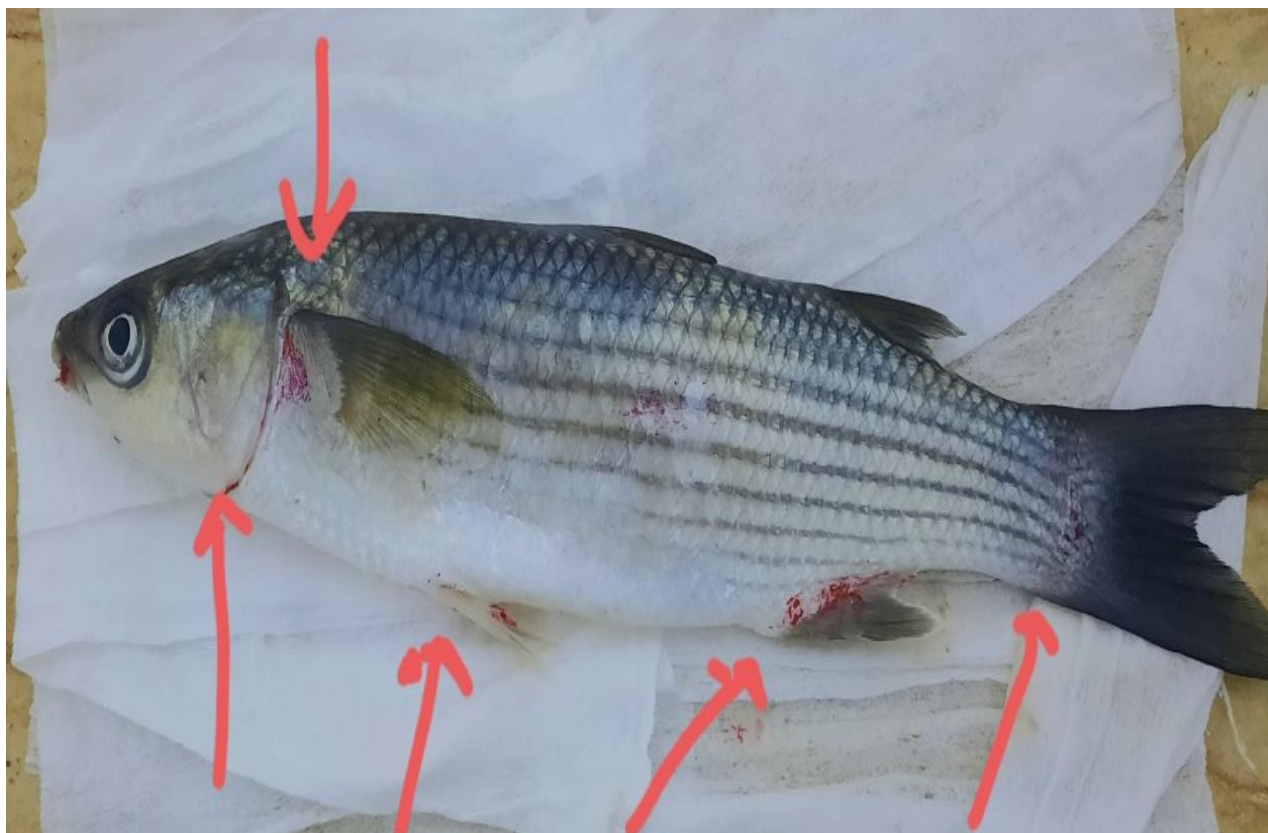


Figure (3) : infected fish (hemorrhage in the body fins and around the operculum)

Table 1: External Lesions Observed in Infected Flathead Grey Mullet (n = 6)

Lesion Type	Number of Fish Affected	Percentage of Infected Fish (%)
Hemorrhages at base of pectoral fin	3	50%
Hemorrhages around operculum	3	50%
Congestion of pelvic fins	2	33.3%
Congestion of caudal fins	2	33.3%
Congestion of anal fins	1	16.7%

Table 2: Internal Lesions Observed in Infected Flathead Grey Mullet (n = 6)

Lesion Type	Number of Fish Affected	Percentage of Infected Fish (%)
Splenomegaly	6	100%
Pale liver	4	66.7%
Congested liver	3	50%
Hemorrhagic liver	2	33.3%
Affected kidney	2	33.3%
Altered swim bladder	1	16.7%

3.2. Bacteriological examination:

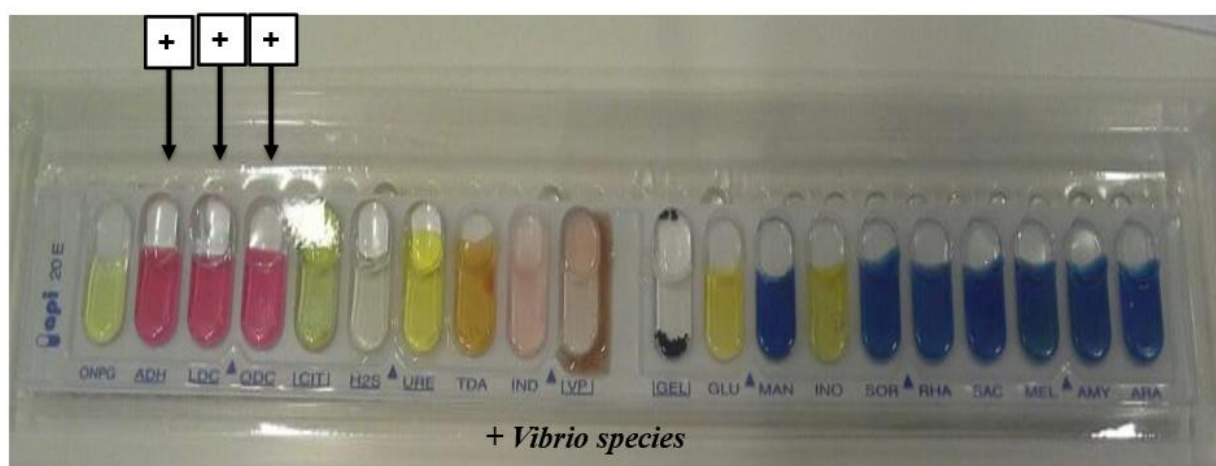
On modified TCBS medium the retrieved colonies appeared as circular green colonies ranging from pinpoint to 2–3 mm characteristic of *Vibrio* spp. . On the MacConkey medium the retrieved colonies were circular colorless colonies ranging from pinpoint to 2 mm, and circular dark pink colonies . Bacteria were Gram-negative curved rods table (3).

Table 3: Phenotypic characters of bacterial isolates from *M.Cephalus* :

Character	Isolates of <i>Vibrio</i>
Gram stain	Negative
Shape	Short-rod
Colonies on TCBS agar	Green
Haemolysis	+
Motility	+
Swarming	+

3.3. Biochemical results :

Based on API 20E profile, the isolate showed characteristics consistent with *Vibrio* species, particularly predatory *Vibrio*. This was supported by a positive oxidase test, green colonies on TCBS agar, and positive reactions to gelatinase, citrate utilization, and glucose fermentation, as well as negative results for indole and lysine decarboxylase figure (4).

**Figure (4) :**API20E result.

3.4. The blood result :

The measured physiological parameters were significantly higher in the infected fish compared to those at the reference site. This suggests that the fish are experiencing physiological stress, likely resulting from environmental pollution, as mullet are found near ports and estuaries where their food (organic matter and algae) is readily available.

Table (4): Mean values of complete blood count of fish samples

Chemical Test	Result
Erythrocyte (RBCs)	2.72±0.11 ($\times 10^6/\mu\text{L}$)
Haematocrit (Ht)	38.90±3.27 (%)
Haemoglobin (Hb)	13.48±1.4 (g/dL)
Platelets (PL)	12.33±2.51 ($\times 10^3/\mu\text{L}$)
Leucocyte (WBCs)	64.25±4.38 ($\times 10^3/\mu\text{L}$)

WBCs : Relatively elevated, which may indicate bacterial or viral infection (potentially due to water pollution) or an immune response to environmental toxins (such as heavy metals).

Platelets : Slightly decreased, possibly reflecting the impact of pollution on the immune system or blood clotting mechanisms.

RBCs : Within the normal range, although the values may suggest the early onset of physiological stress, potentially related to low dissolved oxygen levels or other environmental stressors.

Table (5) : comparative our results with typical reference range :

Parameter	Our Results	Typical Reference Range	Interpretation
RBCs ($\times 10^6/\mu\text{L}$)	2.72 ± 0.11	1.5–3.5	Normal range, but higher end may indicate early stress (e.g., hypoxia from pollution).
Hematocrit (Ht%)	38.90 ± 3.27	30–45	Slightly elevated, possibly due to dehydration or chronic stress.
Hemoglobin (Hb) (g/dL)	13.48 ± 1.4	8–12	Elevated, suggesting compensatory response to low oxygen (e.g., polluted water).
Platelets (PL) ($\times 10^3/\mu\text{L}$)	12.33 ± 2.51	20–100	Critically low, indicating impaired clotting or bone marrow suppression (common in heavy metal toxicity).

In regards to the serological parameters associated with diseased and apparently healthy *M.Cephalus* . The total protein was lower in diseased *M.Cephalus* (2.23±0.17) than apparently healthy *M.Cephalus* (3.72±0.16) . The albumin was normal in the diseased and control groups. The total globulin was lower in the diseased *M.Cephalus* (1.11±0.04) than apparently healthy *M.Cephalus* (1.63±0.05) table (6).

Table (6) : Comparison of Serological Parameters in Diseased and Apparently Healthy *Mugil cephalus*

Parameter	Diseased Fish (Mean $\pm$ SE)	Healthy Fish (Mean $\pm$ SE)	Normal Range (Teleosts)	Remarks
Total Protein (g/dL)	2.23 $\pm$ 0.17	3.72 $\pm$ 0.16	3.0 – 5.0	Lower in diseased fish; indicates possible liver dysfunction or protein loss.
Albumin (g/dL)	3.5 $\pm$ 0.03	3.6 $\pm$ 0.15	1.5 – 3.5	albumin important for osmotic balance.
Globulin (g/dL)	1.11 $\pm$ 0.04	1.63 $\pm$ 0.05	1.0 – 2.5	Lower in diseased fish; may reflect suppressed immune function.

The normal reference ranges for serum total protein, albumin, and globulin in teleost fish, including *Mugil cephalus*, are typically between 3.0–5.0 g/dL for total protein, 1.5–3.5 g/dL for albumin, and 1.0–2.5 g/dL for globulin. These ranges may vary depending on species, season, environmental conditions, and physiological status (Hrubec et al., 2000; Roberts, 2012).

Infected fish showed:

1. A decrease in total protein and globulin

2. This indicates a clear impact of the infection on the immune system and liver function

This is consistent with the external and internal signs of illness you may have noticed, such as bleeding and swelling.

3.5. Statistical Results: -

For hematological parameters, red blood cell (RBC) counts were significantly higher in diseased fish ($p < 0.05$). Hemoglobin and white blood cell (WBC) counts showed a highly significant increase in diseased individuals ($p < 0.001$), while platelet counts were markedly reduced in diseased fish ($p < 0.001$). Hematocrit values did not differ significantly between the two groups ($p > 0.05$).

Table (7) : Complete Blood Count (CBC) in Diseased and Healthy *Mugil cephalus*.

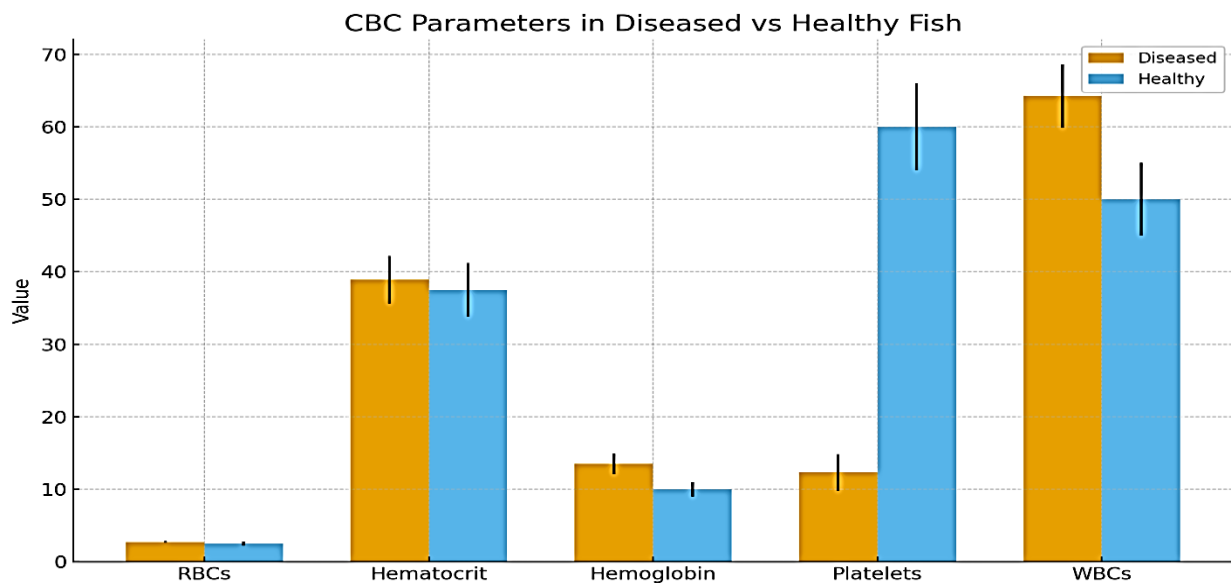
Parameter	Diseased (Mean $\pm$ SD)	Healthy (Mean $\pm$ SD)	p-value
RBCs ($\times 10^6/\mu\text{L}$)	2.72 $\pm$ 0.11	2.50 $\pm$ 0.25	0.025 *
Hematocrit (%)	38.90 $\pm$ 3.27	37.50 $\pm$ 3.70	0.382 (NS)
Hemoglobin (g/dL)	13.48 $\pm$ 1.40	10.00 $\pm$ 1.00	<0.001 **
Platelets ($\times 10^3/\mu\text{L}$)	12.33 $\pm$ 2.51	60.00 $\pm$ 6.00	<0.001 **
WBCs ($\times 10^3/\mu\text{L}$)	64.25 $\pm$ 4.38	50.00 $\pm$ 5.00	<0.001 **

NS = Not significant.

* = Significant at $p < 0.05$.

** = Highly significant at $p < 0.001$.

Figure (5) : Bar chart showing of the statistical analysis diseased and apparently healthy *Mugil cephalus*. Serum.

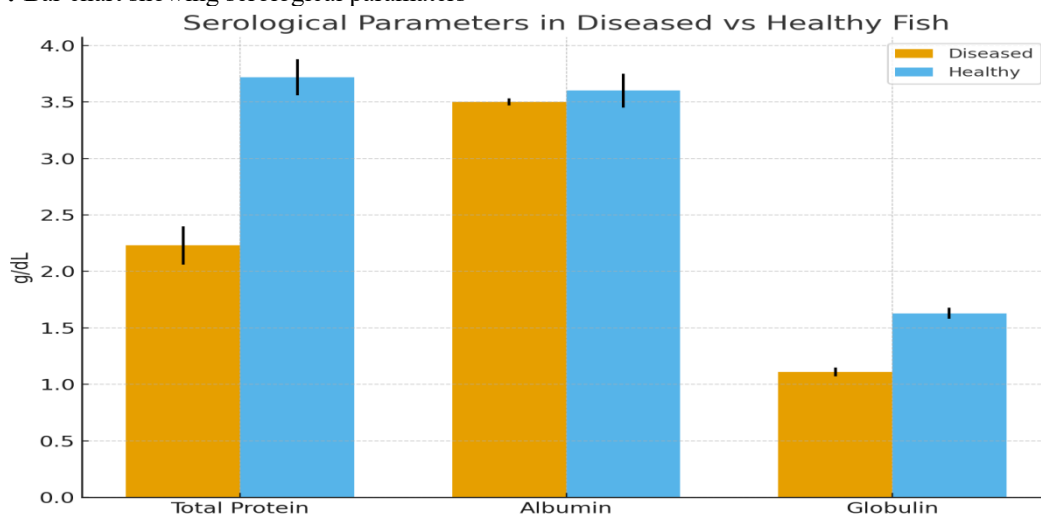


The statistical analysis revealed significant differences between diseased and apparently healthy *Mugil cephalus*. Serum total protein and globulin levels were significantly reduced in diseased fish compared to healthy ones ($p < 0.001$), while albumin levels showed no significant difference ($p > 0.05$).

Table (8) : Serological Parameters in Diseased and Healthy *Mugil cephalus*.

Parameter	Diseased (Mean ± SD)	Healthy (Mean ± SD)	p-value
Total Protein (g/dL)	2.23 ± 0.17	3.72 ± 0.16	<0.001 **
Albumin (g/dL)	3.50 ± 0.03	3.60 ± 0.15	0.066 (NS)
Globulin (g/dL)	1.11 ± 0.04	1.63 ± 0.05	<0.001 **

Figure (6) : Bar chart showing serological paramaters



These findings indicate that *Vibrio* infection in mullet induces marked alterations in both hematological and serological parameters, reflecting systemic stress, impaired liver function, and immune suppression.

5. DISCUSSION

Environmental conditions in the marine ecosystem have a significant effect on fish health. Nonetheless, disease development as a result of the influence of three main elements: the pathogen, the host, and environmental stressors (Austin, 2011). These pathogenic bacteria are commonly associated with the consumption of undercooked fish (Essa et al., 2015). *Vibrio* species are serious pathogens capable of causing fatal diseases in both aquatic animals and humans. In this study, relatively low prevalence rates of *Vibrio* bacteria were found with multiple external pathological lesions during the period from September 2024 to August 2025. Our data revealed that approximately 30% of the examined *Mugil cephalus* collected from the port of Benghazi exhibited signs (lesions) typical of severe *Vibrio* infection, which coincided with the bacteriological results. Our results are in line with those obtained by (Mohamed et al., 2019) who reported that 60% of the examined fish exhibited pale and hemorrhagic livers, hemorrhagic kidneys, ulcerative lesions, and focal hemorrhages. Moreover, fish displaying one or more pathological lesions consistently yielded positive cultures for *Vibrio* species. This observation is also in agreement with previous findings observed by (Elgendy, M. Y., et al., 2015) who mention that approximately 35% of *Mugil cephalus* specimens exhibited external lesions such as skin ulcers and hemorrhages at the base of the fins. Bacteriological analysis confirmed the presence of *Vibrio alginolyticus* and *Vibrio vulnificus* in the affected individuals. The biochemical profiles obtained in our study were in agreement with those obtained previously (Abdel-Tawwab et al., 2019) who found significant increases in WBCs and stress-related parameters in mullet exposed to polluted water and (Authman et al., 2013) who noted that the increases in WBCs count and hematocrit are typical stress responses, and recommended the use of hematological indices as bio indicators for environmental monitoring. The results of the serological examination are consistent with what was reported by (Sun et al., 2024), who observed that fish infected with *Vibrio alginolyticus* exhibited a high decrease in total protein and albumin levels compared to healthy fish, reflecting the impact of infection on hepatic metabolism and immune functions. Several studies have also reported that environmental stress and infection can lead to significant decreases in hematological and serum parameters in fish. For example (Gabriel et al., 2004) observed changes in blood tests in *Clarias gariepinus* exposed to environmental stress, while (Harikrishnan et al., 2003) reported decreased serum protein levels of *Labeo rohita* infected with *Aeromonas hydrophila*, suggesting impaired immune function.

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